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Nedžad Ibrišimović (1940-2011), "The Stairs", 2004, sculpture, polyester.
Sculpture in possession of Ibrišimović family. Photo by Almin Zrno.
Courtesy of Ibrišimović family.

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Epidemiology and prevention of dental caries*

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Dental caries is a chronic disease with low mortality. It is, though, a very common disease, responsible for much suffering, and is one of the most expensive diseases to treat. Within the EU, the cost of dental treatment, most related to dental caries, in 2011 was estimated to be 79 billion euro (1). Dental caries affects all ages; both primary and permanent dentitions. In many European countries, tooth extraction due to dental caries is the commonest reason for general anaesthetics in young children. Yet, it is a preventable disease; its cause is very well known but it is not prevented. Why? Because it is a diet-related lifestyle disease, where prevention requires change to entrenched, and often enjoyable, eating habits. Dental caries was rare before the rise in importation and consumption of sugar. Sugar causes much pleasure but also much disease – and not only dental caries. Strong commercial pressures encourage sugar consumption. However, there are signs that the tide is changing. Medical, dietetic and dental professions now agree on dietary policy. There is much better food labelling now in Europe, with sugars content displayed. In many countries, television advertising of high sugar products cannot be targeted at children, and provision of high sugar foods and drinks is not allowed in schools. Many parents are now well aware of what a healthy diet is and do their best to ensure their children benefit from this knowledge. However, there are many families where life's pressures – financial, availability of time and food choice, and habit – are such that this is not possible. These differences have led to increased social inequality in oral health: the major challenge to those in population oral health. Ironically, it used to be the affluent who had most dental decay; now it is the opposite. This scenario is common to several chronic diseases, often with the same risk factors operating in these several diseases. This requires, what is now well-recognised as the 'common risk

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factor' approach to controlling these costly chronic diseases (2).

Nearly a century ago, classical epidemiology demonstrated the extraordinary ability of fluoride to prevent caries development. By the 1940s, it was clear that the concentration of fluoride naturally present in water was inversely related to the occurrence of dental caries. During the second half of nineteenth century, this property was exploited and many methods developed to ensure that the benefit of fluorides was available as widely as possible. This has radically altered the picture of dental caries both between countries and within countries. In the 1950s and 1960s, dental caries severity was extremely high in northern Europe: now, in younger people, it is low to very low. The present state of dental caries in southern Europe is described in the first article on the prevalence of dental caries in Bosnia and Herzegovina in children and adolescents.

The remarkable decline in caries severity has been due, largely, to widespread use of fluoride. This is well recognised by health authorities and especially by WHO. As an example, at the Sixtieth World Health Assembly in Geneva in May 2007, a Resolution urged Member States to "for those countries without access to optimum levels of fluoride, and which have not yet established systematic fluoridation programmes, to consider the development and implementation of fluoridation programmes, giving priority to equitable strategies such as the automatic administration of fluoride, for example, in drinking water, salt or milk, and to the provision of affordable fluoride toothpaste" (3).

The second article in this Issue discusses possible approaches to prevention. It devotes space to a discussion of nutrition, diet and dental caries, partly because this is the cause of dental caries and partly because this topic is not discussed elsewhere in this Issue. The next four articles are devoted to fluoride and were commissioned by the editors of this Special Issue. The first three cover the main community, or public health, methods of delivering fluoride – through water, salt and milk. These three methods are often considered the 'automatic' methods of fluoride delivery, in that personal effort is not required by the individual and, in this respect, differs from fluoride toothpaste which is purchased by the individual. Because they are community preventive measures, reaching everyone, they are equitable (thus, reducing inequalities in disease) and economic. The next article discusses the 'home-based' use of fluoride, namely in toothpastes and mouthrinses. All of these articles emphasise that the methods described – automatic, home-care, and professional-care delivery are not alternatives, in competition with each other. On the contrary, fluoride toothpastes should be used in areas where drinking water contains the optimum concentration of fluoride – it safe to do so and it will bring added benefit. The many systematic reviews mentioned in these articles indicate the considerable knowledge base now available to ensure appropriate advice to optimise the benefits of fluoride.

The next article by Croatian pharmacological experts describes the cariostatic mechanisms of fluoride action, explaining the systemic and topical effects of fluoride on oral micro-organisms and on the teeth. A Hungarian paper discusses amine fluorides, describing several studies, and reports on excellent results with topical application of amine-fluoride-containing toothpastes and rinses on caries-risk groups in that country.

The assessment of caries risk is an important topic if prevention is to be targeted at those in greatest need, and authors from Bosnia and Herzegovina investigated the efficiency of a multifactorial model in assisting caries prediction. This is followed by a report on the caries-preventive behaviour and oral health attitudes of Czech parents with regard to their pre-school children.

Secondary prevention is important too, and the subject of incipient caries is reported in a Croatian paper concerning current possibilities in occlusal caries management and, from Turkey, a study of the effects of pre-treatment of the enamel surface on success in sealing fissures in tooth surfaces. A Bulgarian paper deals with the development of dental practices in Bulgaria during the last twenty years.

Summarizing, this Special Issue on caries epidemiology and prevention, gives an overview on the different methods of caries prevention and control, from about 10 different countries. The use of fluoride is discussed in greater detail because of its major role in caries prevention. We hope that the information given will stimulate greater effort by all in health care to reduce, if not eliminate, the scourge of oral disease.

Conflict of interest: The authors are Trustees of the Borrow Foundation.

References

1. Patel R. The state of oral health in Europe. Report commissioned by the Platform for Better Oral Health in Europe; 2012 [cited 2013 Jul 31]. Available from: http:// www.oralhealthplatform.eu/sites/default/files/field/document/Report - the State of Oral Health in Europe.pdf.
2. Rugg-Gunn AJ. Preventing the preventable – the enigma of dental caries. Br Dent J. 2001;191:478-88.
3. Petersen PE. World health Organization global policy for improvement of oral health – World Health Assembly 2007. Int Dent J. 2008;58:115-21.

Original article
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Caries prevalence of children and adolescents in Bosnia and Herzegovina

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Objective. The aim of this study was to present epidemiological parameters of caries prevalence in children and adolescents in index age groups on a national and regional level in Bosnia and Herzegovina (BH). **Materials and methods.** The study was conducted during 2004 year for children aged 6 and 12, and during 2007 for adolescents aged 15. An oral health survey was performed on a total number of 1,240 children and adolescents in line with World Health Organization methodology and criteria. Results for caries prevalence and treatment needs were presented and discussed in this paper. **Results.** Mean dmft (decay, missing, filled teeth for primary dentition) for children aged six was 6.7 (SD±3.9) in that the decayed teeth constituted the major part of the index (88.8%), followed by extracted teeth (8.9%) and a small percentage of filled teeth (2.3%). In 12-year-olds DMFT (Decay, Missing, Filled Teeth for permanent dentition) was 4.2 (SD±2.9), Significant Caries index (SiC) was 7.7 (SD±2.9), the decayed teeth constituted the major part of the index (45.4%), followed by 42.1% of FT and 12.5% of extracted teeth. Among 15-year-olds the DMFT was 7.6 (SD±4.1), SiC was 9.2 (SD±1.2), and filled teeth constituted the major part of the index. **Conclusion.** The present study provides some evidence of relatively high caries prevalence and severity in comparison with Western European countries. It is necessary to devote more attention to the oral health of children and adolescents. Community based oral health promotion, preventive programs and preventive oriented public dental health care services should be made available and accessible to all children in Bosnia and Herzegovina.

Key words: Oral health, Caries, Children, Epidemiology.

Introduction

Basic epidemiological data are used to collect information about disease prevalence and/or incidence, treatment needs of a population, and to evaluate or monitor efficiency and changes in healthcare systems. The World Health Organization (WHO) provides a specific methodology to collect epidemiological data on oral health parameters through basic oral health surveys, with clear aims to provide a systematic approach to the collection and reporting of data on oral diseases and conditions, and to ensure that data are comparable with other national surveys (1). WHO recommends conducting periodic national oral health surveys that include monitoring of ten oral health parameters by precisely defined index age groups (1). Most European countries periodically conduct oral health surveys for specific age defined population groups. Recommended age groups in pediatric population to evaluate oral health status are 5 to 6 years, 12 years and 15 years of age (1). Caries, as the most frequent dento-oral disease in children and adolescents, is in the focus of investigations of oral health status for these specific age groups. There is strong agreement that oral health in adults is a reflection of oral health status in childhood (2).

During the past 20 years, many epidemiological studies have revealed a declining trend in the prevalence and severity of oral diseases in Western European countries (3). The first epidemiological study on oral health for the population of Bosnia and Herzegovina (BH) was conducted 27 years ago (4). It was a national oral health survey using WHO methodology, conducted for the population of the former Yugoslav Republics, where BH was included as one of six Yugoslav Republics (4). Due to war and the post-war transitional period in BH, periodic nationwide oral health surveys have not been conducted. Ivankovic (5) conducted an epidemiological study in order to assess the dental health of children aged six and twelve in a part of the region of Herzegovina. There is a lack of data for all three index age groups in BH. Basic epidemiological studies on oral health of the population are necessary to assess morbidity, and to make short-term and long-term plans for the efficient organization and monitoring of the oral-health care system. Previous studies suggested children in BH to be at medium risk for dental caries development, due to bad oral hygiene maintenance, lack of preventive programs, inadequate diet control and frequency, occasional usage of topical fluoridation and changes in living condition (6, 7). Focusing on the oral health of children and adolescents, this study has been conducted to provide baseline epidemiological information of the oral health status of children and adolescents in BH. Partial results of survey have been published previously (6, 8), but results of caries prevalence for all three monitoring groups have not been published before.

The main purpose of this study was to present epidemiological parameters for caries prevalence in children and adolescents on a national and regional level in BH.

Material and methods

Timetable and study groups

The study was conducted during 2004 on children aged 6 and 12 (March, April and May), and during 2007 for adolescents aged 15 (April and May). In BH children are usually enrolled at school by the age of 6 years and have to attend primary school for 9 school years. Therefore, the majority of 12-year -old children were attending the sixth grade. Children aged 15 are in the first or second grade of secondary school, which they have to attend for 4 school years.

Study area

Bosnia and Herzegovina is made up of two entities: the Federation of Bosnia and Herzegovina (FBH) further divided into 10 cantons, and the Republika Srpska (RS). It covers an area of some 51,128 km^2, and has a population of 3,717,130 million people. In 2000, the country had 787 active dentists (6). BH is an area with low natural fluoride content in drinking water (less than 0.1 ppm) and water fluoridation is not yet conducted (6). Fluoride-toothpastes have been available on the market for many years, but studies on the frequency of their usage are lacking.

In 2004, a survey was performed for schoolchildren aged 6 and 12 in 8 locations/cantons of FBH (Sarajevo, Mostar, Gorazde, Siroki Brijeg, Tuzla, Visoko, Sanski Most and Vitez). In the Republika Srpska a survey was performed in Banja Luka in two locations The age 15 study group were children from the capital, Sarajevo, who were examined at three locations in three different secondary schools. Due to a lack of financial funds, this part of the survey was performed only in Sarajevo, considering the capital as representative of the whole country. The survey was carried out with the agreement of local authorities to perform examinations in schools.

Study sample

The oral health survey was performed on total of 1,240 children and adolescents. There were three study groups, following the index age recommended by WHO (1).

Group 1 consisted of 6-year-old children (mean 6.2, SD±0.9) attending the first grade of primary school. A total number of 560 children were examined in the following survey locations: Sarajevo,as the capital (n=160; 40 examinees on four locations), Tuzla as a large town (n=80; 40 examinees at two locations, Banja Luka as a large town (n=80; 40 examinees at two locations); followed by Mostar, Gorazde, Siroki Brijeg, Visoko, Sanski Most as small towns, but also representatives of cantons, with 40 examinees in each location. Group 2 consisted of 12-year-old children (mean 12.2, SD±0.8) attending the sixth grade of primary school. A total number of 560 children were examined, following the same distribution as the first group. Group 3 consisted of 15-year-olds (mean 15.2, SD±0.5), a total number of 120 examinees (40 examinees in three schools).

Schools were randomly selected, the first class was chosen for investigation (designated A or 1), and children from the next class (designated B or 2) were included if necessary. In each school 40 subjects were examined since the 1997 percentage of 12-year-olds without decayed teeth was 6% (5).

Assessment of oral health

One dental team visited primary schools and clinically examined all subjects in line with WHO methodology and criteria, using dental mirrors and standard CPITN-E periodontal probes, under natural light (1). Parameters used to measure oral health status were: DMFT index, presence of sealants, Community periodontal index (CPI) and developmental defects of enamel index. CPI was performed for ages 12 and 15 (1). For age 15, oral hygiene was estimated by the Plaque Index (PI) by Sillness and Loe (1964) (9), using the same index teeth that were used for CPI, and recording values with the highest score, as recommended for CPI recording. Results for caries prevalence and treatment needs were presented and discussed in this paper, as well as the results of CPI, in relationship to oral hygiene status and caries prevalence.

In the Group 1 (6-year-old children), a survey questionnaire was completed by parents/foster parents one day prior to the examination. The questionnaire included: the number of meals per day, sweet intake, and the number of episodes of tooth brushing per day, the time of first dental visits and frequency of dental visits. Answers were coded for further statistical analyses.

Examinations were performed by two investigators, trained and calibrated for recording the parameters of oral health. Training and calibration was performed on twenty-five 12-year-old subjects, who were not included in the final sample. Kappa statistics were used to test the intra-investigator reliability. The Kappa values estimated from repeat examination for the intra-consistency of the fieldwork investigator were k=0.91, and for inter-examiner reliability 0.89. One examiner clinically examined 6 and 15-year-old children, and a second examiner did examinations of 12-year-olds. Periodontal status was recorded by using the CPI, according to WHO recommendations for subjects under 20 years of age (1). Prior to the examinations written approval, from the local authorities, schools heads and parents was obtained.

Statistical analysis

Data were coded, noted on a data sheet and later saved electronically. The Statistical Package for Social Science – SPSS for Windows, version 13.0 (SPSS Inc. Chicago, Ill., USA) was used to analyze the data. Results were analyzed with descriptive and analytical statistics. Mean values, standard deviations, percentages and correlations (Spearman's correlation) were computed. The level of significance was defined as $p<0.05$.

Results

The results of caries prevalence of three study groups are given in Table 1

In a total sample of Group 1 the decay teeth component of dmft constituted the major part of the index (88.8%). 34.1% of examinees needed one surface filling on at least one tooth (T1>1.76.8% of examinees needed two or multi surface filling (T2>0), 17.8% needed endodontic treatment (T5>0), and 64.1% needed extraction due to caries (T6>0). 92.5% of examinees needed some kind of dental treatment (T0>0). Results of the mean dmft values in different survey locations are given in Table 2.

Table 1 Caries prevalence in 6, 12 and 15-year-old children

Study groups (n)	Year of study	Parameters of caries prevalence					
		dmft/DMFT ($\bar{X}$±SD)	dt/DT (%)	mt/MT (%)	ft/FT (%)	SiC ($\bar{X}$±SD)	Caries free (%)
Group 1 (560)	2004	6.7±3.8	88.8	8.9	2.3	-	6.8
Group 2 (560)	2004	4.2±2.9	45.4	12.5	42.1	7.7±2.9	9
Group 3 (120)	2007	7.6±4.0	26.4	14.2	59.3	9.2±1.2	2

dmft=decay, missing, filled teeth for primary dentition; DMFT= Decay, Missing, Filled teeth for permanent dentition; dt/DT= decay teeth; mt/MT= missing teeth; ft/FT= filled teeth; SiC= Caries Significance Index (DMFT for 1/3 examinees with the highest DMFT mean values); Caries free= percentages of examinees without caries.

Table 2 Caries prevalence in 6-year-old children in different survey locations

Survey location	Parameters of caries prevalence			
	dmft index	dt % ($\bar{X}$±SD)	mt % ($\bar{X}$±SD)	ft % ($\bar{X}$±SD)
Sarajevo	6.9 ± 4.2	89.3 (6.2±4.2)	7.2 (0.5±1.4)	3.5 (0.7± 0.5)
Banja Luka	7.1 ± 4	91.8 (6.5±3)	6.7 (0.5±0.9)	1.4 (0.4±0.4)
Sanski Most	6.9 ± 4.1	91.9 (6.3±4.2)	7.3 (0.5±1.2)	0.7 (0.3±0.5)
Tuzla	5.9 ± 3.9	90.2 (5.3±3.9)	9.1 (0.5.±1.4)	0.6 (1±02)
Visoko	6.9 ± 3.3	88 (6.1±3)	11.6 (0.8±0.9)	0.4 (0.1±0.2)
Gorazde	8.6 ± 3.2	92.2 (7.9±3.4)	7.8 (0.7±1.5)	0 (0)
Vitez	7 ± 3.8	82.5 (5.6±3.6)	13.6 (1±1.5)	3.9 (1.7±0.3)
Široki Brijeg	6.1 ± 3.3	84.3 (5.4±3.1)	15.6 (0.9±1.6)	0 (0)
Mostar	5.1 ± 2.9	79.2 (3.9±2.7)	9.4 (0.5±1.3)	11.39 (2.2±0.3)

dmft=decay, missing, filled teeth for primary dentition; dt= decay teeth; mt= missing teeth; ft= filled teeth.

In the group of 6-year-olds (Group 1) first permanent molars (FPM) were analyzed as well. In this group 81.7 % of examinees (n=455, mean 3.6±0.9) had all four FPM erupted at the time of recording the dental status. Mean value of DMFT for FPM was 0.6 ±1.1 with the highest value recorded in Gorazde 1.3±1.3, and the lowest value in Banja Luka 0.3±0.7. Mean values for other locations were within this range. Mean values of DMFT components for FPM in all examined locations were less then 1. The average number of FPM with fissure sealants in BH was 0.2±0.8, with the highest values in Siroki Brijeg (0.8±1.5), Sarajevo (0.4±0.9) and Mostar (0.4±1) at the age of six. 20% of examinees needed one surface filling on at least one FPM, and 2% of examinees needed extraction of at least one FPM at the age of six. Further more, some factors possibly associated with caries were analyzed in this study group.

From the total of 560 completed questionnaires that were returned, 344 were filled out correctly for statistical analysis (response rate of 61%). The most participants had 5-6 meals per day (61%), one sweet meal per day (45%), brushing teeth twice a day (60%), and most of them had the first dental visit between the age of 5 and 7 (48%). Spearman's correlation was used to assess the correlation between dmft and the number of meals per day, sweet intake, number of tooth brushings per day and frequency of dental visits. A weak correlation was found between sweet intake and dmft (r=0.13, p=0.31) and between dental visits and dmft (0.13, p=0.31).

Among 12-year-olds (Group 2) the DT-component (decay teeth) constituted the major part of the DMFT (45.4%) (Table 1). In different locations, DMFT was recorded in a range of 2.7 (SD± 2.2) in Sanski Most to 5.4 (SD±2.8) in Široki Brijeg, but the average value for the DT- component of the index was the lowest for Široki Brijeg (0.9±0.8) as well as the FT- component (filled teeth) being the highest (4.3±2.3) for the same location. Results of DMFT at different survey locations are given in Table 3.

Among the examined children, 5.7% had at least one tooth with a fissure sealant. In the total number of 560 examinees and 15,651 recorded permanent teeth, some kind of dental treatment (T0>0) was needed by 99.8% of examinees, fissure sealing (Tf>0) on at least one tooth was needed by 70.8 % of examinees, one surface filling

Table 3 Caries prevalence in 12-year-olds in different survey locations.

Survey location	Parameters of caries prevalence			
	DMFT index	DT % ($\bar{X}$±SD)	MT % ($\bar{X}$±SD)	FT % ($\bar{X}$±SD)
Sarajevo	3.9 ±2.9	43.9 (1.7±2.2)	12.7 (0.5±0.8)	43.4 (1.7±2.1)
Banja Luka	4.8 ± 2.9	50.9 (2.4±2.7)	10.4 (0.5±0.9)	38.7 (1.8±2)
Sanski Most	2.7 ± 2.2	56.5 (1.5±1.6)	18.5 (0.5±0.9)	25 (0.7±1.4)
Tuzla	3.5 ± 2.6	46.2 (1.6±1.7)	16.4 (0.6.±0.9)	37.4 (1.3±1.9)
Visoko	4.5 ± 2.6	65.7 (2.9±2.5)	20.2 (0.9±1.1)	14 (0.6±1.2)
Goražde	4 ± 3.1	46 (1.8±2.1)	16.8 (0.7±1.0)	37.3 (1.5±2.3)
Vitez	4.8 ± 3	45.4 (2.2±2.5)	11.3 (0.5±0.7)	43.3 (2.1±1.8)
Široki Brijeg	5.4 ± 2.8	16.3 (0.9±1.5)	3.2 (0.2±0.4)	80.5 (4.3±2.3)
Mostar	4.3 ± 3.0	42.4 (1.8±2.8)	9 (0.4±0.8)	48.6 (2.1±1.9)

DMFT=Decay, Missing, Filled teeth for permanent dentition; DT= decay teeth; MT= missing teeth; FT= filled teeth.

Table 4 Assessed periodontal condition of 12 and 15-year-olds using CPI

Study groups (n)	Percentage distribution of subjects according to the highest CPI score					
	Healthy	Bleeding	Calculus	Pockets 4-5 mm	Pockets >6 mm	Excluded sextants
Group 2 (560)	43	43	12	-	-	2
Group 3 (120)	18	38	28	14	1	0

(T1>0) by 48.5%, two or multi surface filling (T2>0) by 27.3%, endodontic treatment (T5>0) 13.9% and extraction due to caries (T6>0) by 18.7%.

Among 15-year-olds, the FT component constituted the major part of DMFT (59.3%) (Table 1). Some kind of dental treatment (T0>0) was needed by 78.8% of examinees, fissure sealing (Tf>0) on at least one tooth was needed by 63.3 % of examinees, one surface filling (T1>0) by 37.5%, two or multi surface filling (T2>0) 29.2%, endodontic treatment (T5>0) by 19.4% and extraction due to caries (T6>0) by 14.8%. Periodontal condition was evaluated for children aged 12 and 15 and the results of CPI are given in Table 4, presented as percentages of examinees.

The mean value of PI for 15-year-olds was 1.2 (SD±4), indicating poor oral hygiene (8). The most frequent CPI score in both groups was score 1 (bleeding on probing), indicating gingivitis due to poor oral hygiene. In order to observe score 1 as an indicator of poor oral hygiene status in 12-year-olds, the correlation between mean values of CPI and mean values of PI were analyzed by Spearman's nonparametric correlation. There was a positive correlation between observed parameters of CPI score 2 and PI (r=0.389, p=0.000), indicating that 43% examinees aged twelve, with recorded CPI score 1, possibly had poor oral hygiene as well.

Discussion

This study is the first to present systematically many parameters associated with caries prevalence for all specific index child population groups of Bosnia and Herzegovina. In the research conducted for some parts of Bosnia and Herzegovina in 1997, mean dmft values in children aged six were 4.8, and DMFT for children aged twelve were 6.2 (5). Comparing these figures with the results of the present survey, there was an increase in dmft from 4.8 to 6.7 in the present study and a decrease of DMFT from 6.2 to 4.2. The increase in dmft within seven years could be explained by reduced sugar consumption during the war and the post-war period, but there is no reasonable explanation for the decrease in 12-year-olds.

According to the results of the investigation of caries risk assessment in Bosnian children aged twelve, the majority of Bosnian children were at medium risk for caries development (72%) with just 20% having low caries risk (9). There were significant differences in caries risk in relation to socioeconomic status, where those with a low socioeconomic background had a higher risk of caries development (9).

In the present study, decay contributed the most to the dmft and DMFT for children aged six and twelve, indicating that untreated caries was a problem for the children investigated. According to treatment needs, there is a lack of preventive and curative treatment in primary dentition. FT were the major part of DMFT in adolescents. This reveals a curative approach to caries treatment that obviously begins after the age of six. Treatment needs for children aged 12 and 15, as well as mean DMFT values, indicated a lack of efficient disease prevention.

According to the results of the questionnaire, most participants had their first visit to a dentist between the ages of 5-7 years, and their dental status indicated the lack of any kind of dental treatment. The answers about dietary and oral hygiene habits were in line with preventive guidelines, but epidemiologic figures show a different picture. Fissure sealants, that have contributed in many countries to a decrease in caries incidence (10), had rarely been applied in our population. The results of treatment needs in the age of twelve and fifteen indicate that most young people would have serious dental problems with very possible tooth loss in early adulthood. Thompson et al. (2) concluded that oral health in adulthood was determined by oral health in children. Bleeding and calculus (CPI 1 and CPI 2) were very frequent, not only in adolescents, but also in 12-year-olds, and the mean value of PI >1 in adolescents indicates that poor oral hygiene maintenance is a common problem in our children. The relationship between CPI, PI and DMFT was tested in order to observe CPI score 1 as a possible indicator of oral hygiene level for children aged twelve. Kukletova et al. (11), in an oral health survey of 13-15-year-old adolescents in the Czech Republic, found a significant relationship between gingival index (GI) and DMFT, particularly in the DT component and between PI and gingivitis.

There were some slight differences in epidemiological figures in different survey locations and they were published previously (6). In all survey locations caries prevalence was high and it was far from acceptable. The first nationwide survey according to WHO standards on the prevalence of dental caries from this region (the region of former Yugoslavia) was carried out in 1986 (4). Results showed the prevalence of dental caries in the Yugoslav child population to be very high, with a mean DMFT for 12-year-olds of 6.1 and 9.6 for 15-year-olds. (4) The WHO Oral health database for DMFT of 12-year-olds showed similar results in the past decade for neighboring countries such as Croatia at 4.9, Serbia 7.8, the Former Yugoslav Republic Macedonia 3.0 and Slovenia 1.8 (12). Slovenia was the only former Yugoslav country where a remarkable decrease in caries prevalence was recorded (12, 13). The notable improvement of dental health in Slovenian children was explained by the estabilishment of preventive programmes, with the stress on supervised teeth brushing with concentrated fluoride gel in primary schools, improved oral hygiene, and a comprehensive programme of applying fissure sealants, particularly on first molars (13).

Declining trends in caries prevalence of children and adolescents have been reported in many European countries (3, 10, 14-16). Following WHO methodology made it possible to compare our findings with other national surveys. In 1979, the World Health Assembly accepted an important goal for oral health in children: the average DMFT in 12-year-old children should be no greater than 3.0, and 50% of children aged six had to be caries free (17). By 1995, the average DMFT for children aged twelve in six Western European Countries (Denmark, Finland; Ireland, the Netherlands, Sweden and the United Kingdom) had fallen below 2.0 (14). Twelve-year-olds represent a standard age category used by WHO to assess and compare dental caries levels in permanent dentition of children worldwide (3, 18). The mean dmft varies between 1.5 in England and Greece and 0.8 in Denmark (3). For the 15-year-olds, mean values of DMFT were in a range from 3.19 in England to 1.48 in Wales, and the lowest percentage of caries free was in Denmark at 42% (3). Findings of the present study were higher than 3.0, and for 12-year-olds at least twice as high as in Western European countries, and the percentage of caries free children aged six was 6.8. Furthermore, the SiC for

12 and 15-year-olds was twice as high as the mean DMFT. By definition, the SiC is part of DMFT, calculated on one third of population with the highest caries scores (17) and it is always higher than DMFT, but in this case it is a cause of great concern. It is obvious that BH is a country with relatively high caries prevalence. Further investigations should concentrate on revealing the specific factors that influence such oral health neglect.

Conclusions

The present study provides evidence of relatively high caries prevalence and severity in comparison with Western European countries. It is urgent to devote more attention to the oral health of children and adolescents. Poor oral health is not only a health problem, it is also a social problem. Realistic goals have to be set and oral health programs should be created and implemented for children and adolescents for the entire population, but also for high risk groups. Community based oral health promotion, preventive programs and preventive oriented public dental health care services should be made available and accessible to all BH children.

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References

1. World Health Organization. Oral Health Surveys Basic Methods, 4th ed. Geneva: WHO; 1997.
2. Thompsom WM, Poulton R, Milne BJ, Caspi A, Broughton JR, Ayers KMS. Socioeconomic inequalities in oral health in childhood and adulthood in a birth cohort. Community Dent Oral Epidemiol. 2004;32(5):345-53.
3. Oulis CJ, Tsinidou K, Vadiakis G, Mami-Homata E, Polychronopoulou A, Athanasouli T. Caries prevalence of 5, 12 and 15 year-old Greek children: A national pathfinder survey. Community Dent Health. 2012;29(1):29-32.
4. Vrbic V, Vulovic M, Rajic Z, Topic B, Tatic E, Malic M, et al. Oral Health in SFR Yugoslavia in 1986. Community Dent Oral Epidemiol. 1987;16:286-8.
5. Ivankovic A, Lukic IK, Ivankovic Z, Radic A, Vukic I, Simic A. Dental caries in postwar Bosnia and Herzegovina. Community Dent Oral Epidemiol. 2003;31:100-4.
6. Arslanagić Muratbegović A, Marković N, Zukanović A, Selimović Dragaš M, Kobašlija S, Jurić H. Oral Health Related to Demographic Features in Bosnian Children Aged Six. Coll Antropol. 2010;34(3):1027-33.
7. Zukanović A, Muratbegović A, Kobašlija S, Marković N, Ganibegović M, Bešlagić E. The relationship between socioeconomic backgrounds, caries associated microflora and caries experience in 12-year-olds in Bosnia and Herzegovina in 2004. Eur J Paediatr Dent. 2008;9(3):118-24.
8. Muratbegović A, Marković N, Kobašlija S, Zukanović A. Oral Health Indices and Molar Incisor Hypomineralization in 12-Year-Old Bosnians [In Croatian]. Acta Stomatol Croat. 2008;42(2):155-63.
9. Zukanović A, Kobašlija S, Ganibegović M. Caries risk assessment in Bosnian children using Cariogram computer model. Int Dent J. 2007;57:177-83.
10. Panagidis D, Schulte AG. Caries prevalence in 12-year-old Cypriot children. Community Dent Health. 2012;29(4):297-301.
11. Kukletova M, Izakovicova Holla L, Musilova K, Broukal Z, Kukla L. Relationship between gingivitis severity, caries experience and orthodontic anomalies in 13-15 year-old adolescents in Brno, Czech Republic. Community Dent Health. 2012;29(2):179-83.
12. WHO Oral Health Country/ Area Profile Programme, Oral health database, MalmoUniversity, Chosen Region: Europe "EURO". [cited 2013 Feb 4] Available from: http://www.mah.se/CAPP/Country-Oral-Health-Profiles/EURO/visited on: 04.02.2013.
13. Vrbic V. Reasons for the caries decline in Slovenia. Community Dent Oral Epidemiol. 2000;28(2):126-32.
14. Mullen J, McGaffin J, Farvardin N, Brightman S, Haire C, Freeman R. Caries status in 16 year-olds

with varying exposure to water fluoridation in Ireland. Community Dent Health. 2012;29(4):293-6.

15. Gorbatova MA, Grjibovski AM, Gorbatova LN, Honkala E. Dental caries experience among 12-old-children in Northwest Russia. Community Dent Health. 2012;29(1):20-4.
16. Davies GM, Jones CM, Monaghan N, Morgan MZ, Neville JS, Pitts NB. The caries experience of 11 to 12 year-old children in Scotland and Wales and 12 year-olds in England in 2008-2009: Reports of co-ordinated survey using BASCD methodology. Community Dent Health. 2012;29(1):8-13.
17. Han DH, Kim JB, Park DY. The decline in dental caries among children of different ages in Korea, 2000-2006. Int Dent J. 2010;60(5):329-35.
18. Agbaje JO, Lesaffre E, Declerck D. Assessment of caries experience in epidemiological surveys: a review. Community Dent Health. 2012;29(1):14-19.

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Dental caries: Strategies to control this preventable disease

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Objective. To provide a brief commentary review of strategies to control dental caries. Dental decay is one of man's most prevalent diseases. In many counties, severity increased in parallel with importation of sugar, reaching its zenith about 1950s and 1960s. Since then, severity has declined in many countries, due to the wide use of fluoride especially in toothpaste, but dental caries remains a disease of medical, social and economic importance. Within the EU in 2011, the cost of dental treatment was estimated to be €79 billion. The pathogenesis is well understood: bacteria in dental plaque (biofilm) metabolise dietary sugars to acids which then dissolve dental enamel and dentine. Possible approaches to control caries development, therefore, involve: removal of plaque, reducing the acidogenic potential of plaque, reduction in sugar consumption, increasing the tooth's resistance to acid attack, and coating the tooth surface to form a barrier between plaque and enamel. At the present time, only three approaches are of practical importance: sugar control, fluoride, and fissure sealing. The evidence that dietary sugars are the main cause of dental caries is extensive, and comes from six types of study. Without sugar, caries would be negligible. Fluoride acts in several ways to aid caries prevention. Ways of delivering fluoride can be classed as: 'automatic', 'home care' and 'professional care': the most important of these are discussed in detail in four articles in this issue of the Acta Medica Academica. **Conclusion.** Dental caries is preventable – individuals, communities and countries need strategies to achieve this.

Key words: Dental caries, Diet, Nutrition, Fluoride, Public health strategies.

Introduction

Dental decay is commonly called dental 'caries', from the Latin word meaning rottenness. It is one of the most common of chronic diseases and is of medical, social and economic importance. Yet its cause is well known and methods of preventing its occurrence and progression have been developed and many are practiced. The preventable is not yet prevented. The aim of this article is to provide a commentary review of strategies to control dental caries.

Size of the problem

While ancient civilisations experienced dental decay, it is largely a 'modern' disease. Dentists are lucky that teeth survive the lon-

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gest of any tissue after death: we do not have to rely on contemporary accounts of disease prevalence, we dig up our ancestors. The results of these archaeological surveys reveal that experience of dental caries was low until the nineteenth century, when it rose sharply in several European countries. This steady increase during the century 1850 to 1960 coincided with increasing importation of cane sugar from the Americas. In Britain, a defining moment occurred around 1900 when 'poor teeth' was the most important cause of rejection of volunteers for military service. This became a 'wake-up call' for those concerned with public health. For much of Europe and other 'developed' countries, 1960 to 1970 became the turning-point; after 1970 the epidemic of dental caries reduced considerably (1). As will be discussed below, this decline has been due largely to the widespread use of fluoride. Although there has been a very welcome decline in the burden of dental caries, it remains one of man's most prevalent diseases (2). Elsewhere in the world, dental caries experience has increased (Figure 1) so that, for children for example, the global average has remained almost unchanged for 30 years.

The effect of dental caries is cumulative with age, and Petersen and colleagues (3) point out that the worldwide average for people aged 65 years or more, is 22 teeth either decayed, missing or filled (out of 32 teeth). Dental caries is the most important oral disease and is of medical, social and economic importance. It is now recognised that dental caries cannot be considered in isolation – its occurrence and control depends on social environment and behaviour, at the levels of the individual and the broader community. It is recognised increasingly that oral diseases have negative impacts on general health. Table 1 shows that, in an area of north-east England without water fluoridation, over a

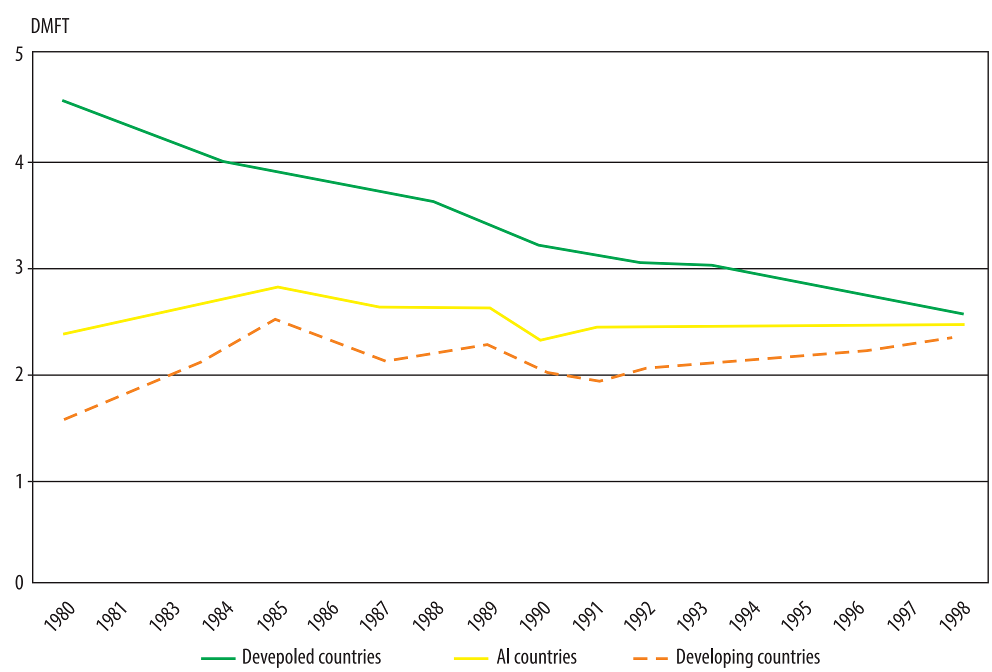

Figure 1 Dental caries severity (decayed, missing and filled teeth) in 12-year-olds between 1980 and 1998, in developed countries (top line), all countries (middle line) and developing countries (bottom line) (2).

Table 1 Percentage of 5-year-old children living in Urban and Rural areas in north-east England in who had (a) one or more dental abscess at the time of examination (point prevalence), (b) lifetime experience of one or more episode of toothache, or general anaesthetic for dental extraction. Data collected in Non-fluoridated (<0.1 mg F/litre) and Fluoridated (1 mg F/litre) communities in 1975 (4)

Experiences	Urban		Rural	
	Non-fluoridated	Fluoridated	Non-fluoridated	Fluoridated
Dental abscess (%)	3	1	5	0
Toothache (%)	40	22	38	17
General anaesthetic (%)	34	18	22	7

third of 5 year-olds had experienced toothache and a quarter to a third had had one or more general anaesthetics for dental extractions due to dental decay (4). In many countries, tooth extraction because of dental caries is the most common reason for general anaesthetics in childhood.

At the Sixtieth World Health Assembly in May 2007, a Resolution, confirmed by the Member States, emphasised that oral disease is a serious public health problem and that its impact on individuals and communities in terms of pain and suffering, impairment of function and reduced quality of life, is considerable (5). Oral disease is the fourth most expensive disease to treat. Globally, the greatest burden of oral diseases lies on disadvantaged and poor populations. The first action point in the Resolution – "urges Member States to adopt measures to ensure that oral health is incorporated as appropriate into policies for the integrated prevention and treatment of chronic noncommunicable and communicable diseases, and into maternal and child health policies." This acknowledges that most oral disease and chronic diseases have common risk factors, for example, unhealthy environments and behaviours, particularly widespread use of tobacco and excessive consumption of alcohol and sugar.

More locally, a recent report on 'The state of oral health in Europe' (6) summarised the impact of oral diseases within the EU. Discussing the economic impact of oral diseases in Europe, the first summary point was: "Oral diseases remain a major public health issue for high-income countries, where expenditure on treatment often exceeds that for other diseases, including cancer, heart disease, stroke, and dementia. This is disturbing, given that much of the oral disease burden in high-income countries is due to dental caries and its complications, and this is preventable through the use of fluoride and other cost effective measures". The report estimates that the annual cost in 2011 of dental treatment within the 27 member states of the current EU was about €79 billion. Dental disease is one of the frequent reasons for absence from school. The aetiology of these diseases is very well known, yet they are not yet prevented because of the seemingly insurmountable hurdles of commercial pressure, politics, local environments and personal behaviour.

Pathogenesis

Ancient civilizations in China, Mesopotamia and Greece believed that dental decay was caused by worms and therefore had to be treated by fumigation. This view persisted up to the time when Antony van Leuwenhoek observed, through his newly developed microscope, little worms (bacteria) in material taken from a carious tooth. Pasteur showed that sugars could be fermented to acids by micro-organisms, and Magitot demonstrated that the acids produced by fermentation of sugars were capable of destroying tooth enamel *in vitro*. In 1890, WD Miller published his chemo-parasitic theory of caries aetiology. This remains the simple explanation of the caries process: bacteria in dental plaque metabolise dietary sugars to acids which then dissolve dental enamel and dentine. A modern refinement is that the process is not continuous but cyclical. Periods of acid attack and mineral loss are interspersed with periods of remineralisation, and major tooth destruction (a cavity) only occurs if mineral loss is greater than healing. The major force to remineralise tooth tissue is saliva, which is supersaturated with calcium. This becomes clinically evident in patients with very low salivary flow (hyposalivation) – perhaps due to radiation to the head and neck or drugs – who suffer rapid, severe dental caries. The most important action of fluoride is to encourage remineralisation of demineralised enamel and dentine. Although the phrase 'dental plaque' is still used extensively, it is more correct to use the term 'dental biofilm' – the important thing is that it is 70% bacteria, forms within days if teeth are not cleaned, and adheres very well to tooth surfaces, thus keeping acid metabolites close to the tooth surface.

Possible approaches to caries prevention

Since the pathogenesis of dental caries is well understood, strategies to prevent caries development would seem to be relatively simple. Either the attacking forces can be reduced or the host resistance can be enhanced. Ways to decrease to attacking forces include: removal of dental plaque (dental biofilm), alteration of dental plaque so that it is less able to metabolise dietary sugars to acids, neutralise the acids within plaque, and remove or reduce dietary sugars. Ways to enhance host resistance include: reduce enamel's solubility in acid, increase the potential for remineralisation of demineralised enamel, cover enamel surfaces so as to put a barrier between dental plaque and enamel. All of these, and more, have been tried.

Toothbrushing is an accepted social habit, making teeth more attractive, removing mouth odours and helping to prevent periodontal disease. If done very well, toothbrushing is able to remove plaque sufficiently to reduce caries development. But the majority of people do not achieve this standard and studies have shown that brushing teeth, *per se*, does not reduce caries development; it is the fluoride in the toothpaste used which has the caries-preventive effect (7) (*vide infra*).

Unlike classical infectious diseases, which are caused by microbial pathogens, dental caries is caused by the resident oral microflora. There are contrasting opinions as to the use of chemical agents in the prevention and treatment of dental caries. Those in favour believe that chemical agents can be an adjunct to toothbrushing and dental flossing, while those opposed argue that such agents may disturb the ecological balance within the oral cavity and that resistant strains may emerge (8). Nevertheless, many chemical agents have been studied, the most successful being chlorhexidine. This cationic antiseptic is bacteriocidal and, being strongly surface-active, is particularly good at preventing microbial colonisation of clean enamel. Commercial mouthrinses commonly contain 0.2% chlorhexidine. Although such mouthrinses are effective in aiding plaque control, they are not used extensively because they discolour teeth and some tooth-coloured restorations, and alter taste sensations.

Other approaches have been the use of probiotics and immunisation. Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" and many species and strains have been studied in relation to oral health (9). Their action is to interfere with attachment and colonisation of cariogenic bacteria and to inhibit sugar fermentation, and there is some evidence that they confer some benefit. The vehicle used is commonly milk and it is fortunate that the action of probiotics and fluoride are additive (10). There was much work on immunisation against dental caries about 30 years ago but virtually none during the past 15 years. Partial protection only was achieved in monkeys following quite extensive courses of inoculation, but the possibility of cross-reaction with heart tissues made further research uninviting. A central problem is that no one plaque organism is uniquely cariogenic. The approach of reducing dietary sugars will be discussed below.

Research into increasing enamel's resistance to dental caries is nearly a century old. Eighty years ago, during the golden era of vitamin research, it was believed that dental caries was a deficiency disease, and dietary vitamins and minerals have been studied extensively (11). There is some evidence that vitamin D is protective and some evidence that caries development is inversely related to hardness of water, but any of these effects is dwarfed by the positive effect of fluoride. The unique ability of fluoride to protect against caries seems to be, in part, because it acts in several ways, all beneficial (12). First, the appropriate dose of fluoride during tooth development in childhood renders the enamel more acid-resistant – fluorapatite is less soluble than hydroxyapatite. Second, there is some evidence that fluoride alters the shape of the fissures in tooth crowns during tooth formation: this is somewhat controversial and likely to be unimportant. Third, an adequate concentration of fluoride within plaque helps to reduce demineralisation and enhance remineralisation of enamel during the dynamic caries process: this ability to encourage remineralisation is now thought to be fluoride's most important action. Fourth, adequate concentrations of fluoride within plaque reduce the ability of plaque to metabolise sugars to acids. The various ways in which fluoride can be used in caries prevention are discussed below.

If you can make saucepans 'non-stick', why not teeth? Of the various 'coatings' studied, 'fissure-sealants' have stood the test of time and are used extensively. The key to their success was the development of the 'acid-etch' technique in the 1950s (13) whereby the enamel surface is etched, washed and dried before resin is flowed onto the etched enamel and set hard. It is only practical to seal the fissures and pits in teeth since these are the most at risk sites. It is an exacting technique carried out by professional staff and is thus relatively expensive (compared with home care or community preventive methods) but the large number of trials indicate high retention rates for sealants and, thus, substantial caries prevention.

In conclusion, a great number of approaches to caries prevention have been tried. At the present time, only three are of practical importance – control of dietary sugars, fluoride and fissure-sealing. Currently, research into genetic factors associated with dental caries is active: certain genes have been shown to be associated with protection against caries (14) but the practical significance of these findings is, as yet, uncertain.

Dietary control of dental caries

Diet advice to prevent and control dental caries has a long history. For example, the ancient Greeks warned against eating over-ripe figs. However, the last century has seen much research in this area.

Dental caries a deficiency disease?

Advice about healthy eating, about fifty years ago, urged mothers to give their young children diets rich in calcium and vitamin D, so that they would form strong healthy bones and teeth: the inference was that these 'strong teeth' would be less likely to decay. Although this is sound advice as far as the skeleton is concerned, there has always been little evidence to substantiate the view that good nutrition in early life helps to prevent dental decay by a systemic effect. This certainly does not mean that good nutrition should be discouraged, it merely reflects the current view that, in developed countries, diet has a much greater effect locally in the mouth on erupted teeth than it does pre-eruptively (11). The evidence supporting the pre-eruptive role of diet now centres around just two aspects – vitamin D and fluoride (11) (*vide infra*). Under-development of salivary glands in malnourished children results in hyposalivation which, in turn, increases risk of dental caries – but this is a post-eruptive effect.

Two key experiments were published in the 1950s. In the first experiment (15), rats were fed a cariogenic (caries-inducing) diet either conventionally or by stomach tube. Those fed by stomach tube did not develop dental caries, even in a sub-group whose salivary glands had been removed, in contrast to extensive caries development in the rats fed conventionally. In the second experiment (16), rats were fed a cariogenic diet but half of them were kept germ-free while the other half lived conventionally with a mixed microbial flora. No caries developed in the germ-free rats in contrast to extensive decay in the conventionally reared animals. Thus, it became quite clear that caries development depended on microflora and food in the mouth. A third important series of experiments was published by Stephan, initially in 1940 (17). Figure 2 shows what has become known as the 'Stephan curve'. Laboratory experiments had shown that enamel dissolves when the pH falls below about 5.5. Stephan showed that the pH within dental plaque falls rapidly after exposure to sugar, from a resting value of about 7, to values below 5.5, taking about 40 minutes to return to its resting value. The slow rise back to the resting value is due to saliva, both removing the sugars and neutralising the acids, as became apparent when salivary glands were cupped. Variations of Stephan's curve experiments are still being used, albeit using more advanced methods of recording pH within plaque, to test the cariogenic potential of foods (*vide infra*).

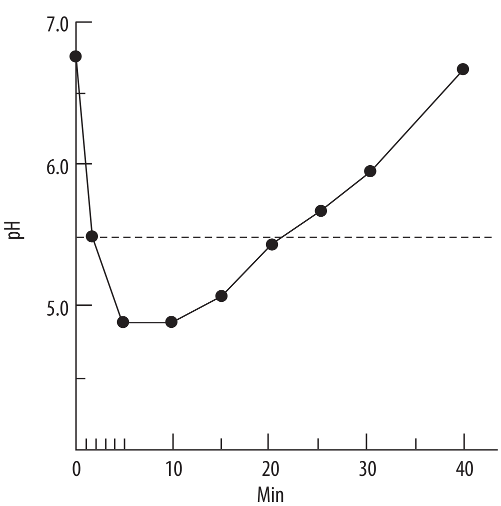

Figure 2 The Stephan curve. Plot of the pH within dental plaque at time-points after exposure to sugar. The interrupted horizontal line at pH 5.5 marks the critical pH below which enamel is at risk of dissolution.

Sugars and dental caries – types of study providing the evidence

One of the key publications which turned opinion away from 'caries is a deficiency disease' to the modern view that dietary sugar is of paramount importance in caries development appeared in 1940 (18). Since then, much research has confirmed this view. Because this has been disputed by the sugar and sugar-related industries, the evidence base will be reviewed briefly. There are six types of study providing evidence on the relationship between diet and the development of dental caries (Table 2). The best source of evidence is studies of the occurrence of caries in human populations in relation to their diet. These studies are either observational (epidemiological) studies or interventional studies. The later provide stronger evidence but are fewer in number because of the practical and ethical difficulties of inviting large groups of subjects to adhere to strict diets for a long enough time for an effect on caries increment to be observed: this time period would normally be two to four years. There have been a large number of observational studies and, since the widespread use of computers, controlling for known confounding factors has become routine. The findings of these studies in human subjects (11) will be summarised.

Table 2 Types of investigation which provide evidence on the relationship between diet and the development of dental caries (11)

Observational human studies
Interventional human studies
Animal experiments
Enamel slab experiments
Plaque pH experiments
Incubation (laboratory) experiments

There are many examples of populations who have traditionally had diets low in sugar but were then exposed to sugar through importation – for example, the Inuit, Bantu, east and west Africa, the island of Tristan da Cunha, and England. Dental caries experience was very low before exposure to sugar but high after exposure. There are groups of people who have had to consume diets low in sugar. An example is those with hereditary fructose intolerance who have to avoid consumption of fructose and sucrose: their caries experience is very low. During the Second World War, consumption of sugar was much reduced in several countries: records show that caries experience fell and then rose in parallel with sugar availability. In contrast, there are groups of people who have habitually consumed diets high in sugar – for example, workers in the confectionery industry, and children taking sugared oral medicines long-term – these have been observed to have high caries experience. There have been a large number of observational studies relating caries experience with diet in free-living children and adults. Most of these have been cross-sectional studies where caries experience and diet have been recorded concurrently once only. This is a relatively weak design since dental caries usually develops slowly over many years and diet may change from the time-period when it was influencing caries initiation to the time, several years later, when the diet was recorded. A better design is a longitudinal observational study (lasting a minimum of two years) where development of caries during that time-period is recorded and compared with diet recorded frequently during the same period of time. Possible confounding factors, such as use of fluorides and socio-economic status, can be recorded and included in data analysis. The vast majority of these studies have recorded positive associations between caries experience or development, and sugar intake – the latter may be specified as weight of sugar ingested, frequency of sugar ingestion, or consumption of specific sugar-rich food groups such as confectionery and sugared drinks.

The Vipeholm study (11) is one of the most famous in dental literature: it was carried out in a mental institute in Sweden between 1945 and 1953. There were eight test groups and one control group – the most aggressive regime was 24 toffees each day. The study would now be considered unethical. Caries development was very rapid in the groups consuming high amounts of sugar confectionery. In contrast, the Turku (Finland) study tested the effect of substituting normal dietary sugar (mainly sucrose) with fructose in one group and with xylitol in another group (11). The study lasted two years, 1972-74, and showed that caries development was virtually absent in the group consuming xylitol compared with the fairly high caries increment in the reference group who consumed their usual diet and the fructose group who developed slightly less decay than the reference group. Both the Vipeholm and the Turku studies are examples of intervention studies (11).

Most of the many animal experiments into the relation between diet and caries development were conducted before 1980. As mentioned above, they provided crucial evidence that sugar in the mouth is essential for caries development. In addition, they indicated that frequency of ingestion of sugars, independent of the amount of sugar per day, is strongly positively related to caries severity. Conversely, they also provided evidence that the amount of sugar ingested per day, independent of frequency, is positively related to caries severity.

'Enamel slab experiments' involve volunteer subjects wearing intra-oral appliances into which are inserted small pieces (slabs) of enamel: caries development in these slabs is measured when the subjects are asked to consume a variety of diets. The advantage is that very early carious changes can be observed (by a variety of instruments) so that experiments last only a few weeks. These experiments have added to the literature on the importance of frequency of exposure, different sugars and sugar-substitutes, and concentration of sugars.

Plaque pH studies are relatively simple to conduct and have been valuable in emphasising the risk of frequent sugar ingestion. They have also shown, for example, how the harmful effect of sugar ingestion might be negated by ingestion of cheese, since ingestion of cheese raises plaque pH rapidly. Fast-flowing saliva is alkaline (~ pH 7.5) and plaque pH experiments have shown that chewing sugarless gum induces salivary flow and raises plaque pH, thus encouraging remineralisation of demineralised enamel.

Incubation experiments are the simplest type of study. They are laboratory-based experiments and valuable in the past for screening foods for their ability to produce acids in the presence of plaque bacteria and the ability of minerals (e.g. calcium) to prevent dissolution of enamel during exposure to acid.

In summary, the evidence that consumption of sugars causes dental caries is overwhelming: several types of study contribute to this body of evidence. Three aspects of sugar-eating will be discussed briefly.

Type of Sugar

The most common dietary sugars are sucrose, glucose, fructose, maltose and lactose. The sugar most commonly associated with dental caries is sucrose and, indeed, it has been labelled "the arch criminal of dental caries" (11). Some, but not all, of the many studies have suggested that sucrose is the most cariogenic sugar; certainly, none is more cariogenic than sucrose. There is extensive evidence that lactose is the least cariogenic sugar, and the cariogenicity of galactose is likely to be similar to lactose. From the practical viewpoint, there is probably little to be gained by substituting glucose, fructose or maltose, for sucrose.

Frequency of consumption and the total amount consumed

There is good evidence that frequency of ingestion of dietary sugars influences the severity of caries development. From the curve shown in Figure 2, it can be understood that demineralisation of enamel can occur each time sugar is ingested – if there are 10 sugar intakes a day, enamel stands to be attacked 10 times a day. The main conclusion from the Vipeholm study (*vide supra*) was that caries severity was strongly related to frequent ingestion of sugar. However, there is also much evidence that the amount of sugar ingested per day is positively related to caries severity. Most of the large number

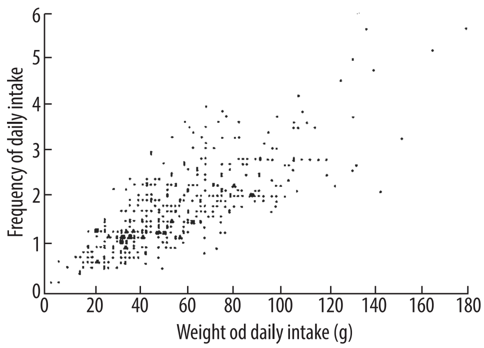

Figure 3 Plot of frequency of intake per day against the weight consumed per day, of confectionery, by 405 12-14-year-old children in north-east England (11).

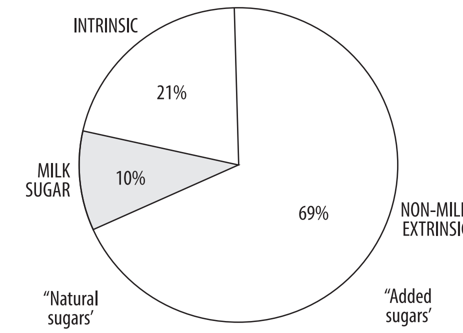

Figure 4 Dietary sugars intake in 12-14 year-old children living in north-east England (21). Mean daily intake of all sugars was 118 g.

of observational studies mentioned above, recorded amount of sugar, or sugar-rich foods, rather than frequency of ingestion. Carefully controlled animal experiments indicate that both variables – frequency and amount – are important. However, from the practical point of view, it probably does not matter if advice is to cut down frequency or amount since, in free-living people, there is a close relation between frequency and amount. Figure 3 is a plot of frequency of daily intake of confectionery against amount of confectionery consumed – the correlation was +0.77.

Sources of dietary sugars

When giving advice about how to reduce sugar consumption, it is useful to know which part of the diet to target. First, it should be appreciated that some foods contain sugars naturally – these include milk (lactose), fruit and vegetables. Much sugar, though, is added to foods. These distinctions are important and have led to the definition of three types of sugar (19): (i) 'intrinsic sugars' – those within the structure of the food – e.g. fresh fruit and vegetables, (ii) 'milk sugars' – lactose, and (iii) 'non-milk extrinsic sugars' – sometimes called 'added sugars' and by WHO 'free sugars' (20). In a study of UK adolescents, over two-thirds of total sugars intake (90 g out of 118 g) were non-milk intrinsic sugars as can be seen in Figure 4 (21).

Table 3 shows where these 90 g of non-milk extrinsic sugars came from: 60% came from just two sources – confectionery and soft drinks. It should be noted that both of these are marketed for frequent consumption (snacking) and it is therefore not surprising that these two are targeted heavily in health promotion.

Table 3 Mean daily intake of non-milk extrinsic sugars (19) ('free sugars' (20); 'added sugars') from various dietary sources (as grams and as percentage) in a survey of 379 12-year-old English children in 1990 (21)

Various dietary sources	Added sugars (g; %)
Confectionery	30 (33)
Soft drinks	24 (27)
Table sugar	11 (12)
Biscuits and cakes	10 (11)
Sweet puddings	5 (6)
Breakfast cereals	5 (5)
Syrups and preserves	2 (2)
Other sources	3 (4)
All sources	90 (100)

Dietary starch and dental caries

It is not uncommon to see advice that dental caries is caused by 'dietary carbohydrates'.

This is convenient for those promoting sugar products, as it takes the spotlight off dietary sugars. Dietary carbohydrates are, in broad terms, sugars and starches (excluding 'fibre'). A review of the evidence shows that dietary starches are not cariogenic, certainly compared with dietary sugars (11). If finely ground and heat-treated, starch can cause dental caries, but the amount is less than that caused by sugars. Cooked staple starchy foods, such as rice, potatoes and bread are of low cariogenicity. This view is supported by the WHO (20) who, when considering the strength of the evidence linking diet to dental caries, stated: (a) the evidence was 'convincing' for 'increased risk' of caries from 'amount of free sugars', and 'frequency of free sugars', and (b) there was 'no relationship' between dental caries and 'starch intake (cooked and raw starch foods, such as rice, potatoes and bread)'. The advice for oral health and general health are in agreement – increase consumption of staple starchy foods and decrease consumption of 'free' ('added' or non-milk extrinsic) sugars.

Fruit and milk

Fruit and milk contain sugars and, thus, could be considered cariogenic. To summarise the evidence (11): as eaten by humans, fresh fruit appears to be of low cariogenicity; sugared, fruit-flavoured drinks when used as a comforter are a significant cause of dental caries in young children; and there is insufficient evidence regarding pure fruit juice and caries, most of the studies looked at sugar-containing fruit-flavoured drinks. The WHO (20) classified the evidence as 'no relation' between 'whole fresh fruit' and dental caries.

Cow's (bovine) milk contains about 4.8 g lactose per 100 g, thus having the potential to cause dental caries. However, milk also contains factors which protect teeth; these are mainly the high calcium content (about 125 mg/100 ml) and proteins, particularly casein. The result is that milk is classed as non-cariogenic. In some experiments, milk has been shown to prevent dental caries and, indeed, WHO (20) classifies the strength of evidence relating milk to 'decreased risk' of caries as 'possible'. Thus, neither fruit nor milk are seen as a threat to oral health, and this is the reason for classifying them separately (as 'intrinsic' and 'milk' sugars) from non-milk extrinsic sugars.

Non-sugar sweeteners

A number of confectionery companies have tried to overcome the problem of sugar-containing (and therefore cariogenic) products by substituting non-sugar sweeteners for sugars. Foremost amongst these have been manufacturers of chewing gum. The sweeteners used include sorbitol, xylitol, mannitol and maltitol (11). Evidence indicates that these sweeteners are non-cariogenic. It would appear that xylitol has better dental properties compared with the other sweeteners (22). The dental benefits of chewing sugarless gum (commonly containing xylitol) have been investigated extensively in Scandinavia, particularly Finland (23). This is promoted because the chewing stimulates saliva flow, thus encouraging remineralisation of dental enamel. WHO (20) classifies the strength of evidence for 'decreased risk' of caries for 'sugars-free chewing gum' as 'probable'. Manufacturers of carbonated soft drinks have marketed 'sugar-free' or 'no calorie' drinks for many years. These contain intense sweeteners, which are non-cariogenic (11). However, these drinks are acid and are strongly linked to erosion of dental enamel.

Summary and implications for health policies

It is clear from the above brief review that what we eat dictates whether or not we develop dental caries. Consumption of foods and drinks rich in sugar increases risk considerably. Staple starchy foods, fresh fruit, vegetables and milk are not a threat to teeth. Dietary fat and protein are not metabolised to acids within the mouth. Thus, dietary advice for oral health is completely compatible with dietary advice for general health (20). There is growing evidence that non-milk extrinsic sugars increase risk of diseases other than dental caries, either independently or via increased risk of obesity (20). Strategies to reduce sugar consumption need to be robust because there are strong forces encouraging consumption of foods and drinks high in sugar: the advertising budgets for the confectionery and soft drinks manufacturers are very large. There are signs that diets of children are improving (24) and it is worth mentioning the following initiatives. First, food and drink in school should conform to standards: confectionery is not sold and the only drinks allowed are water, milk and pure fruit juice. Second, advertisements on television displayed at times when children may be watching, should not encourage purchase and consumption of high sugar foods and drinks. Third, foods and drinks for sale must be labelled for nutrient content including sugars content. In addition, manufacturers should agree to their food products carrying 'traffic-light' labels, indicating whether a product is high (red) or low (green) in fat, salt, sugar, and energy. A tax on products high in sugars has been discussed but not yet implemented. Further information may be obtained from published reviews of diet and dental disease (25, 26).

Fluoride

It was stated in the first section of this article that, in many countries, caries severity reached its zenith around the 1950s and 1960s, and then declined, markedly so in several countries. This decline was almost certainly due to the increased use of fluoride, particularly the introduction of fluoride toothpastes. The story of the recognition and exploitation of fluoride's ability to prevent, partially, caries development is long and interesting (12). The 1930s and early 1940s was a time when the inverse relationship between fluoride concentration in drinking water and dental caries experience became apparent, and the first water fluoridation scheme began in the USA in 1945. The subsequent story of water fluoridation is the subject of an article in this issue (27).

It wasn't long before fluoride was added to vehicles other than water, leading to much research and the production of many effective fluoride agents. The course of research was not always easy, as the first fluoride-containing toothpastes were ineffective due to the incompatibility between the added fluoride (as sodium fluoride) and the calcium-based abrasives (12). Alternative abrasives and fluoride compounds (e.g. sodium monofluorophosphate) overcame these difficulties. Another line of research was the application of concentrated solutions to tooth surfaces to make them more resistant to caries attack. At the same time, the success of water fluoridation led to experiments adding fluoride to domestic salt, milk, flour and even sugar – only salt and milk have stood the test of time, and these are discussed in detail in later articles in this issue (28, 29).

Table 4 lists the fluoride vehicles currently in use: these are grouped as those which provide fluoride 'automatically', those suitable for home care by the individual, and those which require application by a health professional. Water, salt and milk fluoridation are suitable for community prevention. Their advantages include low cost and little if any personal effort by the individual benefitting from the programme. To some extent, they can be targeted at communities most in need. These advantages have been a tremendous help in trying to combat health inequalities. The concentration of fluoride in water, salt or milk is decided taking background fluoride exposure and climate (since water consumption increases in hot climates) into account.

Table 4 Methods of delivering fluoride

Automatic	Home care	Professional care
Water	Toothpaste	Solutions
Salt	Mouthrinse	Gels
Milk	Tablets	Varnishes
-	-	Slow-release devices

These three vehicles differ from the other vehicles (Table 4), in that the fluoride in water, salt and milk is ingested. This may bring added benefit but it means that only one of these so-called 'systemic' methods should be used in any community. An example of this, and the way they can be used appropriately on a population basis, can be seen in Chile. Here, the national policy is for water fluoridation and 70% of the Chilean population receives fluoridated water. In many rural areas, water fluoridation is not technically possible, and the national policy is for children in these communities to receive fluoridated milk in school. In this way, the whole population is covered. In addition, of course, the whole population is encouraged to use fluoride-containing toothpastes, since the separate preventive effects of fluoride in water and fluoride in toothpaste are additive.

The groupings in Table 4 are somewhat flexible. For example, fluoride mouthrinsing has been used, and still is, in several countries as a community preventive measure – children rinsing daily or weekly in school. Likewise, there are school-based toothbrushing programmes. School-based fluoride tablet programmes were common, especially in Eastern Europe, but there are few such programmes now.

Professional application of fluoride has a long history (12). Advantages are that applications are infrequent (two to four applications per year), and you know that the application has been done. Disadvantages include: the cost is high as professional time is used, and effort to attend the appointment is needed. Professional fluoride applications, therefore, tend to be targeted at those in greatest need. Home-based and professional fluoride use will be discussed in a later article in this issue (30).

Concluding comments

From the above discussions, it can be seen that dental caries can be prevented. The fact that it remains a prevalent, expensive disease, of medical and social importance, is deeply frustrating. This preventable disease is not yet prevented. In theory, dietary control of sugar could assign caries to the 'rare disease' category, as it was in millennia past. But sugar consumption has become integral to our daily life, encouraged by massive marketing. But progress is being made: health promotion in many countries has made people aware of the desirability of reducing sugar intake and it is now less socially-acceptable to 'take sugar'. An important step was that relevant health professionals – medical, dental and dietetic – agreed what dietary messages should be. Governments have made well-documented and authoritative statements about nutrition and diet, which have been applied at national, community and individual levels. For once, the sugar industries are 'on the back foot'. Progressive, coordinated effort will be needed to continue progress to better diets in many countries.

The picture of dental caries in the 1950-60s was bleak, particularly in northern Europe and Australia; the wave continued in other European countries and South America. The widespread use of fluoride has much reduced this epidemic. This issue will discuss its use and its future. There is no doubt that fluoride is underused. Toothpaste use is less than half what would be required if all dentate people brushed their teeth twice a day. Water fluoridation is a low-cost, very effective, socially-equitable preventive measure yet, for a variety of reasons, it is very much underused in Europe. It is important that each country has a strategy to decrease the burden of dental caries through nutritional policy and appropriate use of fluorides.

Nutritional policy and appropriate use of fluorides is best decided at the national level. Nutritional policy for oral health is wholly compatible with nutritional policy for general health, as indicated above, and it is important that national authorities deciding such policy include expert(s) on nutrition and oral health. Part of that policy should be to ensure that dental personnel in that country are fully aware of policy, their role in promoting good nutrition in the population and know how to give nutritional advice to patients of all ages. The success of these policies needs to be monitored by regular surveillance of activities of dental personnel and by assessing the dietary habits of the population.

Decisions regarding national fluoride policy will depend on disease levels, existing fluoride exposure and resources available: these need to be assessed. Countries with large urban populations usually have centralised piped water supplies, and these would be more economic to fluoridate than water supplies for small, rural communities. The implementation of fluoridation of water, salt and milk, and the resources needed for each, will be discussed in subsequent articles in this issue. When community preventive schemes are introduced, it is important that adequate resources are identified to measure the effectiveness of these programmes: WHO recommends that 10% of the budget is devoted to evaluation. It is important to recognise that efforts to improve nutrition and maximise the appropriate use of fluoride are not alternatives; both are important to the improvement of oral health.

Conflict of interest: The author declares that he has no conflict of interest.

References

1. Marthaler TM. Changes in dental caries 1953-2003. Caries Res. 2004;38:173-81.
2. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century – the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol. 2003;31(Suppl 1):3-24.
3. Petersen PE, Kandelman D, Arpin S, Ogawa H. Global oral health of older people – call for public health action. Community Dent Health. 2010;27(Suppl 2):257-68.
4. Rugg-Gunn AJ, Carmichael CL, French AD, Furness JA. Fluoridation in Newcastle and Northumberland: a clinical study of 5-year-old children. Br Dent J. 1977;142:395-402.
5. Petersen PE. World Health Organization global policy for improvement of oral health – World Health Assembly 2007. Int Dent J. 2008;58:115-21.
6. Patel R. The state of oral health in Europe. Report commissioned by the Platform for Better Oral Health in Europe; 2012. [cited 2013 January 25]. Available from: http://www.oralhealthplatform.eu/sites/default/files/field/document/Report%20-%20the%20State%20of%20Oral%20Health%20in%20Europe.pdf
7. Nyvad B. The role of oral hygiene. In: Fejerskov O, Kidd EAM, editors. Dental caries. Oxford: Blackwell Munksgaard; 2003. p. 171-7.
8. Scheie AAa. The role of antimicrobials. In: Fejerskov O, Kidd EAM, editors. Dental caries. Oxford: Blackwell Munksgaard; 2003. p. 179-88.
9. Meurman JH. Probiotics and oral health. In: Wilson M, editor. Food constituents and oral health. Cambridge: Woodhead; 2009. p. 421-32.
10. Petersson LG, Magnusson K, Hakestam U, Baigi A, Twetman S. Reversal of primary root caries lesions after daily intake of milk supplemented with fluoride and probiotic lactobacilli in older adults. Acta Odont Scand. 2011;69:321-7.
11. Rugg-Gunn AJ. Nutrition and dental health. Oxford: Oxford Medical Publications; 1993.
12. Murray JJ, Rugg-Gunn AJ, Jenkins GN. Fluorides in caries prevention. 3rd ed. Oxford: Butterworth-Heinemann; 1991.
13. Rugg-Gunn AJ. Dental caries. In: Welbury RR, editor. Paediatric dentistry. 2nd ed. Oxford: Oxford Medical Publications; 2001. p. 95-114.

14. Wang X, Willing MC, Marazita ML, Wendell S, Warren JJ, Broffitt B, et al. Genetic and environmental factors associated with dental caries in children: the Iowa fluoride study. Caries Res. 2012;46:177-84.
15. Kite OW, Shaw JH, Sognnaes RF. The prevention of experimental tooth decay by tube-feeding. J Nutrit. 1950;42:89-103.
16. Orland FJ, Blayney JR, Harrison RW, Reyniers JA, Trexler PC, Wagner M, et al. Use of the germ-free animal technic in the study of experimental dental caries. J Dent Res. 1954;33:147-74.
17. Stephan RM. Changes in hydrogen-ion concentration on tooth surfaces and in carious lesions. J Am Dent Ass. 1940;27:718-23.
18. Jay P. The role of sugar in the etiology of dental caries. J Am Dent Ass. 1940;27:393-6.
19. Department of Health. Dietary sugars and human disease. Report on health and social subjects 37. London: HMSO; 1989.
20. World Health Organization. Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consultation. WHO technical report series 916. Geneva: WHO; 2003.
21. Rugg-Gunn AJ, Adamson AJ, Appleton DR, Butler TJ, Hackett AF. Sugars consumption by 379 11-12 year old English children in 1990 compared with results in 1980. J Hum Nutr Diet. 1993;6:419-31.
22. Maguire A, Rugg-Gunn AJ. Xylitol and caries prevention – is it the magic bullet? Br Dent J. 2003;194:429-36.
23. Mäkinen KK. Oral care gum products. In: Wilson M, editor. Food constituents and oral health. Cambridge: Woodhead; 2009. p. 433-54.
24. Fletcher ES, Rugg-Gunn AJ, Matthews JNS, Hackett A, Moynihan PJ, Mathers JC, et al. Changes over 20 years in macronutrient intake and body mass index in 11- to 12-year-old adolescents living in Northumberland. Br J Nutr. 2004;92:321-33.
25. Moynihan PJ. Dental Disease. In: Geissler CA, Powers HJ, editors. Human nutrition. 11th ed. Edinburgh: Elsevier; 2005. p. 461-76.
26. Moynihan PJ. Dietary advice in dental practice. Br Dent J. 2002;93:563-8.
27. Harding MA, O'Mullane DM. Water fluoridation and oral health. Acta Med Acad. 2013;42:131-39.
28. Marthaler TM. Salt fluoridation for the prevention of dental caries – its role and potential. Acta Med Acad. 2013;42:140-55.
29. Bánóczy J, Rugg-Gunn AJ, Woodward M. Milk fluoridation for the prevention of dental caries. Acta Med Acad. 2013;42:156-67.
30. Rugg-Gunn AJ, Bánóczy J. Fluoride dentrifices and fluoride mouthrinses for home use. Acta Med Acad. 2013;42:168-78.

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Water fluoridation and oral health

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Water fluoridation, is the controlled addition of fluoride to the water supply, with the aim of reducing the prevalence of dental caries. Current estimates suggest that approximately 370 million people in 27 countries consume fluoridated water, with an additional 50 million consuming water in which fluoride is naturally occurring. A pre-eruptive effect of fluoride exists in reducing caries levels in pit and fissure surfaces of permanent teeth and fluoride concentrated in plaque and saliva inhibits the demineralisation of sound enamel and enhances the remineralisation of demineralised enamel. A large number of studies conducted worldwide demonstrate the effectiveness of water fluoridation. Objections to water fluoridation have been raised since its inception and centre mainly on safety and autonomy. Systematic reviews of the safety and efficacy of water fluoridation attest to its safety and efficacy; dental fluorosis identified as the only adverse outcome. **Conclusion:** Water fluoridation is an effective safe means of preventing dental caries, reaching all populations, irrespective of the presence of other dental services. Regular monitoring of dental caries and fluorosis is essential particularly with the lifelong challenge which dental caries presents.

Key words: Water fluoridation, Effectiveness, Dental caries, Fluorosis.

Introduction

Dental Caries (tooth decay); a transmissible infectious disease with a multifactorial aetiology has affected individuals for centuries at least since the seventh century and the Iron Age (1). Change in the distribution of dental caries intra-orally has occurred, as has the dominant paradigm in managing dental caries. A much greater emphasis is now being encouraged towards prevention rather than curative care. Community factors are currently the focus of considerable research internationally. Social gradients in caries are demonstrated and neighbourhood factors such as where we live and the foods and the fluorides we have access to, all exert an impact on the development of the disease (2). Dental caries is still a major oral health problem in many established economies, affecting 60-90% of schoolchildren and the vast majority of adults. It is also a prevalent oral disease in several Asian and Latin-American countries, while it appears to be less common and less severe in many African countries (3). In the US it has been described as the most common chronic disease of childhood (4). Untreated dental caries can lead to pain, infection, impaired function, poor aesthetics, and diminished quality of life, which equate to a significant human, financial, psychological and emotional cost.

Water fluoridation is described as the controlled addition of fluoride to the water supply with the aim of reducing the prevalence of dental caries. Fluoride can also occur naturally in some water supplies. Current estimates are that 370 million people in 27 countries are currently supplied with artificially fluoridated water and 50 million around the world are drinking naturally fluoridated water (5).

This paper will discuss water fluoridation under the following headings: Background, the mode of action, the effectiveness, the risks and benefits, the monitoring of water fluoridation and the legislative nature of providing communities with water fluoridation.

In the review baseline and subsequent national oral health surveys conducted in Ireland are included to demonstrate the effectiveness of water fluoridation and the challenges to water fluoridation; in the Republic of Ireland (RoI) 73% of the population presently benefit from water fluoridation. Thus providing an appropriate example for Europe (6, 7, 8).

The terms part per million, ppm and mg/l are used rather than the SI unit for fluoride in water μg/ml, to conform to previous research.

Background

Water fluoridation is an ideal public health measure in reducing dental caries; since its effectiveness does not require conscious daily cooperation from individuals (9) The beneficial effects of natural water fluoridation in caries prevention was identified in the first part of the 20th century and is undoubtedly a significant landmark in dentistry (10, 11, 12), culminating in the introduction of artificial water fluoridation to the pioneering public health city of Grand Rapids, Michigan (13). In the second part of the 20th century, to address the high prevalence of dental caries water fluoridation was introduced to many countries, including Ireland, Australia, Hong Kong, Israel, New Zealand, Singapore, and the UK.

Mode of Action

The mode of action of fluoride in the prevention of dental caries is predominantly post-eruptive; however, the pre-eruptive effect of ingested fluoride is also important. Findings from Australia, the Netherlands and Maryland support the pre-eruptive effect of fluoride in reducing caries levels in pit and fissure surfaces of permanent teeth. Research has also indicated that exposure to fluoridated water from birth produces the maximum benefit (14, 15). What is clear is that a constant low level of fluoride ion in saliva and plaque fluid reduces the rates of enamel demineralisation during the caries process and promotes the remineralisation of early caries lesions (16, 17). Fluoride concentrated in plaque and saliva inhibits the demineralisation of sound enamel and enhances the remineralisation of demineralised enamel.

The effectiveness of water fluoridation

The Centers for Disease Control and Prevention (CDC) have recognised water fluoridation as one of the ten great public health measures of the twentieth century (12). The extensive international research demonstrating the effectiveness of water fluoridation is summarised in a number of important texts (18, 19), recently Rugg-Gunn and Do (20) presented the international studies attesting to the effectiveness water fluoridation published between 1990 and 2010, the reader is referred to these sources for a review of the many international studies. The number of studies which were conducted since 1990 has declined; newer studies have tended to be pragmatic with the statistical analyses taking account of confounding factors (20, 21). Despite an overall reduction in the number of countries and studies represented the number of studies from Brazil and Australia had increased, both countries having extensive water fluoridation (5, 20). All studies demonstrate a similar positive reduction in per cent caries reduction.

Sources suggest that water fluoridation is not only effective in childhood but also into adulthood (22, 23). Water fluoridation combined with toothpaste use could be more effective than either alone (24).

Water fluoridation in Ireland

The fluoridation of water supplies in Ireland is indicative of the effectiveness, the benefits of, the required monitoring and challenges that may occur after implementation (25). In the mid twentieth century the RoI required a solution to the effects of widespread dental caries and introduced water fluoridation to Dublin on July 15th 1964, and to Cork in May 1965 the planned introduction being delayed by some four years due to legal challenges in both the High and Supreme Courts (26). By 1970 the majority of cities and larger towns were fluoridated. Under the legislation directing water fluoridation (27) provision was made that, before implementation of the Act a baseline survey of caries levels among children and adolescents would be undertaken (6). The Act also importantly stipulated that regular caries surveys be undertaken "whenever and as often as the Minister requires" to monitor the effectiveness of fluoridation of water supplies in controlling dental caries.

The baseline surveys conducted prior to water fluoridation indicate a high caries experience; this was recorded as the number of teeth which were decayed, missing or filled because of tooth decay. They were recorded using the dmf/DMF index for both the primary (baby teeth) (dmf), and permanent (adult) (DMF) dentitions in 5-year-old to 15-year-old children (6, 28) (Table 1). Once the fluoridation of water supplies commenced the concentration of fluoride in water was set in the range 0.8 to 1.0 ppm, with a target of 0.9 ppm.

National survey of children's oral health (Republic of Ireland) – 1983-84

In 1982 the Department of Health in the RoI commissioned a National Survey of Children's Dental Health, the primary aim of which was to measure the effectiveness of water fluoridation on a countrywide basis, it was also decided that levels of enamel fluorosis would be recorded, using internationally accepted indices (28, 29). Random sam-

Table 1 Mean dmft* in five-year-olds, and DMFT* in 15-year-olds, in fluoridated communities (full Fl) in the Republic of Ireland in 1984 and 2002, and in non-fluridated communities (non Fl) in the Republic of Ireland and Northern Ireland in the 1960s, 1983-84 and 2002 (6, 7, 8)

Year	5-Year-Olds			15-Year-Olds		
	Full Fl	Non Fl		Full Fl	Non Fl	
	RoI	RoI	NI	RoI	RoI	NI
1960	-	5.6	4.8	-	8.2	10.6
1983 – 1984	1.8	3.0	4.5	4.1	5.4	9.2
2002	1.3	1.7	1.8	2.1	3.2	3.6

Fl = fluoridated; RoI = Republic of Ireland; NI = Northern Ireland; dmf = decayed missing filled primary (teeth). DMF refers to permanent teeth.

ples of children who were lifetime residents of either fluoridated or non-fluoridated areas and aged five, eight, 12 or 15 years-old were examined by 10 examiner/recorder teams (7). The criteria adopted for dental caries examination were similar to those used in the baseline studies of 1961-1963 (6) thus permitting comparison. The results indicated a decline in caries levels for children in both fluoridated and non-fluoridated areas; the decline being considerably greater in fluoridated areas, fluorosis was measured using Dean's index of fluorosis, the teeth scored for fluorosis were the upper permanent incisors (29). The children who were resident in non-fluoridated areas had a significantly higher dmf/ DMF than those in fluoridated areas (Table 1).

The observed downward trend in dental caries has been noted in many international studies; the advent of fluoridated toothpastes in the 1970's providing a valued contribution (30). In the national survey in 1983-'84 (7) the prevalence of fluorosis was low, with 94% of children in fully fluoridated communities having normal enamel according to Dean's Index (29), compared with 98% among eight-year-old children in non-fluoridated communities (Table 2). Only fluorosis grades of 'questionable' and 'very mild' were recorded in the survey (7, 8, 31).

The North south survey of children's oral health – 2002

In 2000 under a contract for the evaluation of oral health services the Department of Health commissioned a further national survey of children's dental health, with the aim of monitoring the effectiveness of water fluoridation (8).The study included a contemporaneous survey of children's dental health in Northern Ireland (NI), where water fluoridation has not been introduced (31). The diagnostic criteria for both caries and dental fluorosis were the same as used in the 1984 study (7). It was seen that in the period from 1983-1984 to 2002 there was a substantial reduction in dental caries in both fluoridated and non-fluoridated communities in the RoI, and in the non-fluoridated population of NI; the reduction in the period from 1983-'84 to 2002, is greater in fluoridated communities. In the five-year-old age group, the mean dmft among the lifetime residents of fluoridated communities in the RoI declined from 1.8 in 1983-'84 to 1.3 in 2002, the corresponding figures for five-year-old children in non-fluoridated areas in the RoI were 3.0 and 1.7, and in NI were 4.5 and 1.8 respectively. Similar trends are apparent in the figures recorded for caries among 15-year-olds in both jurisdictions (Table 1).

Table 2 Dean's Index of Fluorosis*- % of eight-year-olds affected according to fluoridation status in the Republic of Ireland and Northern Ireland in 2002 and 1984 (7, 8)

Eight-Year-Olds	Full Fl	Non Fl	Full Fl	Non Fl	Non Fl
	RoI	RoI	RoI	RoI	NI
	1984 (a)	1984 (b)	2002 (c)	2002 (d)	2002 (e)
Normal	94	98	76	90	90
Questionable	5	2	11	7	6
Very Mild	1	0	8	2	3
Mild	0	0	4	0	0
Moderate	0	0	0	0	0
Severe	0	0	0	0	0

*Scores relate to permanent maxillary incisor teeth; RoI= Republic of Ireland; NI= Northern Ireland; The difference between a and c, c and d and c and e were significant ($p<0.0001$).

The inverse occurred with the prevalence of dental fluorosis and fluorosis increased in the RoI between 1983-1984 and 2002, particularly in residents of communities with water fluoridation. In 1983-'84, ninety-four percent of children residing in fluoridated communities in the RoI had normal enamel; this figure had reduced to 76% in 2002 (Table 2). The figures for 'questionable', 'very mild' and 'mild' fluorosis in 1983-1984 were 5%, 1% and zero, respectively; these figures had increased in 2002 to 11%, 8% and 4%, respectively. The increasing prevalence of fluorosis was also identified internationally (32, 33).

The benefit of water fluoridation

Water fluoridation has been the subject of rigorous reviews of late and has been recognised as safe and as the most effective method of reaching the whole population irrespective of access to dental services in this way each individual can benefit without the need for active participation (24, 34, 35). In addition the review of McDonagh et al. (24) suggested water fluoridation conferred a benefit over and above the use of fluoride containing toothpastes alone. The process of water fluoridation has been endorsed by the world's leading science and health organisations including the WHO (36), IADR (International Association of Dental Research) and FDI (World Dental Federation), with the benefits available to both child and adult (22, 23). Griffin et al. (37) showed that for the US water fluoridation delivered significant cost savings.

The risk of water fluoridation

Dental fluorosis is recognised as a consequence of consuming fluoridated water supplies.

It arises as a result of a long-term intake of fluoride during the preeruptive development of teeth. It is a hypomineralisation of enamel characterised by an increased surface and subsurface porosity causing opacity, pitting or staining of the enamel (38).

However water fluoridation since its initiation has attracted hostile publicity, those who do not put a value on water fluoridation caution that it is; costly and not effective, that it impacts negatively on general health; causes objectionable dental fluorosis and that it is a violation of medical ethics and the rights of the individual (39). Thus the very core of its capacity to promote prevention to the whole population is challenged.

All of the reviews conducted on water fluoridation have acknowledged that fluorosis occurs to some degree with water fluoridation, and fluorosis was identified as the only adverse effect of fluoridation (33). The likelihood of fluorosis occurring was identified at the very outset, for it was McKay's observation of the Colorado 'brown stain' that led to the identification of the beneficial effect of fluoride in the prevention of dental caries and was acknowledged in the work of Dean and the '21 cities study' (10). The environmental concerns which have been raised with regard to fluoride were recently addressed in the report of the European Commission's Scientific Committee on Health and Environmental Risks (SCHER) (40). The committee did not identify any evidence of negative environmental impacts from community water fluoridation. Ethical concerns were addressed by the Nuffield Council on Bioethics (41, 42, 43). Reviews conducted in the US, Australia, and Canada arrived at similar conclusions (35, 44, 45). Nevertheless it is crucial that on-going surveillance of general health be maintained in fluoridated and non-fluoridated communities. The structured use of health registers, for example cancer and hip fracture registers, are an important source of information for this purpose.

Monitoring

The studies conducted in Ireland (7, 8) established there was a decline in dental caries after the fluoridation of water supplies and also an increase in dental fluorosis. Good practice recommends the recording of the fluoride concentration in water supplies on a regular basis, daily, weekly, monthly and strategies must be in place to notify the relevant authorities of the measurements that are recorded. Audit is possible when the agency fluoridating supplies is not the same agency. In Ireland the sanitary authorities have responsibility for the addition of fluoride to water supplies while the health authorities and environmental protection agency have responsibility for monitoring the concentration of fluoride in supplies (26, 46). This also ensures agencies are compliant with legislation and regulation.

Regular monitoring has led to changes in fluoride concentration internationally. When the prevalence and severity of fluorosis between the two national surveys (7, 8) was compared in Ireland (7, 8), (Table 2), the prevalence had increased. Consequent on these findings in 2007 the level of fluoride in drinking water was reduced from a range of 0.8 to 1.0 ppm, with a target of 0.9 ppm, to a range of 0.6 to 0.8 ppm, with a target of 0.7 ppm (47). In addition, recommendations for the use of fluoride toothpaste by infants and young children were also introduced (34). Recommendations with regard to toothpaste were made as the inappropriate use of fluoride toothpaste in young children who may not be able to expectorate it adequately is a major risk factor in fluorosis (38, 48, 49).

A downward revision of the concentration of fluoride in water supplies has occurred in other jurisdictions to balance the availability of fluorides from other sources, such as fluoridated toothpastes. The Department of Health and Human Services in the US has recommended water fluoridation at 0.7 mg/l (ppm), rather than the previous range 0.7mg/l – 1.2 mg/l, (ppm) to take account of other sources from which communities may receive fluorides (50). In Canada the concentration of 0.7 mg/l (ppm) of fluoride has been set moving from the previous range of 0.8 to 1.0 mg/l (ppm) (51) while in Australia, levels have remained unchanged, since the current research in Australia into caries prevention and fluorosis suggests maintaining the status quo. Some Asian, tropical and sub-tropical regions have reviewed the concentrations at which water is fluoridated and have agreed an upper and lower limit of 1mg/l and 0.5 mg/l (ppm) respectively.

Naturally occurring high fluoride water supplies occur around the world and defluoridation if required is possible (18), some of the methods which can be used for defluoridation are to blend waters with high fluoride concentration with waters of low concentration in addition technologies such as reverse osmosis, electrodialysis and distillation are available in the market. The fluoridation plants must have an effective fail-safe system with well-defined limits for the precision of measurements (52). A concentration of 1.5mg/l (1.5 ppm) is accepted as the Maximum Acceptable Concentration (MAC) of artificially fluoridated supplies.

Legislation

Legislation providing for water fluoridation can be of two types. It may be mandatory, requiring a ministry of health or communities of a certain size to fluoridate their public water supplies if it is below the accepted fluoride level; this is the type of legislation in Ireland. Alternatively, it may be of the permissive or enabling type, empowering the ministry of health or a local government to institute fluoridation. Some countries and jurisdictions require consultations with the community and to consider such consultations prior to proceeding, such as in the UK.

Discussion

In this article the authors have brought together the experience of and the challenges to water fluoridation using the experiences in the RoI for examples. They have also considered why it remains an effective component of prevention and oral health policy. Emerging evidence suggests that the declining caries levels which excited oral health professionals through the nineties and early 2000's may have plateaued. Internationally established economies are tending towards a more energy dense, refined carbohydrate diet, which may become more challenging in the delicate balance in preventing dental caries and dental fluorosis, and promoting oral health. The National Health and Medical Research Council in Australia (35) concluded: the existing body of evidence strongly suggests that water fluoridation is beneficial at reducing dental caries'. For most studies the consistent measure of effect to indicate the effectiveness of water fluoridation is the dmf/DMFT index (20), scientifically this makes sense and permits comparison with relative ease. Perhaps going forward ways of demonstrating effectiveness in terms of the distress and misery avoided, capturing children's ability to develop a positive association with oral health should be considered. The emotional impact of dental caries is significant and apparent on a daily basis to a significant number of families and dental personnel.

Conclusion

Water fluoridation is an effective safe means of preventing dental caries, reaching all populations, irrespective of the presence of other dental services. The monitoring of dental caries and dental fluorosis is the cornerstone of good public health practice and is essential particularly when the lifelong challenge which dental caries presents is considered. Future research must consider the challenges in reporting appropriate outcomes for both dental caries and dental fluorosis and the means of overcoming the challenges in the design, conduct and reporting of future work.

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References

1. Moore WY. The distribution of dental caries in ancient British populations. Caries Res. 1973;(7):139-53.
2. Sgan-Cohen HD, Evans RW, Whelton H, Villena RS, Mac Dougall M, Williams D. IADR-GOHIRA Steering and Task Groups. IADR Global Oral Health Inequalities Research Agenda (IADR-GOHIRA®). A call to action. J Dent Res. 2013;(92):209-11.
3. World Health Organisation. Oral disease burdens and common risk factors: WHO; 2012. [cited 2013 Jan 31]. Available from: http://www.who.int/oral_health/disease_burden/global/en/index.html.
4. Oral Health in America. A Report of the Surgeon General. Rockville, MD: US Dept. of Health and Human Services, National Institutes of Health, National Institute of Dental and Craniofacial Research; 2012. p. 2.
5. British Fluoridation Society (BFS): One in a million – the facts about water fluoridation, 3rd ed. [cited 2013 Jan 19]. Available from: http://www.bfsweb.org/onemillion/onemillion2012.html.
6. Minister for Health: Reports on the incidence of dental caries in school children and on the analyses of public piped water supplies in the different counties, 1961-1965. Dublin: Stationery Office; 1965.
7. O'Mullane DM, Clarkson J, Holland T, O'Hickey S, Whelton HP. Children's Dental Health in Ireland 1984. Dublin: Stationery Office; 1986.
8. Whelton H, Crowley E, O'Mullane D, Harding M, Guiney H, Cronin M, et al. North South Survey of Children's Oral Health in Ireland 2002. Dublin: Brunswick Press Ltd; 2006 [cited 2013 Jan 20]. Available from: www.dohc.ie/publications/oral_health.html.

9. Centers for Disease Control and Prevention (CDC). Populations receiving optimally fluoridated public drinking water-United States. MMWR Morb Mortal Wkly Rep. 2002;(51):144-7.
10. Dean HT, Arnold FA, Elvove E. Domestic water and dental caries. Additional studies of the relation of fluoride domestic waters to dental caries experience in 4,425 white children aged 12-14 years of 13 cities in 4 states. Public Health Rep. 1942;(57):1155-79.
11. Dean HT, Arnold FA, Jay P, Knutson JW. Studies on mass control of dental caries through fluoridation of the public water supply. Public Health Rep. 1950;(65):1403-8.
12. Centers for Disease Control and Prevention (CDC). Achievements in public health, 1990-1999: fluoridation of drinking water to prevent dental caries. MMWR Morb Mortal Wkly Rep.1999;(48):933-40.
13. Centers for Disease Control and Prevention (CDC). CDC Honours 65 Years of Community Water Fluoridation; 2010. [cited 2013 Jan 16]. Available from: http://www.cdc.gov/fluoridation/65_years.htm.
14. Groeneveld A, Van Eck AA, Backer-Dirks O. Fluoride in caries prevention: is the effect pre- or post- eruptive? J Dent Res. 1990;69 Spec No: 751-5:820-3.
15. Singh KA, Spencer AJ, Brennan DS. Effects of water fluoride exposure at crown completion and maturation on caries of first permanent first molars. Caries Res. 2007;(41):34-42.
16. Fejerskov O, Thylstrup A, Larsen MJ. Rational use of fluoride in caries prevention: A concept based on various cariostatic mechanisms. Acta Odontol Scand. 1981;(39):241-9.
17. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol. 1999;(27):31-40.
18. World Health Organisation. Appropriate use of fluorides for human health. Geneva: World Health Organisation; 1986.
19. Murray JJ, Rugg-Gunn AJ, Jenkins GN. Fluorides in caries prevention, 3rd ed. Oxford: Butterfield-Heinemann; 1991.
20. Rugg-Gunn A and Do L. Effectiveness of water fluoridation in caries prevention. Community Dent Oral Epidemiol. 2012;40(Suppl 2):S55-64.
21. O'Mullane DM, James P, Whelton H, Parnell C. Methodological issues in oral health research: intervention studies. Community Dent Oral Epidemiol. 2012;40(Suppl 1):S15-20.
22. Neidell M, Herzog K, Glied S. The Association between community water fluoridation and adult tooth loss. Am J of Pub Health. 2010;(100):1980-5.
23. Slade GD, Sanders AE, Do L, Roberts-Thomson K, Spencer AJ. Effects of fluoridated drinking water on dental caries in Australian adults. J Dent Res. 2013;(92):376-82.
24. McDonagh MS, Whiting PF, Wilson PM, Sutton AJ, Chestnutt I, Cooper J, et al. Systematic review of water fluoridation. BMJ. 2000;321(7265):855-9.
25. O'Grady P. In the public good. J Ir Dent Assoc. 2012;58(3 Suppl)S4-5.
26. Clarkson J, McLoughlin J, O'Hickey S. Water fluoridation in Ireland – A success story. J Dent Res. 2003;(82):334-7.
27. Health (Fluoridation of Water Supplies) Act, 1960. Dublin: Stationery Office; 1960.
28. Klein H, Palmer E, Knutson JW. Studies on Dental Caries: Dental status and dental needs of elementary school children. Public Health Rep. 1938;(53):751-65.
29. Dean HT. The investigation of physiological effects by the epidemiological method. In: Moulton FR, ed. Fluorine and dental health. Washington DC: American Association for the Advancement of Science; 1942. p. 23-71.
30. Petersson G, Bratthall D. The caries decline: A review of the reviews. Eur J Oral Sci. 2007;(104):436-43.
31. Whelton H, Crowley E, O'Mullane D, Donaldson M, Cronin M, Kelleher V. Dental caries and enamel fluorosis among the fluoridated population in the Republic of Ireland and non-fluoridated population in Northern Ireland in 2002. Community Dent Health. 2007;(23):37-43.
32. Fomon SJ, Ekstrand J, Ekhard E, Ziegler D. Fluoride intake and prevalence of dental fluorosis: Trends in fluoride intake with special attention to infants: Review and commentary. J Pub Health Dent. 2000;(60):131-9.
33. Pendrys DG, Haugejorden O, Bardsen A, Wang NJ, Gustavsen F. The risk of enamel fluorosis and caries among Norwegian children: implications for Norway and the United States. J Am Dent Assoc. 2010;(141):401-14.
34. Report of Forum on Fluoridation. Dept. of Health. [cited 2013 Feb 11]. Available from: http://www.dohc.ie/publications/pdf/fluoridation_forum.pdf.
35. National Health and Medical Research Council. A systematic review of the efficacy and safety of fluoridation. Canberra, Australian Government; 2007. [cited 2013 Feb 11]. Available from: http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh41_1.pdf.
36. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century – the approach of the WHO Global Oral

Health Programme. Community Dent Oral Epidemiol. 2003;(1):3-23.

37. Griffin SO, Jones K, Tomar SL. An economic evaluation of community water fluoridation. J Public Health Dent. 2001;(61):78-86.
38. Browne D, Whelton H, O'Mullane D. Fluoride metabolism and fluorosis. J Dent. 2005;(33):177-86.
39. Melbye MLR, Armfield JM The dentist's role in promoting community water fluoridation: A call to action for dentists and educators. JADA. 2013;(144):65-75.
40. EU Scientific Committee on Health and Environmental Risks (SCHER). Critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water; 2011 [cited 2013 Feb 4]. Available from: http://ec.europa.eu/health/scientific_committees/environmental_risks/index_en.htm.
41. Nuffield Council on Bioethics. Public Health: Ethical Issues. London: Nuffield Council on Bioethics; 2007.
42. Mullen J. History of water fluoridation. BDJ. 2005;(199);1-4.
43. Mullen J. Defending fluoridation. J Ir Dent Assoc. 2012;58(3 Suppl):S9.
44. Centers for Disease Control and Prevention CDC Oral Health Division. Oral Health Resources; 2013. [cited 2013 Feb 3]. Available from: http://www.cdc.gov/oralhealth/.
45. Guidelines for Canadian Drinking Water Quality. Guideline Technical Document Fluoride. Federal-Provincial-Territorial Committee on Drinking Water of the Federal-Provincial-Territorial Committee on Health and the Environment. Ottawa, Ontario; 2010. [cited 2013 Feb 3]. Available from: http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/water-eau/2011-fluoride-fluorure/2011-fluoride-fluorure-eng.pdf.
46. European Communities (Drinking Water) (No.2) Regulations S.I. No. 278 / 2007). Government of Ireland; 2007. [cited 2013 Aug 07]. Available from: http://www.irishstatutebook.ie/2007/en/si/0278.html.
47. Fluoridation of Water Supplies Regulations. S.I. No. 42/2007. Government of Ireland; 2007. [cited 2013 Jan 24]. Available from: http://www.irishstatutebook.ie/2007/en/si/0042.html.
48. Pendrys DG, Stamm JW. Relationship of total fluoride intake to beneficial effects and enamel fluorosis. J Dent Res. 1990;69(Special Issue):529-38.
49. Harding MA, Whelton H, O'Mullane DM, Cronin M, Warren JJ. Primary tooth fluorosis in 5-year-old schoolchildren in Ireland. Eur J Paediatr Dent. 2005;(6):155-61.
50. Centers for Disease Control and Prevention (CDC), Division of Oral Health, Changes proposed to national fluoridation level. [cited 2013 Feb 11]. Available from: http://www.cdc.gov/oralhealth/.
51. Findings and recommendations of the Fluoride Expert Panel. Health Canada; 2007. [cited 2013 Feb 2]. Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/2008-fluoride-fluorure/index-eng.php.
52. Fawell J, Bailey K, Chilton J, Dahi E, Fewtrell L, Magara Y. Fluoride in drinking water. WHO. London: IWA Publishing; 2006.

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Salt fluoridation and oral health

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The aim of this paper is to make known the potential of fluoridated salt in community oral health programs, particularly in South Eastern Europe. Since 1922, the addition of iodine to salt has been successful in Switzerland. Goiter is virtually extinct. By 1945, the caries-protective effect of fluorides was well established. Based on the success of water fluoridation, a gynecologist started adding of fluoride to salt. The sale of fluoridated salt began in 1956 in the Swiss Canton of Zurich, and several other cantons followed suit. Studies initiated in the early seventies showed that fluoride, when added to salt, inhibits dental caries. The addition of fluoride to salt for human consumption was officially authorized in 1980-82. In Switzerland 85% of domestic salt consumed is fluoridated and 67% in Germany. Salt fluoridation schemes are reaching more than one hundred million in Mexico, Colombia, Peru and Cuba. The cost of salt fluoridation is very low, within 0.02 and 0.05 € per year and capita. Children and adults of the low socio-economic strata tend to have substantially more untreated caries than higher strata. Salt fluoridation is by far the cheapest method for improving oral health. **Conclusions.** Salt fluoridation has cariostatic potential like water fluoridation (caries reductions up to 50%). In Europe, meaningful percentages of users have been attained only in Germany (67%) and Switzerland (85%). In Latin America, there are more than 100 million users, and several countries have arrived at coverage of 90 to 99%. Salt fluoridation is by far the cheapest method of caries prevention, and billions of people throughout the world could benefit from this method.

Key words: Fluoride, Prevention, Dental caries, Salt.

Introduction

Fluorides are the main factor leading to the decline in the prevalence of dental caries, a decline which has surpassed all expectations. When "artificial" water fluoridation was introduced in 1945 in the USA and Canada, it was expected that caries prevalence would be reduced by as much as 50 percent. In the next two decades this prediction was found to be correct in many countries. The topical effects of fluorides, particularly when contained in dentifrices, have brought about a further caries decline in the industrialized countries. However, the fact that the lower social strata consistently show the highest levels of caries in the population has so far remained an unsolved problem. In analogy to fluoridated water, which confers partial protection independent of social conditions, salt with added fluoride was assumed to be as useful a carrier for human consumption as iodine has proved to be in the case of goiter since the early 1920s. It is the aim of this paper to update the scientific basis and the beneficial effect of fluoridated salt for human consumption.

Iodized salt as a precursor of fluoridated salt

In several valleys throughout the Alps, endemic goiter has been prevalent since prehistoric times. Between 1910 and 1920, insufficient intake of iodide was finally identified as the cause of endemic goiter. The key paper on the preventive effect of iodized salt was published by Marine and Kimball in 1917 (1). At that time, two local Swiss physicians started to add a few milligrams of iodide per kg of salt for human consumption in communities where goiter was prevalent. The success of this intervention was visible already after one year. The newborn children exposed to iodized salt had no symptoms of goiter, demonstrating the preventive effect of iodide added to salt and providing further evidence of the role of insufficient intake of iodide in goiter.

In 1922, the main Swiss salt factory developed the apparatus to add potassium iodide to salt. In the following years five of the 26 Cantons (which at that time still had a Cantonal Monopoly on the salt) decided to use exclusively iodized salt. By 1942, the number of cantons in which less than 50% of the salt was iodized had dwindled to seven (2). Goiter is now virtually extinct in Switzerland.

Dr. Hans J. Wespi, the son-in-law of the physician who had added iodide to the sacks of salt in 1918, was then Director of the Clinic for Gynecology and Obstetrics in the Cantonal Hospital at Aarau in 1946. He started to add sodium fluoride to already iodized salt which he gave to his patients who had or were expecting children. He did this on the hypothesis that soluble fluoride in salt would be as effective against caries as fluoride added to the drinking water. Salt fluoridation in Switzerland was therefore based on 25 years of successful iodization, since at that time scientific evidence was not available that fluoride has a cariostatic effect when contained in salt. The medical profession, however, was aware of the fact that salt had been shown to be a valuable vehicle for the micronutrient iodide. In the former "goiter areas", the public, as well as the medical profession, had noted that goiter became less severe, less frequent or disappeared altogether. It was logical that Wespi published his early papers (3, 4) on fluoridated salt not in a dental journal, but in the leading Swiss medical journal.

Fluorides and teeth, early findings in North America

From 1900 to 1938 research regarding the effects of fluoride on teeth – predominantly carried out in the USA – focused on gross malformations of tooth crowns. Affected crowns had brown or black stains and multiple fractures of the enamel. The malformations occurred endemically. Therefore, many dentists believed that the cause was to be found in the local drinking water. In fact, there were three publications, which, in 1931, identified high concentrations of fluoride in the drinking water as the cause of defective tooth crowns, then often called "mottled" teeth (now called enamel fluorosis).

On the other hand, Dean (5) found that children aged 9 years living in various counties in the State of North Dakota with 1.7 to 2.5 ppm F in the drinking water (where enamel fluorosis was frequent but not severe) had only 2.0 decayed teeth, while those in counties with 0.6 to 1.5 ppm F in water had 4.2 decayed teeth. In 1939, Dean et al. (6) examined hundreds of children in 4 Illinois cities where drinking water contained either 0.2 ppm F or else 1.7-1.8 ppm F. Again, the children who had been consuming high fluoride water had less decayed teeth, namely 2.0 as opposed to 4.0 to 4.6. Between 1941 and 1948, at least 10 reports confirmed the caries-protective effect of 1.0 ppm F in the drinking water. In these papers, the severity of caries was expressed as the number of Decayed, Missing, or Filled Teeth per individual, abbreviated to DMF-teeth, and this is still the best measure expressing the prevalence of caries.

In 1947, Arnold (7) compiled data on fluoride concentrations in drinking water and the averages of DMF-teeth in 12 to 14 year-old children in 27 Mid-Western US cities. These data documented that under conditions of 0.9 to 1.3 ppm F in the drinking water, the number of DMF-teeth was halved when compared to the teeth of children on water with 0.1 to 0.6 ppm F. On the other hand, (mild) enamel fluorosis was present in 12 to 33% of the children on 0.9 and 1.3 ppm F in the drinking water. With fluoride concentrations below 0.7 ppm F enamel fluorosis was far less frequent (0 to 11%). The protective effect was shown to last at least until the age of 15 years.

In 1945, artificial fluoridation was started, with water treated to contain 1.0 milligrams of fluoride per liter, or 1.0 ppm F, in three cities of the USA and Canada. For each fluoridated city a reference city was selected. After five years, the number of DMF-teeth in children aged six to nine was reduced by 26 to 60% in the three fluoridated cities, whereas in the three reference cities the number of DMF-teeth remained more or less constant. Such experiments were repeated in several other cities with similar results: children who benefited from drinking water containing 1 ppm fluoride had lower DMF-averages by approximately 50%, and the protective effect was maintained at least up to the age of 15 years.

The reports on reduced caries prevalence in children after only five years of fluoridation induced many scientists to believe that early or even prenatal fluoridation would be indispensable to obtain the full protective potential of fluoride. In view of the almost complete absence of fluoride research in Continental Europe until the late 1950s, it is understandable that the American opinion prevailed for some years.

On the other hand, fluoride tablets, available since 1954 in Switzerland, were widely used for caries prevention. Many dentists working with the school dental services noted that caries incidence was also reduced in children who were already five to nine years old when they started to take the fluoride tablets. Swiss and German data became available providing evidence that fluoride tablets, given to six year-old children each school day, reduced dental caries experience in school aged children by 25 to 50%.

Changing concepts regarding the protective effect of fluoride

In the period from around 1955 to 1970, several developments improved the prospect for salt fluoridation.

- Use of fluoride in Switzerland began with program of daily intake of fluoride via tablets. After 2 to 3 years many dentists already noted a rapid decrease in caries incidence, not only in pre-school but also in schoolchildren. The idea that fluoride intake during the first two or three years of life is important for caries prevention was gradually abandoned.
- With the increasing duration of clinical studies on water fluoridation, it was observed that there must a considerable topical protective effect of fluoride on erupted teeth, even at relatively low fluoride concentrations around 1 ppm F. With increasing duration of water fluoridation it emerged that, for instance, 14-year-old children who had not consumed fluoridated water during their

first five or six years of life had only slightly higher caries levels than children exposed to fluoridated water since birth.
- A considerable number of well-controlled clinical trials comparing caries increments in children using fluoridated toothpaste with those from children using the same toothpaste, but without fluoride, supported the opinion that the topical effect might even be the main anti-caries factor.
- Biochemical research based on analytical chemistry and enamel microhardness studies showed that within the 24-hour cycle there are hours during which submicroscopic demineralization dominates. This occurs during and after eating sugared food, which is fermented to lactic and other acids by the biofilm on the teeth, or dental plaque. Alternatively, there are hours during which remineralisation occurs, and this "healing" process of the slightly demineralized enamel is greatly enhanced by fluoride. In this dynamic situation, the presence of fluoride in the oral fluid is very important, as it catalyzes remineralisation of the enamel as well as dentine. Today, this mechanism is regarded as the main reason for the tooth-protecting effectiveness of fluoride.
- As time went by, it had to be accepted that with the existing network of drinking water in Switzerland, water fluoridation is difficult or impossible to realize for technical and legal reasons related mainly to a multitude of small water supply systems. A political move for the introduction of water fluoridation was successful only in Basel, serving 0.3% of the Swiss population from 1962 to 2003 (8).

Clinical caries studies with fluoridated salt in Western Switzerland

In Western Switzerland, the Canton of Vaud, which has its own salt factory, decided to add fluoride to the salt. This Canton (half a million inhabitants in 1970) had had a scheme of daily distribution to schools of one 1.0 mg fluoride tablet since the early 1960s. On the advice of Prof. Mühlemann of the Zurich University Dental School, the Cantonal Salt Commission of Vaud decided in 1970 to add 250 mgF to salt for human consumption and recommended that fluoride tablets should not be used any longer in the Canton. Since 1970 salt with 250 ppm F has been used in households, bakeries and all institutions running their own kitchens. Accordingly, fluoridated salt has been virtually the only salt sold in the Canton of Vaud. In the course of time with mobility and rural shopping centres on the increase, the rather strict situation regarding the use of the 250

Table 1 The number of DMF-teeth per 12-year-old child in the three reference communities with no or limited exposure to F-salt and in the three communities of the Canton of Vaud where the use of fluoridated salt began in 1970

Year	Reference limited F-exposure	Using 250 ppm F, F-salt, VD	% lower under F-salt
1970	6.97	5.61	20
1974	5.24	4.30	18
1978	4.47	3.93	12
1982	4.87	2.92	40
1986	3.17	2.12	30
1990	2.29	1.88	18

VD= Canton of Vaud; Smallest numbers of children: 34, 41, 58, all other sample sizes between 63 and 189. Source of data: (9).

ppm F salt in Vaud was "diluted" to some extent. Nevertheless, surveys studying urinary excretion in the main city of Lausanne confirmed that fluoride concentrations were still similar to those found in regions with fluoridated water in similar climates. Table 1 shows the results obtained in children at the age of 12 years (9).

The children from the three Vaud communities close to the cantonal border had consistently lower averages of DMF-teeth than those in the three reference communities outside the Canton of Vaud. Regarding the initial examinations in 1970, lower caries experience was to be expected in view of the fact that the Vaud children had been given 1 mg fluoride via tablets distributed daily at school for many years. In all six surveys from 1970 to 1990, carried out at four-year intervals, the Vaud children had consistently lower DMFT-averages when compared to the non-Vaud children, as indicated by the statistical significance in the sign test. The DMFT counts of the 8 and 10-year-old children resulted in the same pattern: the eight averages from Vaud were consistently lower than those of the reference children. In addition, 14-year-old children were examined in Yverdon, another town in the Canton of Vaud with a population of about 20,000. The average number of DMF-teeth was 8.47 (number of children examined: 68) in 1970 but decreased to 2.99 (N=101) in 1988.

With respect to the caries decline in the reference communities, several factors are to be considered. Within the school dental service, comprising one free dental examination per year and subsidized restoration of cavities, school committees implemented educational program of various intensity:

- Regular toothbrushing exercises with topical fluoride were organized.
- Information on prevention was made available through leaflets.
- In some of the communities, fluoride tablets were distributed until 1983.

Accordingly, the reduction in the caries levels in the reference communities is easily explained by the educational program in school.

In 1974, a second epidemiological long-term study was begun in the Canton of Glarus. It lasted until 1996 and confirmed the cariostatic effectiveness of fluoride when added to salt (10). The long term results were summarized (11). The Swiss Dental Association and subsequently the Swiss Academy of Medical Sciences then agreed that for mass prevention of caries in Switzerland, the fluoride concentration in the domestic salt should be raised to 250 ppm F (250 mg fluoride per kg of salt). This is equivalent to 1.0 mg of fluoride contained in 4 grams of salt. The respective resolutions in the involved committees and by the Health Ministry were passed in 1980 to 1982. Interestingly, there was little opposition to the higher concentration. In fact the market share of fluoridated salt in Switzerland has since increased consistently (12).

In 1992, the marketing department of the main salt factory started to reduce the package size of domestic salt from the usual 1 kg to 500g packages. Within three months after this change of policy, the market share dropped from 75% (September 1992) to 46% (November 1992). After this serious setback, it was decided that the 250 ppm F salt should be available in all package sizes up to 1 kg whereas the unfluoridated varieties were restricted to the 500 gram packages. In January 1994, immediately after re-introduction of the 1 kgF salt package, the market share started to rise again and by 1998 leveled out at 86% (12).

Evidence for the caries-preventive effect of fluoridated salt in adults

It is generally accepted that the cariostatic action of fluorides continues during adult life. In this case it is exclusively the local mode of action of fluoride (the accelerated remineralisation of demineralized enamel

Table 2 Swiss Army Recruits subdivided according to the level of previous prevention

Lines 1-3: 3 different levels of OPS within the existing school dental service

Line 1: no or only a few communities with OPS in the Cantons

Line 2: about half of the communities with OPS in the Canton

Line 3: most communities with OPS in the Canton

Line 4: closely supervised OPS in the Canton of Zurich

Line 5: universal fluoridation via salt, Canton of Vaud, VD; 250 ppm since 1970 when the later recruits were 5 years old

Line 6: fluoridated water, Canton of Basel City (1.0 ppm F since 1962, which was 13 years before the later recruits were born)

Prevention level	n of recruits	Caries experience, average per recruit	
		DMFT	DFS
1 No prevention in school	157	11.3	21.3
2 Little prevention in school	302	10.9	20.3
3 Good prevention at school	123	9.1	17.0
4 Canton of Zurich, ZH	92	9.3	17.3
5 Canton Vaud, VD, F-salt	56	7.1	11.6
6 Ct. Basel, F-water	23	9.0	15.4
All recruits	753	10.1	18.8
Stand. dev. N=753		4.9	12.5
	Results of the analyses of variance F-tests between/within groups		
F: between/within, p	<0.001	<0.001	<0.001

OPS=Organized Preventive Service; *DFSrad: DF-counts in approximal surfaces of premolars and molars (24 surfaces examined). Source of data: (13).

and dentine) which leads to the protective effect. Epidemiological data are available from Switzerland and Hungary.

In 1985, 753 recruits of the Swiss army (most of them 20 years old) were examined. A total of 57 recruits had grown up in the Canton of Vaud, where the salt has contained 250 ppm fluoride since 1970. Table 2 shows they had an average of 7.1 DMFT. The recruits from water-fluoridated Basel had an average of 9.0 DMFT, while all others had between 9.3 and 11.3 DMFT, depending on whether or not preventive program had been implemented during school age (13).

Table 3 shows that the DMFT of the Vaud recruits was significantly lower than

Table 3 Confidence limits (LowL and UppL) of DMF-findings and t-tests between Vaud and the other groups exposed to different levels of prevention or to fluoridated water (see Table 2)

Line Table 2. level of prevention	n	Mean DMFT	LowL	UppL	Comp.	Difference	% diff.	p
1. No or few OPS	157	11.3	10.5	12.1	vs VD	-4.20	-4.20	>0.001
2. OPS for about 50%	302	10.9	10.3	11.5	vs VD	-3.80	-3.80	>0.001
3. OPS for majority	123	9.1	8.2	10.0	vs VD	-2.00	-2.00	>0.001
4. Canton of Zurich. good OPS	92	9.3	8.3	10.3	vs VD	-2.20	-2.20	>0.001
5. Canton of Vaud. VD. F-salt	56	7.1	5.8	8.4	-	-	-	-
6. Ct. Basel. BS. F-water	23	9.0	7.0	11.0	vs VD	-1.90	-1.90	≈0.001

Source of data: (13). Menghini et al. 1991; see also Table 2.

that of any of the other groups, including the 23 recruits from water-fluoridated Basle. This study shows clearly that the supply of fluoride, used by the entire population, has resulted in an improved level of dental health which was maintained into young adulthood.

Salt fluoridation and enamel fluorosis in Switzerland

In Switzerland, the sale of fluoride tablets started in 1953. A large number of dentists used the tablets in their own families with great success. Several small cantons decided to distribute fluoride tablets on each school-day. High exposures to fluoride occurred in connection with the early recommended dosage of fluoride (first year of life: 0.25 mgF, then increasing each year by 0.25 mgF to 1.0 mgF from the fourth year of life onwards) and resulted in slight and sometimes easily visible enamel fluorosis. New recommendations for a lower dosage were made in 1960: from the eruption of the first primary tooth until 23 months of age: 0.25 mg F; 24 months to 48 months: 0.5 mg F; 48 months to 71 months: 0.75 mg F; from age 6 to adulthood: 1.0 mgF. After introduction of the lower dosage, children with easily visible enamel fluorosis became increasingly rare but studies recording the actual prevalence were not carried out in those years.

In the context of the epidemiological caries studies in Vaud, De Crousaz (14) assessed enamel fluorosis frequencies of the upper incisors among children aged 6-8y and 9-13y in water-fluoridated Basel (fluoride added from 1962 until 2003), in the Canton of Vaud and in two reference towns outside Vaud. The sensitive index of Thylstrup and Fejerskov (15) was used. Mouth frequencies of enamel fluorosis were at 36% at age 6-8 and at 38% at age 9-13. In the Canton of Vaud (universal salt fluoridation) mouth frequencies were at 37% and 38% and in the reference children at 25% and 26%, respectively. Non-fluoride opacities occurred in 18% of the Basle children, in 7% of the Vaud children and in 25% of the reference children. In the entire survey, only one child (among a total of 1242) showed a TF-score 3 on the left central incisor.

Studies between 1982 and 1996 in five different locations resulted in frequencies between 13% and 28% (16). In their own extensive study, Steiner and Menghini (16) assessed enamel fluorosis in 1087 schoolchildren (age 9-10y) and 964 military recruits (age 19-21y) in the years 1995-69 and in 2004-2005. The subjects took part in epidemiological surveys for dental caries organized by the Clinic for Preventive Dentistry, Periodontology and Cariology, Dental Centre, University of Zurich. In their summary the authors stated: "Fluoride-associated opacities of TF score 2 were only observed in approximately 1% of the participants. One examiner noted a TF score 3 in a single individual. Fluoride-associated opacities therefore are not a cosmetic problem and certainly not a public health concern".

To complete the picture it is important to know that in Switzerland fluoride tablets are still on sale in pharmacies without prescription, but only very few families still use them. Fluoride toothpastes (up to 1500 ppm F) are regularly used. For small children up to six years, toothpastes with less than 500 ppm F are recommended. Daily fluoride rinses are used by 20% of Swiss children; another 20% brush their teeth with concentrated fluoride gels (1.25%F) once a week. Respective information is given in the schools at regular intervals. Urinary excretion studies in the Canton of Vaud showed that fluoride levels in urine obtained from adolescents were in the optimal range in terms of both fluoride concentrations and fluoride excretion due to the universal use of fluoridated salt (17). Children at age 10-14y in Lausanne (the Capital of the Canton of Vaud) had urinary concentrations of 1.3 ppm F after lunch, 1.1 ppm F at night and 0.8 ppm F in the morning. Enamel fluorosis is certainly not a problem in Switzerland.

Fluoridated salt in other European countries

In 1968, K. Toth of Szeged (a city in Southern Hungary), Professor of Conservative Dentistry, started clinical studies in several towns, using salt containing 200 or 250 mgF/kg (18). Since 1972, two further communities were added and were provided with salt containing 350 ppm F. After 1981, all salt in the fluoridated towns contained 350 ppm F. The last DMFT-averages are presented in Table 4. They provide further evidence for the cariostatic effect of fluoride added to salt.

Radnai and Fazekas (19) examined the teeth of Hungarian adults in 1991. The reference subjects (group A) consisted of subjects who had had no significant fluoride exposure. The second group of adults, B, had been living in the towns and villages which were provided with fluoridated salt from 1966 to 1985 (initially with 250 or 200 ppm; after 1972 increasingly, and from 1979 to 1985 consistently, with 350 ppm F) (18, 19). Group C had been lifetime residents in a town with the naturally optimal content of 1.1 ppm F in the drinking water (Kunszentmarton). The total number of subjects examined in the age group 28-37 years in the three groups was 205. Reference group A had 16.5 DMF-teeth, group B 13.4.The subjects using 1.1 ppm F water since birth had only 9.7 DMFT-teeth (group C). They had received the supplemental fluoride in the water during their whole life, whereas in group B the fluoride intake from salt started only at age 3 to 12 years and lasted only 19 years. In spite of the shorter exposition to fluoride, group B had 3.1 less DMFT when compared to the 16.5 DMFT in the reference group A.

In the youngest age group (18-27 years), the DMFT averages for groups A, B and C were 13.8, 7.9 and 5.4, respectively. The mean DMFT of group B was much closer to the mean of group A. This would be expected since these young adults had exposure beginning at birth or at the age of three years at the latest. These young adults did not receive fluoridated salt during the six years prior to the dental examination in 1991 because the production of fluoridated salt was stopped in 1985.

The Swiss and Hungarian surveys both support the hypothesis that the beneficial effect of fluoride in salt extends into adulthood, and that immigrants into salt fluoridated communities also benefit from fluoride. In spite of comprehensive research in and around Szeged and several political moves to introduce salt fluoridation for the whole country, this measure has not been implemented in Hungary.

France was the second European country adopting salt fluoridation in 1986. The resolution for this measure was in part based on a series of publications in French on fluori-

Table 4 Average counts of diseased teeth per child in Southern Hungary and changes from 1966-1982

Group (Age)	Initial Average caries	Use of F-salt 1966-1982	Final Average caries	Lower 16 years later (%)
5-6, dmft (primary teeth)	5.19*	No	6.70	-
	6.78	Yes	3.16	53
13-14, DMFT	7.33*	No	10.27	
	8.21	Yes	3.29	60

*Children from 3 reference villages: Age 2-6 and 12-14 from Table 82, all other data from Table 111 (18).

dation in Switzerland (Frey and Maeglin ed. 1982: *Fluoruracion du sel pour la prevention de la carie*, Revue mens. suisse Odonto-stomatol. Vol. 92, No. 4, April 1982, 130 pages), and on other evidence of the cariostatic effect of fluoride when added to salt. Due to a nationwide campaign for its use, the market share in France attained 50% in 1991 and even 60 % in 1993 (20). However, due to the lack of continued promotion, the market share dropped to 27% in 2003 and was below 10% in 2010. In terms of the French population of now roughly 60 million this means that after 1993, when there were 36 millions users of fluoridated salt, their number has dwindled to less than six million by 2012. Lack of continued promotion of fluoridated salt was the main reason for the decreasing market share.

Germany started to import fluoridated salt from France in 1991. After a few years, the government authorized production of F-salt by German refineries. The salt industry promoted the switch from iodized salt to domestic salt with both added iodide and fluoride. The information campaign included dietary advice for the prevention of caries and recommendations regarding oral hygiene. A committee of university professors and practicing dentists met once a year with a public relations agency, who produced and updated leaflets and other material promoting dental health.

The cost of the promotion in Germany has been in the order of magnitude of 100,000 € per year. On the other hand, the cost of adding fluoride may be assumed to be 200,000 € per year (as explained at the end of this paper). In markets and shops, the price of fluoridated salt in Germany is 5 or 10 cents higher per package of 500 g of salt. Due to the continued well-planned information campaigns, including general rules for oral hygiene and dietary measures, the market share of F-salt rose from approximately 20% in 1997 to 50% in 2000 and, since 2007, it has leveled out just below 70% (21). In comparison with France's breakdown of fluoridated salt usage, the German campaign has been a real success.

In the Czech Republic, it was reported that 35% of the domestic salt is now fluoridated (22). This is somewhat surprising since the price of fluoridated salt there is almost twice as high as that of the unfluoridated salt. Part of the fluoridated salt sold in the Czech Republic is imported from neighbouring countries, mainly Germany.

In other countries (for example Austria, Spain, Slovakia) fluoridated salt is used on a very limited scale. The sale of fluoridated salt would be legal in Greece and in the Netherlands (22) but there seems to be no interest in this preventive method. On the other hand, the vast majority of dentifrices are fluoridated all these countries. While the health authorities seem to have some knowledge regarding the benefits of fluorides, they appear to be unaware of their full cariostatic potential. Unfortunately, many dental experts are of the opinion that fluoridated toothpastes are sufficient to provide protection from caries. From a public health point of view, however, additional measures are necessary, above all in order to reach socially disadvantaged or remote groups in many countries. Of the world's current population of seven billion, three to five billion people have no or very limited access to prevention or, more specifically, to caries-preventive fluorides. Caries is still destroying teeth in children, adults and old people world-wide - only in minors and young people of a minority of countries has caries prevalence been reduced on a public health scale.

Salt fluoridation in the Americas

The "First International Symposium on Salt Fluoridation" took place in 1977 in the city of Medellin, Colombia. Participants came from South and North America and a few from Europe, mainly Switzerland. The Scientific Publication No. 501 "Salt Fluoridation" (23) documents the early interest in salt fluoridation in this part of the world. The Symposium site was well-chosen: in this country, a clinical trial had compared the effect on caries of three fluoride regimens:

- Fluoride in water (NaF)) at 1.0 ppm F (in San Pedro);
- Sodium fluoride (NaF) added to salt at 200 ppm F (in Armenia);
- Calcium fluoride (CaF_2) added to salt at 200 ppm F (in Montebello);
- No fluoride added, reference (Don Matias).

The initial dental examinations in 1964 showed that in all four towns chosen for the study, caries experience was similar (24, see Table 5). Addition of fluoride to salt was undertaken in a laboratory at the University of Antioquia, which also supervised the examinations for caries (age group 6 to 14 y) and assessed urinary fluoride concentrations. The study was carried out under the guidance of the local dental staff of the University of Antioquia NIDR-USPHS, and Dr. G. Gillespie of the Pan-American Health Organization (the American Regional Office of the World Health Organization in Geneva). Table 5 shows the initial (1964) and final (1972) DMFT averages in the 10 and 12 year old children. The reductions obtained were close to 50%, ranging from 47 to 58% (24).

The fluoride concentrations in urine ranged between 0.50 and 0.80 ppm F in the CaF2-group (Armenia), and between 0.64 and 0.87 in the NaF group (Montebello). This was somewhat below the concentrations in water-fluoridated San Pedro (0.9 to 1.2 ppm F). In the reference town Don Matias, the concentrations were at 0.18 and 0.27 ppm F. There were no differences between the cariostatic effectiveness between the chemicals used for fluoridation. Notably, caries was equally inhibited whether the rapidly soluble fluoride (NaF) or the almost insoluble compound (CaF2) was added to food. In the reference community, there was virtually no change in caries prevalence.

In the Colombian towns, domestic salt was under government control at that time and very little industry-processed food existed, whereas today salt is often added to food before it is offered to the customer. Such pretreated food was then simply not available or on sale only in cities. Thus, virtually all of the salt ingested was fluoridated in the two respective groups. The Colombian results became rapidly known in Latin American states, and several countries began to add fluoride to salt for human con-

Table 5 DMFT-averages per child in the 4 Colombian towns and percent reductions of DMF-averages in 1964 (when addition of fluoride to water and salt started) until 1972

Age	1964	1972	Reduction (%)
10 years			
F-water, NaF	6.21	2.91	53
NaF in salt	5.33	2.23	58
CaF2 in salt	5.70	2.47	57
No added fluoride	6.96	6.91	-
12 years			
F-water, NaF	9.48	4.10	57
NaF in salt	8.59	4.50	48
CaF2 in salt	9.44	4.96	47
No added fluoride	10.72	11.03	-

Source of data: (24).

sumption. Costa Rica and Jamaica were the first to start in 1996/1997.

In Jamaica, which as an island has a partially isolated population, there is only one factory which refines the raw sea salt purchased from several nearby Caribbean islands. Addition of iodide had been introduced already in the 1970s, and goiter disappeared from the island. The refinery built a fluoridation apparatus which spread a concentrated fluoride solution (containing also iodide) on the salt on a conveyor belt passing underneath. At the Health Ministry, there was a debate as to whether salt without fluoride should be on sale besides the salt with both iodide and fluoride. After some discussion, the manager of the salt refinery suggested fluoridating all salt for human consumption when a consensus was reached that fluoridated salt was the better product from a standpoint of public health and marketing. Accordingly, the decision was that the regular salt should contain both micronutrients. The factory manager then conceded that for anyone insisting on using unfluoridated salt for cooking, the enterprise would keep unfluoridated salt on stock, but that the customary distribution channels would be uniformly provided with the fluoridated (and iodized) salt.

Caries levels in Jamaica were assessed in 1986 and 1995 (25). In 12 year-old children, the number of DMF-teeth was reduced from 6.7 to 1.1. A reduction of 69% was even found in 15 year old children who had started to benefit from fluoride after the age of seven or eight years. Investigations regarding factors that may have influenced the caries experience of Jamaican children led to the conclusion that the fluoride in the salt must have been the main reason for the dramatic decrease in caries prevalence on the island (26). In 2008, follow-up assessments of urinary fluoride in children at age three to five were made. Urinary fluoride concentrations in nocturnal collections were 1.33 ppm F (urban children) and 1.26 ppm F (rural children) and at daytime 1.28 and 1.26 ppm F, respectively (27). From a large number of studies it is known that under conditions of optimal levels of fluoride in drinking water, urinary fluoride concentrations are in the range 0.9 to 1.2 ppm F. Fluoride excretions per hour and per 24 hours were also within the optimal range.

The following favourable external circumstances facilitated the introduction of universal salt fluoridation in Jamaica:
- Universal iodization had been introduced before 1980;
- There is only one salt refinery on the island (=country in this case);
- Fluoride concentrations were below 0.3 ppm F in 95% of the drinking water;
- All drinking water had concentrations below 0.7 ppm F;

Table 6 Average number of DMF-teeth per child in four American countries before and 8 to 12 years after the start of salt fluoridation

Country, age of children	First survey		Last survey		Reduction of DMFT in %
	Year	DMFT	Year	DMFT	
Jamaica, 12 years	1986	6.7	1995	1.1	84
Jamaica, 15 years	1986	9.5	1995	3.0	69
Costa Rica, 12 years	1987	8.4	1999	2.5	72
Mexico, 12 years	1988	4.4	1997	2.5	44
Uruguay, 11-14 years	1991	4.1	1999	2.4	42

Source of data: (22).

- There was literally no opposition to the addition of fluoride to salt.

Countries with similarly high coverage of almost the entire population are Colombia, Costa Rica, Mexico and Uruguay; the population of these four countries is approaching 160 million. These countries have now up to 20 years of documented use of fluoridated salt. Results regarding dental caries are shown in Table 6.

Fluoridated salt is also available in countries such as Belize, Bolivia, Cuba, Dominican Republic, Ecuador, Peru, Venezuela; a few other countries are in the process of implementing salt fluoridation. In Latin America, bakers mostly use the same small salt packages of 0.5 kg as the households. Restaurants also frequently use small salt packages. That means that even when only the salt in small packages is fluoridated, it provides fluoride beyond the households, which enhances the effectiveness against caries. Details of the situation in the Americas up to 2008 were presented in detail by Estupinian (28).

Approximate costs of salt fluoridation

General problems to be considered for making plans prior to the introduction of a salt fluoridation scheme have been discussed extensively in a previous publication. Initial information available in each country on salt production and distribution is relatively easy to obtain:

- Is there already a program of salt iodization?
- How many major producers of salt are there in the country?
- How much of the salt is imported? From where?
- How many drinking water systems with fluoride concentrations above 0.8 ppm F exist in the country?

In addition to these basic questions, many details must be clarified. These are listed in the respective paper (11). On the basis of this information, cost estimates can be made. The cost of stainless batch mixers for one ton of salt amounts to tens of thousands of € (Euro). Local solutions for mixers, particularly smaller ones, may be constructed at lower prices. For sufficient capacity, for instance for one million users, two or three mixers may be necessary, which may cost 100,000 €. For quality control, a laboratory equipped for simple analytical chemistry is also necessary. It may be integrated in the laboratory for control of iodization. These costs are necessary only in the first year.

The cost of the fluoride (using sodium fluoride, NaF) necessary for one ton of salt lies between 1.38 and 2.76 €. Further costs are for technical and laboratory personnel, needed for operating the mixer and working in the analytical laboratory for quality control and technical maintenance. For a population of one million, which "uses" or "consumes" approximately six kg salt per year and capita, the calculations indicate a total cost between 0.023 and 0.038 € per year and person. Details of the respective calculations were presented in an earlier paper (29).

Promotion costs for implementing F-salt instead of unfluoridated salt are variable. As indicated above, about 100,000€ per year is spent to convince roughly two-thirds of the German population to use fluoridated salt. 100,000 € divided by a population of 80 million is equivalent to 0.00125 €, or 0.125 €-cents per capita and year. This is roughly 4 times the cost of F-salt production and quality control. The example of the dormant salt fluoridation in France shows that promotion costs are indispensable, although they exceed the cost of production costs during the initial years. The total cost for a successful salt fluoridation scheme, while maintaining free choice, would thus range between 0.024 (=0.023+0.00125), and 0.04 €-cents (or 0.039 €-cents; =0.038+0.00125). In these estimates, the cost of building the fluoride addition apparatus is not considered. It is evident that salt fluoridation is by far the cheapest method of caries prevention.

Universal salt fluoridation has only been implemented in Jamaica (close to 100%) and in Uruguay (95%). In the majority of iodization program, such a high coverage is the rule. This is justified because the consequences of insufficient iodide intake are very serious: disfiguring goiter, oligophreny, idiocy. One may point out, on the other hand, that progressive destruction of the teeth and their roots can lead to situations which are potentially dangerous for health in general: infections, abscesses, tooth loss and bacteraemia with unforeseeable consequences affecting work and income. In view of the public health aims, use of universal salt fluoridation is clearly indicated: it is a safe, effective and extremely cheap health measure. On the other hand, restoration of decayed teeth and tooth replacements is very expensive, amounting to about 10% of all medical costs.

In Switzerland there is free choice between "pure" salt and salt with iodine, fluoride or both micronutrients. However, it has been the aim of both the Swiss health authorities and the Swiss Dental Association to motivate the greatest possible proportion of the population to use the iodized and fluoridated salt regularly. Since 1998, 85% of the domestic salt has been fluoridated. The following factors may have played a role in this success:

- Salt iodization was a great success in Switzerland for both the medical community and the public at large.
- The idea of salt fluoridation was closely connected with the idea of using salt as a carrier of a micronutrient, as in the case of iodide.
- A few middle-sized food chains offer only F-I-salt since the other varieties are purchased by less than 10% of the customers.
- The price of salt is the same, regardless of whether it contains additives or not.

Contrary to the situation in "water fluoridation countries", opposition against fluoridated salt has been minor and has been almost totally absent for 30 years. When the fluoride concentration was increased from 90 to 250 ppm F, the Swiss Dental Association created a simple leaflet stating that people should continue to use the "new" fluoridated salt, in view of its stronger anticaries effect. The population followed this advice and used fluoridated salt increasingly.

High caries prevalence in the low social strata

Caries prevalence has consistently been highest in the lowest social strata. Could salt fluoridation be the right vehicle to improve this deplorable situation? It is a fact that in the low social strata, preventive recommendations and practices are not adopted to a sufficient degree. For example, if only 40% of domestic salt is fluoridated, the greatest part of this salt will be consumed by the population in the upper social strata. In order to attain benefit for all social levels, the great majority of the population must become regular users of fluoridated salt. In order to reach the lower strata, a percentage of fluoridated salt of at least 67% is necessary. In Europe only two countries, Switzerland and Germany, have attained a coverage exceeding two-thirds of the population. In these two countries, part of the lower strata population actually uses fluoridated salt, particularly in Switzerland where approximately 85% of the domestic salt is fluoridated. In the other European countries, where the use of fluoridated salt is below 50%, the beneficial effect on a public health scale must be considered to be minimal. It should be noted that EU-regulations permit addition of fluoride to salt (and to water) (22).

Obviously the overall situation is unsatisfactory in most European countries. The great challenge for each country would be to find means and ways to increase the percentage of people using – exclusively or at least predominantly – the fluoridated variety of salt. In addition to the promotion of public health campaigns on all levels, further possibilities should be considered:

- Use of fluoridated salt for bread. This single step would increase the number of daily contacts of fluoride with the teeth; whenever a piece of bread is eaten, the fluoride concentration in the saliva is raised to approximately one ppm F, whereas in resting saliva it is only at about 0.02 ppm F.
- Use of fluoridated salt in restaurants, canteens and institutions for the aged. This would specifically increase the number of "fluoride contacts" in the adults.
- Put fluoridated salt on sale in all current package sizes, so that the housewife is not tempted to buy unfluoridated salt because she does not like a certain package size; this measure increased the market share of F-salt from 58% in October-November-December 1993 to 84% in April-May-June 1994 (see Fig. 2 in Marthaler (17)).
- Limited access to unfluoridated salt: the "official" or "regular" salt would be fluoridated: this situation actually exists in several Latin American countries, for example in Jamaica, Costa Rica and Colombia. Legislation may be passed allowing unfluoridated (and/or uniodized) salt to be available exclusively in pharmacies or else in organic food shops.
- Higher concentrations of fluoride in the salt. This would also increase the effectiveness of an already functioning salt fluoridation scheme, but it would not increase the percentage of those who use the fluoridated salt. The possibility of covering the highest possible percentage, however, is a primary aim of any project. Toth (18) for example was aware that 250 ppm F is too low when fluoride is added exclusively to domestic salt. In the final years of his studies, he used exclusively salt with 350 ppm F.

These changes could be attained at minimal cost. Increasing the percentage of users by using public relations firms, as in Germany, would be much more costly. It is indispensable for the medical community to receive excellent documentation on the benefits and safety of the fluoride added to salt. In view of the trend for lowering salt intake, the public should be informed clearly that fluoridated salt is the only type of salt recommended for use in all normal households. In highly industrialized countries, an increasing percentage of food is industrially processed whereby salt is often added; in addition, bouillon cubes and many other spices replace simple cooking salt. In fact, the consumption of plain salt is decreasing slowly but steadily.

Conclusion

Fluoridated salt has enormous potential in controlling dental caries. It is cheaper than any other method of caries prevention applicable to millions. Nevertheless, the current trend in academic dentistry is to rely almost exclusively on the topical effectiveness of fluoride-containing dentifrices to attain better dental health. However, only one or at most two billion of the world's population have access to high quality dentifrices, can afford them and have sufficient instruction and discipline in toothbrushing. The fluoride in dentifrice will reach the tooth surfaces to be protected only when a certain level of oral hygiene is obtained. For the majority of people in the world, this situation is unlikely to change in the next decades.

The cost of a fluoride-containing dentifrice is at least 5 € per year and person (the cost of the toothbrush may be categorized as being paid for by maintaining gingival and periodontal health). For fluoride reaching the tooth from salt in food, the cost is less than 0.05 €, a ratio of 1/10,000. Assuming that fluoride in dentifrices is four times more effective than salt fluoridation, the cost ratio is still favorable: 1/2,500. Of course, fluoridation of salt and dentifrices are not alternatives – they should be used in combination. It is well documented that 55-60 million Germans and Swiss and more than 160 million people in Latin America benefit from the combined use of both fluoridated salt and fluoride toothpastes.

Salt fluoridation is legal in the EU, and is suggested by WHO (Resolution WHO 60-17) when water fluoridation cannot be implemented for whatever reason. In continental Europe, there are 25 EU countries with a total population of around 330 million (Great Britain and the Irish Republic both have water fluoridation policies). Eight of these countries, with a total population of 200 millions, already have at this time the technical means to produce fluoridated salt. Accordingly, the total number of 200 million inhabitants of these countries could all at once benefit from the already existing machinery. While the oral health situation is slowly improving in some of the modernized countries, the introduction of salt fluoridation in combination with fluoride-containing dentifrices would accelerate this favorable development.

Dental treatments are still expensive. The cost of dentistry has hardly been reduced, even in countries where the decline in caries began 30 years ago. Extension of preventive dentistry is still indispensable for improving oral health, also from a financial viewpoint. Last but not least, salt fluoridation offers a realistic potential to ameliorate dental health in the poorer regions of the world.

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References

1. Marine D, Kimball OP. The prevention of simple goiter. J Lab and Clin Med. 1917;3:40-5.
2. Wespi HJ. 40 years of goiter prevention with fluoridated salt [40 Jahre Kropfprophylaxe mit jodiertem Salz]. Bulletin des Eidg. Gesundheitsamtes 1962; Beilage B, Nr.2
3. Wespi HJ. Some thoughts about optimal nutrition during gravity [Gedanken zur Frage der optimalen Ernährung in der Schwangerschaft] Schweiz. Med Wochenschrift. 1948;78:153-7.
4. Wespi HJ. Fluoridated salt for caries prevention [Fluoridiertes Kochsalz zur Cariesprophylaxe]. Schweiz med Wochenschr. 1950;80:561-4.
5. Marthaler TM, Steiner M. Cariostatic results in the Canton of Glarus 4 years after the introduction of fluoridated salt [In German]. SSO Schweiz Monatsschr Zahnheilkd. 1981;91(1):9-20.
6. Dean HT. Endemic fluorosis and its relation to dental caries. Public Health Rep. 1938;53:1443-52.
7. Dean HT, Jay P, Arnold FA Jr., McClure FJ, Elvove E. Domestic water and dental caries, pincluding certain epidemiological aspects of oral L. acidophilus. Public Health Rep. 1939b;54:862-88.
8. Arnold FA Jr. Fluoride in drinking water: Its effect on dental caries. J Am Dent Assoc. 1948;36:28-36.
9. Marthaler TM, Meyer J, Bürgi H. The change from water to salt as the main vehicle for community-wide fluoride in Basle. Community Dent Oral Epidemiol. 2003;31:401-2.
10. DeCrousaz P, Marthaler TM, Menghini G, Steiner M. Fluoridation of salt in Switzerland [Fluoration du sel alimentaire en Suisse]. Réal Clin. 1993;4:343-50.
11. Marthaler TM, Steiner M. Caries-statistical results in the Canton of Glarlus, four years after the introduction of fluoridated salt [Kariesstatistische Ergebnisse im Kanton Glarus, vier Jahre nach Einführung fluoridierten Salzes]. Schweiz Monatsschr Zahnheilk. 1981;91:9-20.
12. Marthaler TM, Petersen PE. Salt fluoridation – an alternative in automatic prevention of dental caries. Int Dent J. 2005;55:351-8.
13. Marthaler TM. Overview of salt fluoridation in Switzerland since 1955, a short history. Schweiz Monatsschr Zahnmed. 2005;115:651-5.
14. Menghini G, Marthaler TM, Steiner M, Bandi A, Schürch E. Caries prevalence and ginigval inflammation in recruits in 1985; effects of preven-

tion [Kariesprävalenz und gingivale Entzündung bei Rekruten im Jahre 1985: Einfluss der Vorbeugung]. Schweiz Monatsschr Zahnmed. 1991;101:1119-26.

15. De Crousaz P. Observations regarding enamel opacities in Switzerland related to fluoridation of drinking water and alimentary salt [Beobachtungen über Schmelzopazitäten in der Schweiz im Zusammenhang mit der Trinkwasserfluoridierung oder der Salzfluoridierung]. Schweiz Monatsschr Zahnheilk. 1982;92:332-44.
16. Thylstrup A, Fejerskov. Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. Comm Dent Oral Epidemiol. 1978;6:315-8.
17. Steiner M, Menghini G. Asssessment of dental fluorosis prevalence in Swiss populations. Schweiz Monatsschr Zahnmed. 2010;120:12-20.
18. Marthaler TM, Steiner M, Menghini G, Sener B, De Crousaz P. Urinary Fluoride Excretion in Children With Low Fluoride Intake or Consuming Fluoridated Salt. Caries Res. 1995;29:26-34.
19. Toth K. Caries prevention by domestic salt fluoridation. Budapest: Akademiai Kiado; 1984.
20. Radnai M, Fazekas A. Caries prevalence in adults seven years after previous exposure to fluoride in domestic salt. Acta Med Dent Helv. 1999;4:163-6. [in: Schweiz Monatsschr Zahnmed Vol. 109, No. 10, October 1999]
21. Tramini P. Salt Fluoridation in France since 1986. Schweiz Monatsschr Zahnmed. 2005;115:656-8.
22. Schulte AG. Salt fluoridation since 1991. Schweiz Monatsschr Zahnmed. 2005;115:659-62.
23. Marthaler TM, Gillespie G, Götzfried F. Salt fluoridation in Europe – with potential worldwide. Kali und Steinsalz. 2011;Heft 3:2-15
24. Gillespie G, Roviralta G, eds. Salt fluoridation. Scientific Publication No. 501 of the Pan American Health Organization. Washington D.C.: the Regional Office of the World Health Organisation; 1986.
25. Mejia R, Espinal F, Velez H, Aguirre M. Resultados obtenidos de 1864-1972, Fluoruracion de la sal. Scientific and Technical Publication No. 615 of the Pan American Health Organizaion. Washington D.C.: the Regional Office of the World Health Organization; 2005.
26. Estupinan SR, Baez RJ, Horowitz H, Warpeha R, Sutherland B, Thamer M. Salt fluoridation and dental caries in Jamaica. Community Dent Oral Epidemiol. 2001;29:247-52.
27. Warpeha R, Beltran-Aguilar E, Baez R. Methodological and Biological factors Explaining the Reduction in Dental Caries in Jamaican School Children between 1984 and 1995. Pan American Journal of Public Health. 2001;10(1):37-44.
28. Baez RJ, Marthaler TM, Baez MX, Warpeha RA. Urinary fluoride levels in Jamaican children in 2008, after 21years of salt fluoridation. Schweiz Monatsschr Zahnmed. 2010;120:21-8.
29. Estupinan S. Promoting Oral Health: the use of salt fluoridation to prevent dental caries. Scientific and Technical Publication No. 615 of the Pan American Health Organizaion. Washington D.C.: the Regional Office of the World Health Organization; 2005.
30. Gillespie GM, Marthaler TM. Cost Aspects of Salt Fluoridation. Schweiz Monatsschr Zahnmed. 2005;115:778-84.

Review article
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Milk fluoridation for the prevention of dental caries

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The aim of this review is to give an overview of 55 years experience of milk fluoridation and draw conclusions about the applicability of the method. Fluoridated milk was first investigated in the early 1950s, almost simultaneously in Switzerland, the USA and Japan. Stimulated by the favourable results obtained from these early studies, the establishment of The Borrow Dental Milk Foundation (subsequently The Borrow Foundation) in England gave an excellent opportunity for further research, both clinical and non-clinical, and a productive collaboration with the World Health Organization which began in the early 1980s. Numerous peer-reviewed publications in international journals showed clearly the bioavailability of fluoride in various types of milk. Clinical trials were initiated in the 1980s – some of these can be classed as randomised controlled trials, while most of the clinical studies were community preventive programmes. **Conclusion.** These evaluations showed clearly that the optimal daily intake of fluoride in milk is effective in preventing dental caries. The amount of fluoride added to milk depends on background fluoride exposure and age of the children: commonly in the range 0.5 to 1.0 mg per day. An advantage of the method is that a precise amount of fluoride can be delivered under controlled conditions. The cost of milk fluoridation programmes is low, about € 2 to 3 per child per year. Fluoridation of milk can be recommended as a caries preventive measure where the fluoride concentration in drinking water is suboptimal, caries experience in children is significant, and there is an existing school milk programme.

Key words: Caries prevention, Fluoride prevention, Milk fluoridation, Caries reduction, Community programmes.

Introduction

The aim of this article is to describe the history of milk fluoridation and its place in caries prevention. Individual studies have not been referenced in this review as they are listed in the WHO publication 'Milk fluoridation for the prevention of dental caries' (1).

Early investigations into fluoridated milk

The idea of milk fluoridation emerged, at about the same time, in Japan (1952), in Switzerland (1953) and the USA (1955). Early investigations showed that fluoride added to milk does not change its taste or other characteristics, is absorbed well, although slower

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than from fluoridated water. It was considered advantageous that fluoride is added to an important food for infants and small children, and that consumption of fluoridated milk is not mandatory for everybody, only for those who need it most and agree to receive it. The caries preventive effect of fluoride can even be enhanced by the milk vehicle, due to the cariostatic properties of the mineral, protein and fat content of milk.

The first clinical results were reported by Imamura in 1959, after a five-year study of Yokohama schoolchildren. Milk or soup, containing 2.0 to 2.5 mg sodium fluoride, was consumed at lunch-time, 150 to 180 days per year, by 167 children. Compared with the control group, 29 to 34% caries reductions were observed in the permanent dentition. In Baton Rouge, Louisiana, USA, Rusoff and co-workers reported in 1962 on 3.5 years' results in 129 (65 test and 64 control) children. In children consuming fluoridated milk at school meals, 35% less caries was recorded than in the control children; in those who were 6 years old at the beginning, the reduction was even larger at 70%. In Winterthur, Switzerland, Ziegler and Wirz reported a study where 0.22% sodium fluoride solution, prepared by pharmacies in plastic bottles, was added by the parents within the home to milk consumed by children. Participants were 749 test and 553 control children who were 9 to 44 months old at the start of the programme. In 1964, after six years, caries reductions were 17% for the deft index (number of decayed, extracted or filled primary teeth) and 30% for the defs index (number of decayed, extracted or filled primary tooth surfaces) in the primary dentition, and 64% for DMFT (number of decayed, missing or filled permanent teeth) and 65 for DMFS (number of decayed, missing or filled permanent tooth surfaces) in permanent molars. The proportion of caries-free children increased significantly in the fluoridated milk group.

The Borrow Foundation

The establishment of a charity in England by Edgar Wilfred Borrow (1902-1990) for the promotion of milk fluoridation in order to prevent dental caries in children, brought important progress in the field of research and clinical studies. E.W. Borrow (Figure 1), a wealthy farmer and mechanical engineer in south England, constantly interested in the technical aspects of fluoridation of milk, set up a charity in 1971, named the "Borrow Dental Milk Foundation", for the above purposes.

The aims, summarized in 12 points, were mainly "to promote and support research of fluoridated milk for human consumption by the help of grants, equipment, lectures, scientific publications, and to disseminate knowledge about this method". The aims of the original 'Trustees' deed' were extended in 1993 to include "the support of activities on health promotion and education,… and on healthy nutrition, including milk and milk products".

Figure 1 Edgar Wilfred Borrow – the founder of "The Borrow Foundation".

The name of the foundation was changed in 2002 to 'The Borrow Foundation' (www.borrowfoundation.org). In recognition of his humanitarian services, E.W. Borrow received an Honorary Doctorate from Lousiana University, USA, in 1983. Two of the authors of this present review are two of the five "Trustees" of The Borrow Foundation.

The results of clinical and basic research studies, supported by The Borrow Foundation, have made the creation and extension of milk fluoridation programmes possible in numerous countries of the world. Based on discussions initiated in the 1980s between The Borrow Foundation and the World Health Organization (WHO), the Bulgarian milk fluoridation programme was initiated, and a 'Memorandum of Understanding' was signed by The Foundation and WHO in 1991; this has been renewed every three years. As a result of this collaboration, a book was published in 1996 (2), summarizing the studies of basic and clinical research into milk fluoridation, and a revised edition was published in 2009 (1).

Theoretical considerations

Concerning the pathomechanism of fluorides, it is accepted that elevated fluoride ion concentrations at the dental plaque/enamel border decrease the rate of demineralisation, increase remineralisation, and reduce acid production of dental plaque. However, the use of milk as a vehicle, generated questions concerning possible chemical reactions between milk and fluoride ions, bioavailability of systematically administered fluoride in milk, and interactions involving fluoride in the oral cavity (enamel, saliva, plaque and caries).

The results of basic studies on milk fluoridation have been published in more than 100 peer-reviewed papers, with increasing frequency in the last 20 years. Based on these studies, according to recent knowledge, the greater part of fluoride added to milk, forms a soluble complex with the protein fraction of milk, from which the fluoride can be liberated in ionic form, so that it is bioavailable. The absorption of fluorides with simultaneous food consumption is slower than for fluoride without food, and the proportion absorbed depends on the calcium content of the diet. Different types of milk are drunk in communities around the world – whole milk or low-fat milk, fresh, pasteurised or sterilised milk, liquid or dried milk. The bioavailability of added fluoride has been investigated in all of these, on the day of milk processing and after several days' storage, and shown to be satisfactory.

Because urine is the main vehicle for excretion of fluoride, analysis of 24 hour urine excretion is presently the best marker of fluoride intake (3). Recording of fluoride excretion in urine over 24 hours has been recommended before and after introduction of fluoride-based community preventive programmes. A WHO document, published in 1999, offers detailed guidelines for the method and calculations: based on these, the optimal fluoride concentration in milk and the appropriate intake of fluoride can be determined.

The systemic effect of fluorides in milk is supported by numerous experimental data. However, by the 1980s, the opinion as to how fluoride acts to prevent dental caries was going through a change: even with the use of systemic fluoride agents, topical effects were considered more important. The consumption of fluoridated milk incorporated into dental enamel inhibited demineralisation and promoted remineralisation. In addition, 30-60 minutes after ingestion of fluoridated milk, both the levels of fluoride in whole saliva and dental plaque increase as a consequence of the presence of fluoridated milk in the mouth and increased concentrations of fluoride in salivary secretions following the absorption of ingested fluoride. Thus, fluoride in milk acts both systemically and topically, in the same way as fluoride in water.

Clinical evaluations

Long-term human studies with fluoridated milk on children, undertaken in about twelve countries have been reported in numerous peer-reviewed papers. Only some of these studies can be classified as RCTs (randomised controlled trials) according to the criteria used in evidence-based medicine; the others can be classed as community-based programmes. In the following paragraphs, the main features of the evaluations of these milk fluoridation programmes in different countries of the world will be summarized, but without the detailed numerical results which can be found in the relevant literature (1, 4, 5) (Table 1).

Scotland: Glasgow

Due to the strong criticism of the early clinical studies (for example, small numbers of participants, lack of baseline examinations, etc.), Stephen and colleagues initiated in Glasgow in 1976, a double blind clinical trial on 4 ½ and 5 ½ year old schoolchildren. The group of test children consumed 200 ml milk each school day (about 200 days per year), containing 1.5 mg fluoride, while the control group received plain milk. The results published in 1984, after five years, reported a 36% reduction in DMFT and a 48% reduction in DMFS values for the first permanent molars which were not yet erupted at baseline in the test group compared with the control group. Fluoride excretion in urine was monitored constantly during the study. (1) This evaluation (together with the Volgograd programme by Maslak: see later) is one of the programmes accepted as an RCT by the Cochrane Centre for Systematic Reviews.

Table 1 List of published reports of studies into the effectiveness of milk fluoridation

Study	Year of study	Authors	Caries prevention in:	
			Primary teeth	Permanent teeth
Yokohama, Japan	1952 – 1956	Imamura, 1959	-	+
Baton Rouge, USA	1955 –1959	Rusoff et al., 1962	-	+
Winterthur, Switzerland	1958 – 1964	Wirz, 1964; Ziegler, 1964	+	+
Agudos, Brazil	1976 – 1979	Lopes et al., 1984	-	-
Glasgow, UK	1976 –1981	Stephen et al, 1981; Stephen et al., 1984	-	+
Fót, Hungary	1979 –1990	Bánóczy et al., 1983; Bánóczy et al., 1985; Gyurkovics et al.,1992	+	+
Louisiana, USA	1982 – 1985	Legett et al., 1987	-	+
Bethlehem, Israel	1983 –1986	Zahlaka et al., 1987	+	+
Asenovgrad, Bulgaria	1988 – 1993	Pakhomov et al., 1995; Atanassov et al., 1999	+	+
Codegua, Chile	1994 –1999	Mariño et al., 2001	+	-
Voronezh, Russia	1994 – 2004	Pakhomov et al., 2005	+	-
Wirral, UK	1995 – 2003	Riley et al., 2005	-	+
Beijing, China	1997 –1999	Bian et al., 2003	+	-
Knowsley, UK	1997 – 2001	Ketley et al., 2003	-	-
Volgograd, Russia	1998 –2002	Maslak et al., 2004	+	+
Araucania, Chile	1999 – 2002	Weitz and Villa, 2004	-	+
Umeå, Sweden	2006 – 2007	Steckén-Blicks et al., 2009	+	-
Ljungby, Sweden	2006 – 2008	Petersson et al., 2011	-	+

Hungary: Fót

In the 'Children's City' of Fót, a milk fluoridation programme was initiated by Bánóczy, Zimmermann and colleagues in 1979, involving about 1000 children aged 2 to18 years (1). The results were published after 2, 3 and 10 years (1982-1992). The children drank for breakfast milk or cocoa, containing 0.4 mg fluoride for kindergarten children and 0.75 mg fluoride for the schoolchildren. The sodium fluoride solutions were prepared by the Pharmacy of Semmelweis University in closed glass bottles, then added to the milk in the kitchen of the home, stirred thoroughly for 15 minutes, and consumed within 30 minutes by the children. After five years, in the test group (165) children compared with a control group, a considerable caries reduction was observed in both the primary and permanent dentitions. In 7 to 10 year old children, these percentage reductions were 54% in DMFT and 53% in DMFS values. The reduction in the total permanent dentition was 60% for DMFT and 67% for DMFS; the highest reductions were found in the children who had consumed fluoridated milk from 2 to 3 years of age. The difference between the caries prevalence of the test and control groups was, in spite of loss of children from the study, still statistically significant after 10 years.

USA: Lousiana, Baton Rouge

In the second Lousiana community programme, begun in 1982, schoolchildren consumed fluoridated milk, containing cocoa and sugar, for lunch for two or three years. After two years, a significant caries reduction was observed in the permanent dentition: however, due to the loss of children, three year results could not be evaluated (1).The organiser of the experiment, Legett, planned also to establish a research institute for milk fluoridation which, however, could not be realised.

Israel: Bethlehem

Zahlaka and colleagues reported in 1987 the results of a study on 273 children who were aged 4 to 7 years at baseline and who had consumed fluoridated milk for three years. The fluoridated milk was produced from milk powder, and the dissolved milk contained 1 mg fluoride per litre. A 63% caries reduction was observed in both the primary and permanent dentitions after three years (1).

Bulgaria: Asenovgrad

One of the most extensive milk fluoridation programmes was initiated by Pakhomov and colleagues in Bulgaria in 1988 with the support of WHO (1). The objective was to see if such a programme was feasible under everyday life conditions. Bulgaria seemed to be an excellent choice for this community-based programme due to the regular consumption of milk and milk products (for example, yoghurt) by children. The city Asenovgrad in south Bulgaria was selected as the test community and the nearby city of Panaguriche as the control community; later, Karlovo became the control community. The fluoridated milk was produced and transferred from the Plovdiv dairy, in plastic bags for each child containing 1 mg fluoride per day. About 3,000 children aged 3 to 10 years entered the programme in Asenovgrad (Figure 2).

The caries examinations at baseline and after 3 and 5 years were performed by dentists calibrated by a WHO epidemiologist. Urine monitoring was carried out regularly. After five years, mean dmft values were 52% lower in the test group children aged 6 ½ years and 40% lower in the 8 ½ year olds. The reductions in mean DMFT in these two age groups were 89% and 79% – statistically highly significant. After 10 years of the programme, Atanassov and colleagues recorded further significant differences in the proportion of caries-free children and in

Figure 2 Bulgaria program: kindergarten children drinking fluoridated milk.

mean DMFT (1) values of the test and control groups. In some communities there is a preference for fluoridated yoghurt.

Brazil and Peru

From Agudos in Brazil, Lopes and colleagues reported in 1984 a small milk fluoridation study lasting 16 months. However, due to the short period, the results were not significant. In Peru, a milk fluoridation programme started in the early 2000s, based on the government programme 'vaso de leche', which provides one glass of milk for children each day. The programme was controlled by the University of Trujillo. The children received their milk in 'Mother's clubs', where a fluoride solution prepared by the pharmacies was added to fresh milk brought in by farmers, stirred thoroughly for 15 minutes, and consumed shortly after (1) (Figure 3).

However, the programme was stopped after a few years because of the expanding use of fluoridated salt in that community, before any evaluation was made. The encroachment of fluoridated salt was detect-

Figure 3 Peru program: fluoridated milk –after stirring- distributed at the "mothers club".

ed before the community was aware of the presence of fluoridated salt, by monitoring of urinary fluoride excretion.

Chile: Codegua and Araucania

The Chilean milk fluoridation programmes possess two features which differ from other programmes. First, instead of using sodium fluoride they use sodium monofluorophosphate which, according to Villa and colleagues, has good bioavailability and other technical advantages and, second, the fluoride is added to powdered milk.

The first investigation took place in Codegua involving infants and young children, and took advantage of the 'national nutrition complementing programme' (PNAC) which has been in existence for more than 50 years. Under this scheme, every Chilean child, from birth to two years of age, receives two kilogrammes of milk powder every month, while children aged 2-6 years receive monthly one kilogramme of milk powder with cereals. The PNAC programme covers 90% of the child population. The fluoridated milk pilot programme was organised and evaluated by Villa, Mariño and colleagues in 1994 in the rural areas of Codegua (test) and La Punta (control). Children between 0 and 6 years of age consumed daily, for four years, 0.25, 0.5 or 0.75 mg fluoride mixed into the milk powder, according to their age-group. Fluoride-containing toothpaste was available and urine monitoring for fluoride excretion was performed regularly. After five years, the proportion of caries-free children was higher in Codegua than in the control La Punta, and mean dmfs values showed significant reductions in children in Codegua compared with children in La Punta. However, examinations performed three years after cessation of the program showed very small differences, pointing to the necessity of continuous maintenance of caries preventive programmes.(1).

In the IXth region of Chile, a new fluoridated milk programme started in 1999 with about 35000 children aged 6 to 14 years who were participating in the national powdered milk programme (see above). In the community of Araucania, 6, 9 and 12 years old children received milk powder containing sodium monofluorophosphate, while the control children received milk powder without added fluoride. The control children were already participating in a community preventive programme in which they received applications of a high-fluoride gel. Examinations showed, historically, reductions in caries of 24 to 27% in children aged 9 and 12 years, which was similar to the results of the fluoride gel programme. Because the fluoride gel programme was difficult to administer (it involved gel application by health professionals), the milk-powder fluoridation programme has now been introduced into the majority of the Chilean regions as part of the caries preventive programme for 6-14 year old children living in rural communities. While the main cities in Chile receive optimally fluoridated water as a public health measure, milk fluoridation is provided in the rural areas where water fluoridation is technically not possible, in order to ensure equity.

China: Beijing

Due to the increasing caries prevalence in some parts of China, a milk fluoridation programme was introduced between 1994-1997 for Chinese kindergarten children in a district of Beijing. An evaluation showed no effect, probably due to the high amount of sugar (7-10%) added to milk. In the second phase of the programme, therefore, no sugar or only small amount of sugar was added to the pasteurized milk which contained 2.5 ppm fluoride and which was consumed everyday in kindergartens. In addition, children brought home fluoridated milk for weekends. Dentists calibrated to WHO standards examined the children after 21 months, recording also arrested caries. The mean dmft value in the test group showed a 69% reduction compared with the control (1). These results showed that fluoridated milk, when consumed daily, was able to prevent caries in the primary dentition and stop active dentinal caries from progressing, probably due to the topical effect of fluoridated milk. The study may also indicate the importance of not adding sucrose to milk (or other drinks).

United Kingdom: Knowsley and Wirral

A milk fluoridation programme was launched in 1997 in Knowsley by Ketley and colleagues, where 4060 three to five year old children (mean age 4.7 years), consumed, each day, milk containing 0.5 mg fluoride; the control children in Skelmersdale drank plain milk. The number of days the children received milk was about 180 days per year. Caries evaluation was made, based on BASCD (British Association for the Study of Community Dentistry) criteria. After four years, no statistically significant differences in dmft and dmfs values of the two groups were found. The DMFT and DFS values were slightly, but not statistically significantly, smaller in the 7 to 9 year old children of the test group, than in the control. The assumption for these results was that the dose of fluoride in the milk was too low and that the period of consumption was not long enough to show an effect.

In a second evaluation in the Wirral region of north-west England, examinations, using the same BASCD criteria, were made by Riley and colleagues in 2003 on 5700 children who were at least 5 years old when they entered the fluoridated milk programme. Data for the four permanent molars were compared between 773 children who had been drinking fluoridated milk for six years at least, and 2052 children from Sefton, who had received milk without added fluoride. Caries prevalence in the test group was 13% less in the primary dentition and 16% less in the permanent dentition. The mean DMFT value showed a reduction of 31%, and the mean DFS a 37% reduction, compared with the control (1).

Russia: Volgograd and Voronezh

Milk fluoridation programmes in Russia started in 1993 as a collaboration between the WHO and The Borrow Foundation, with participants initially in three communities – Voronezh, Maykop and Smolensk – and later on in Volgograd and several communities in Tatarstan (Figure 4). Kouzmina and colleagues evaluated three year results in 1999 for 15000 participating children, and reported caries reductions between 55 and 68%.

Figure 4 Measurement of the fluoride solution in the Russian program.

The second milk fluoridation programme in Russia was in Volgograd, and this was evaluated by Maslak and colleagues in a three year study involving children who were caries-free when entering the programme at 3 years of age. In this double-blind evaluation, undertaken by examiners calibrated according to WHO criteria, on 75 test and 91 control children, statistically significant reductions were recorded, both in dmft and DMFT values, and in longitudinal as well as cross-sectional comparative analyses. According to the evaluation by the Cochrane Centre for Systematic Reviews, this study, as well as that of Stephen and colleagues in Scotland, is accepted as an RCT and as evidence for the effectiveness of milk fluoridation.

In the town of Voronezh, the effect of a 10 year milk fluoridation programme was evaluated on 15000 kindergarten children in two horizontal comparative analyses. Pakhomov and colleagues compared data from 335 test and 175 control children after three years, and revealed a statistically significant reduction in dmft values and an increase in caries-free children in the test group. In a second analysis, data from 3, 6, 9 and 12 year old children were compared cross-sectionally with baseline data, and a statistically significant caries reduction was observed. Urinary fluoride monitoring showed that the daily consumption of 200 ml milk containing 2.5 ppm fluoride is an effective caries preventive method and that the fluoride intake corresponded to physiological norms (1).

Thailand: Bangkok and other communities

A well-organized milk fluoridation programme for children started in Thailand in the year 2000 with the help of The Royal Chitralada Projects a unique centre for agricultural and research development, initiated by His Royal Highness King Bhumibol Adulyadej. An evaluation is in progress. The project now includes all schoolchildren in Bangkok, and seven other provinces in Thailand, reaching nearly a million children in total (Figures 5 and 6).

Figure 5 Thailand program: schoolchildren consuming fluoridated milk during a break.

Former Yugoslav Republic of Macedonia

In October 2009 the Ministry of Health introduced a milk fluoridation programme in the Former Yugoslav Republic of Macedonia which was promoted as one of the measures to be applied under a national strategy for prevention of oral diseases in children aged 0 to 14 years.

The scheme was established through the kindergarten system, and involved approximately 7,700 children aged 3 to 5 years, who received 200 ml fluoridated UHT milk on school days. Although the programme ceased in 2011 when Government funding for school milk was decentralised to local

Figure 6 Thailand program: schoolchildren consuming fluoridated milk during a break.

municipalities, there is a will for the programme to be reinstated as part of a wider health promoting project, "Healthy Food for Healthy Childhood".

Sweden: Umeå and Ljungby

Stecksén-Blicks and colleagues (4) carried out an evaluation of the effect of supplementing milk with fluoride and probiotic bacteria which was provided to pre-school children in day care centres: the results were published in 2009. It was conducted near Umeå, northern Sweden, where there is a culture of using probiotics for general health benefits. 248 children aged 1 to 5 years attending 14 day care centres entered the study. The centres were randomly assigned to test and control. Children in the test group received 150ml of milk containing 2.5 mgF/litre and probiotic bacteria while the control group received standard milk. The double-blind intervention lasted 21 months when a per cent caries reduction of 75% was recorded. Although there is some evidence elsewhere that probiotics confer some caries-preventive effect, the majority of this large effect is likely to be due to the addition of fluoride.

The second Swedish trial investigated prevention of dental caries in root surfaces of teeth in older people. The main outcome of this trial by Petersson and colleagues (5) and published in 2011, was the healing (remineralisation or hardening) of early lesions. Again, addition of fluoride and probiotic bacteria to milk was investigated but, unlike the Umeå study, there were four parallel groups, so that the independent effects of fluoride and probiotics could be investigated. 160 healthy subjects aged 58 to 84 years took part: the study period was 15 months. The quantity of milk drunk was 200ml per day and the level of fluoride supplementation was 5.0 mg F/litre. Although some benefit was recorded from consumption of probiotics, this effect was not statistically significant and the effect was much less than the statstically significant effect of fluoride.

Safety considerations

Numerous studies in several countries have demonstrated that ingestion of fluoride added to milk is well within WHO guidelines for young and older children: this conclusion is based on WHO guidelines for urinary fluoride excretion (6). One of the advantages of milk fluoridation is that a precise amount of fluoride is added to milk and provided to children each day. Follow up studies, by Mariño and colleagues (7), of children who took part in the Codegua study in Chile (see above) showed no adverse effect on the appearance of permanent front teeth which had been forming at the time the children were receiving their fluoridated milk.

Cost of milk fluoridation programmes

There have been several economic evaluations of the milk fluoridation programme in Chile (8, 9). These conclude that the programme costs about €1.20 to €2.40 per child per year. This figure is very similar to the figure of £1.25 (€1.50) per child per year in the UK milk fluoridation programme and 34.06 Thai baht (€ 0.86) per child per year in Thailand.

Conclusions

There are now over a million children receiving fluoridated milk (Table 2).

The effectiveness of milk fluoridation in preventing dental caries is supported by about 18 clinical studies reported in numerous papers. Of these, nine demonstrated caries prevention in primary teeth and 12 in the permanent dentition (Table 1). Two studies showed no effect in either dentition. An evaluation after cessation of a pilot milk fluoridation programme in Chile, caries incidence increased (10). Four RCTs showed caries reductions, and evaluations of the several community programmes pointed to the feasibility of the method under real life conditions. This evidence from clinical studies is underpinned by much research which demonstrates the bioavailibility of fluoride added to milk and the biological plausibility of milk fluoridation. Milk fluoridation is safe and the cost is low.

Based on these published studies, it seems that to obtain good results with milk fluoridation, even in the primary dentition, the programmes should start early, possibly

Table 2 The international programme

Country	Year F milk programme began	Number of participating children	Age-range of children (y)	Amount of milk (ml)	Amount of fluoride (ml)
Bulgaria*	1988	31000	3 to 7	100	0.5
				150	0.75
				200	0.5
United Kingdom	1993	32000	3 to 11	189	0.5
Russian Federation	1994	39000	3 to 11	200	0.5
Chile	2000	220000	6 to 14	200	0.85
Thailand	2000	982188	3 to 12	200	0.5
Former Yugoslav Republic of Macedonia†	2009	7700	3 to 6	200	0.5

*As at 2008; programme under review; †As at 2011; programme under review.

before the age of four years. In order to protect the permanent molar teeth, consumption of fluoridated milk is necessary during and after their eruption too. The amount of fluoride added to milk is decided depending on age and background exposure to fluoride: the amount is commonly about 0.5 mg per day for young children and around 1.0 mg per day for older children. The introduction of milk fluoridation programmes should be considered where the fluoride content of drinking water is low, where a regular school milk system is working and where the children are able to consume the fluoridated milk for at least 200 days in a year.

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References

1. Bánóczy J, Petersen PE, Rugg-Gunn AJ, editors. Milk fluoridation for the prevention of dental caries. 2nd ed. Geneva: World Health Organization; 2009.
2. Stephen KW, Bánóczy J, Pakhomov GN, editors. Milk fluoridation for the prevention of dental caries. Geneva: World Health Organization/Borrow Dental Milk Foundation; 1996.
3. Rugg-Gunn AJ, Villa AE, Buzalaf MAR. Contemporary biological markers of exposure to fluoride. In: Buzalaf MAR, editor. Fluoride and the oral environment. Monogr Oral Sci. Basel: Karger; 2011. p. 37-51.
4. Stecksén-Blicks C, Sjöström I, Twetman S. Effect of long-term consumption of milk supplemented with probiotic lactobacilli and fluoride on dental caries and general health in preschool children: a cluster-randomized study. Caries Res. 2009;43:374-81.
5. Petersson LG, Magnusson K, Hakestam U, Baigi A, Twetman S. Reversal of primary root caries lesions after daily intake of milk supplemented with fluoride and probiotic lactobacilli in older adults. Acta Odontol Scand. 2011;69:321-7.
6. Marthaler TM. Monitoring of renal fluoride excretion in community prevention programmes on oral health. Geneva: World Health Organization; 1999.
7. Mariño R, Villa A, Weitz A, Guerrero S. Prevalence of fluorosis in children aged 6-9 years-old who participated in a milk fluoridation programme in Codegua, Chile. Community Dent Health. 2003;20:143-8.
8. Mariño R, Morgan M, Weitz A, Villa A. The cost-effectiveness of adding fluorides to milk-products distributed by the National Food Supplement Programme (PNAC) in rural areas of Chile. Community Dent Health. 2007;24:75-81.
9. Mariño R, Fajardo J, Morgan M. Economic evaluation of dental caries prevention programs using milk and its products as the vehicle for fluorides: cost versus benefits. In: Watson RR, Gerald JK, Preedy VR, editors. Nutrients, dietary supplements, and nutriceuticals: cost analysis versus clinical benefits. New York: Springer Science; 2011. p. 143-60.
10. Mariño RJ, Villa AE, Weitz A, Guerrero S. Caries prevalence in a rural Chilean community after cessation of a powdered milk fluoridation program. J Public Health Dent. 2004;64:101-5.

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Fluoride toothpastes and fluoride mouthrinses for home use

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Objective. To provide a brief commentary review of fluoride-containing toothpastes and mouthrinses with emphasis on their use at home. Toothpastes and mouthrinses are just two of many ways of providing fluoride for the prevention of dental caries. The first investigations into incorporating fluoride into toothpastes and mouthrinses were reported in the middle 1940s. Unlike water fluoridation (which is 'automatic fluoridation'), fluoride-containing toothpastes and fluoride-containing mouthrinses are, primarily, for home use and need to be purchased by the individual. By the 1960s, research indicated that fluoride could be successfully incorporated into toothpastes and clinical trials demonstrated their effectiveness. By the end of the 1970s, almost all toothpastes contained fluoride. The widespread use of fluoride-containing toothpastes is thought to be the main reason for much improved oral health in many countries. Of the many fluoride compounds investigated, sodium fluoride, with a compatible abrasive, is the most popular, although amine fluorides are used widely in Europe. The situation is similar for mouthrinses. Concentrations of fluoride (F), commonly found, are 1500 ppm (1500 μg F/g) for toothpastes and 225 ppm (225 μg F/ml) for mouthrinse. Several systematic reviews have concluded that fluoride-containing toothpastes and mouthrinses are effective, and that there is added benefit from their use with other fluoride delivery methods such as water fluoridation. Guidelines for the appropriate use of fluoride toothpastes and mouthrinses are available in many countries.**Conclusion.** Fluoride toothpastes and mouthrinses have been developed and extensive testing has demonstrated that they are effective and their use should be encouraged.

Key words: Toothpaste, Mouthrinse, Fluoride, Dental caries prevention, Oral health.

Introduction

An oft-quoted observation is that increasing consumption of sugar has been responsible for the rise in dental caries experienced by much of the world, while the fall in dental caries observed in many countries has been due to the use of fluoride. This epidemic of dental decay and its partial cure was discussed more fully in an earlier article in this issue of the Journal (1). In the UK, the first

*The authors are Professors emeritus, Semmelweis and Newcastle Universities, respectively. No support was provided for the preparation of this paper.

signs of improving dental health became evident in the late 1970s (2, 3). Surveys in other countries recorded similar improvements, and a conference was convened in Boston, USA in 1982, to explore possible reasons for this unexpected occurrence (4). The summary of the report included the following: "All 16 speakers agreed that the prevalence of dental caries has declined substantially in several countries represented in areas both with and without organised preventive programmes or fluoridation. Most agreed that fluoride in toothpastes, rinses and community water supplies provides the best single association and that there were many factors, including unknowns, which might be involved." Further data were collected and a second conference and report (5), under the auspices of the WHO and World Dental Federation, concluded that "the most probable reasons for the decrease in dental caries in children in the developed countries were considered to be associated with: (i) the widespread exposure to fluoridated water and/or fluoride supplements, especially the regular use of fluoride toothpaste.... The factors common to all countries with a substantial reduction in caries was fluoride, either as fluoridated water or toothpaste." Evidence accumulating over the past twenty years confirms the above views.

Table 1 Estimate of the number of people throughout the world using various types of fluoride therapy in 1990 and 2000. Numbers in millions (27).

Type of fluoride therapy	Period (years)	
	1990	2000
Water fluoridation (n)	210	300
School fluoridation (n)	0.2	0
Fluoridated salt (n)	4	97
Fluoridated milk (n)	0.1	0.2
Drops/tablets (n)	20	15
Mouthrinses (n)	20	100
Clinical topicals (n)	20	30
Toothpastes (n)	450	1500

Although there are several ways of delivering fluoride (1), the number of people using each type of fluoride therapy differs considerably (Table 1).

It can be seen that fluoride toothpastes are by far the most important way of providing fluoride worldwide. This has been recognised by WHO for many years (6), not least by the World Health Assembly 2007 which urged Member States to "... consider ... the provision of affordable fluoride toothpaste". Previous articles in this issue of the Journal have considered fluoride in water, salt, and milk; this article will provide a brief commentary review (7) of the development, effectiveness and use of fluoride-containing toothpastes and mouthrinses. These products are used almost exclusively in the home, in comparison with fluoride in water, salt or milk, which are suitable for community preventive programmes. Details of the very many clinical trials will not be given in this review and the reader is referred to a textbook (8) and the numerous reviews of the Cochrane Collaboration, mentioned below, for further information. By tradition, the common way of expressing fluoride concentration in toothpastes and mouthrinses (and other vehicles such as water) is 'parts per million' or 'ppm': this is equivalent to µg/g (µgF/g toothpaste) or mg/kg for toothpaste, and approximately mg/l for mouthrinse.

The aim of this article is to give a narrative account of the history and development of these products, and recommendations for their use.

History of the development of fluoride-containing toothpastes and mouthrinses

Toothpastes

The remarkable caries-inhibiting effect of fluoride had become clear by the early 1940s and this knowledge led to the introduction of water fluoridation in 1945 and publication of the first trial of a fluoride-containing toothpaste, also in 1945 (9). Formulating an effective fluoride toothpaste is not easy and it took 25 to 30 years before they were widely available. In 1970, less than 5% of toothpastes sold in the UK contained fluoride; by 1976, over 90% contained fluoride. Issues dominating early research were: the incompatibility of abrasives included in toothpastes, different fluoride compounds, effective concentrations, and stability of fluoride compounds in toothpastes during storage. Fluoride compounds that have been tested for caries-inhibitory properties when incorporated into toothpastes include: sodium fluoride, acidulated phosphate fluoride, stannous fluoride, sodium monofluorophosphate, and amine fluoride.

Sodium fluoride (NaF) is the simplest fluoride compound and is very soluble in water. It was added to water in the first water fluoridation scheme and was the first fluoride compound to be added to toothpaste. The first trial, lasting two years, found no preventive effect (9). The abrasive in the toothpaste was calcium carbonate, which was the abrasive usually used at that time. Three more trials of sodium fluoride, using the same or different abrasives, were published up to 1961, all reporting a lack of caries prevention. In 1961, Ericsson, in Sweden, concluded from laboratory research (10) that sodium bicarbonate would be a suitable compatible abrasive, and a subsequent trial in Sweden demonstrated the effectiveness of this formulation (11). A few years later, another Swedish trial showed a toothpaste containing sodium fluoride with an inorganic (acrylic bead) abrasive to be very effective – a 40 to 48% reduction in caries development during the three year trial (12). The formulation was expensive, though. Subsequently, almost all toothpastes containing sodium fluoride included a silica abrasive, and these toothpastes were shown to be effective.

Early laboratory experiments showed that more fluoride was incorporated into enamel when the fluoride-containing solution was acidified. The most promising compound was 'acidulated phosphate fluoride' (APF). Four trials of toothpastes containing APF were published between 1966 and 1972, but the results were mixed and this line of research was not pursued. It should be noted, however, that APF has been the compound of choice in fluoride-containing gels used for topical application in clinics.

Following the initial negative results with sodium fluoride, attention turned to other readily soluble fluoride compounds. Laboratory experiments showed that stannous fluoride could be suitable, and the results of a one year trial of a toothpaste containing stannous fluoride with a calcium pyrophosphate abrasive were published in 1955, showing a substantial caries-preventive effect (13). This was marketed with the name 'Crest' in the USA in the same year. Subsequently, some 40 clinical trials of toothpastes containing stannous fluoride have been published, almost all using the same pyrophosphate abrasive system, although effectiveness was less than that reported in the first 1 year results. There were, however, two problems. First, was the poor stability of stannous fluoride, which tended to hydrolyse to ineffective stannic fluoride; and, second, that use of stannous fluoride toothpastes resulted in teeth accumulating some dark stain. After nearly 30 years, the manufacturers of 'Crest' changed to a sodium fluoride, silica abrasive formulation.

Another compound showing promise in laboratory and animal experiments was sodium monofluorophosphate (MFP). The first clinical trial of a toothpaste containing MFP (with an insoluble metaphosphate abrasive) was published in 1963 (14). This two-year trial demonstrated the clinical effectiveness of this formulation, as has been the case in over 30 subsequent trials testing MFP with a variety of abrasives including chalk, alumina and silica. The way MFP exerts its effect has been debated for nearly 50 years; some research supports the view that the main effect is by direct incorporation of MFP into the apatite lattice, while other research emphasises the importance of enzymatic hydrolysis of MFP within dental plaque.

The protection of enamel from acid dissolution by aliphatic monoamines in laboratory experiments led Swiss researchers, certainly by 1960 (15, 16), to test the hypothesis that the detergent action of these organic compounds could be combined with the action of fluoride to give increased protection from acid attack. These organic compounds were superior to more widely used inorganic compounds in laboratory experiments. It was also suggested that amine fluoride compounds had a strong affinity for enamel and had a direct anti-enzymatic effect on microbial activity within plaque. An impressively long trial in Switzerland (17) demonstrated the effectiveness of an amine fluoride toothpaste. Subsequent trials in Germany, USA and Hungary (18) confirmed the caries-preventive effect. At the end of the 1980s, amine fluoride was combined with stannous fluoride; several studies providing evidence of an increased effect of this compound on dental plaque and enamel fluoride accumulation (18, 19).

Mouthrinses

As with most of the early investigations into fluoride and caries, the first trials of fluoride mouthrinses were carried out in the USA. Since then, results of clinical trials have been reported in at least 14 countries. The first publication of a clinical trial appeared in 1946 (20) – a year after publication of the results of the first trial of a fluoride-containing toothpaste (*vide supra*) – and the second trial in 1948. Both trials were of short duration (12 mo) and did not demonstrate effectiveness; perhaps, also, because the rinse was acidified to pH 4.5. At that time dental caries experience was very high in Sweden and a number of Swedish researchers took investigations into fluoride mouthrinses a stage further: they saw the potential for fluoride mouthrinsing by children in school to be an alternative to water fluoridation since this was unlikely to happen in Sweden. These Swedish trials, lasting two or three years resulted in substantial reductions in dental caries (11). Supervised school-based mouthrinsing programmes became popular in Sweden and some other countries and were credited with a substantial improvement in the oral health of Swedish children.

As with water fluoridation, toothpastes and topically applied solutions, sodium fluoride was the compound of choice in the early mouthrinse trials. In 1972, the first of many trials was published, testing a mouthrinse containing acidulated phosphate fluoride (APF). This followed the pattern of research on fluoride compounds in toothpaste: mouthrinses containing stannous fluoride were tested (first publication in 1973), a trial of a mouthrinse containing amine fluoride was published in 1979, and a trial of a mouthrinse containing ammonium fluoride was published in 1977 – all demonstrated effectiveness in caries prevention. The possibility of enhancing the effectiveness of NaF-containing mouthrinses by the addition of ions such as Al, Mn, Fe, Mg, Zr and K was studied by Swedish researchers for over 20 years. Although some of the results suggested that effectiveness may be increased by the presence of various combinations of the above ions, their superiority to simple NaF mouthrinses was not established.

Effectiveness and recommendations for use of fluoride-containing toothpastes

A very large number of clinical trials of toothpastes containing compounds mentioned above have been published during the past 65 years. These have usually lasted for three years and have involved several hundred subjects. They have been, therefore, expensive, and it is testament to the manufacturers of these toothpastes that they have been prepared to invest heavily to improve their products. This has been highly successful, with the four major global manufacturers working ethically together, with the result that oral health has improved very significantly in most of the world. There have been scientific disagreements between manufacturers, for example over the degree of clinical effectiveness of stannous fluoride toothpastes, but these have been rare. Four aspects of the effectiveness and use of fluoride-containing toothpastes will now be considered.

Type of fluoride compound

The incompatibility of sodium fluoride with commonly used, and cheap, abrasives, led to the development of toothpastes containing other fluoride compounds. Stannous fluoride had drawbacks of stability and staining (*vide supra*) and was abandoned: despite manufacturer's claims, stannous fluoride was never shown to be superior to sodium fluoride or sodium monofluorophosphate (MFP). Presently, results of trials indicate that the clinical effectiveness of toothpastes containing sodium fluoride, MFP, and amine fluoride are similar. In some trials, toothpastes contained both NaF and MFP, but any clinical advantage was small. Additions aimed at increasing effectiveness of toothpastes, such as calcium glycerophosphate, have been tested but, again, any additional benefit was small. During the past twenty years, toothpastes which reduce 'tartar' (dental calculus), reduce gum inflammation (gingivitis), reduce dentine sensitivity, and whiten teeth have been developed. It has been important to show that these additions did not reduce the caries-preventing role of fluoride – it would appear that caries prevention has not been compromised. It should be noted that toothpastes containing stannous fluoride are available, marketed mainly for their protection against plaque, gingivitis and tooth sensitivity.

A Cochrane Collaboration review of the effectiveness of fluoride toothpastes in preventing dental caries (21) gave the prevented fraction, pooled from over 42,000 children in the 70 studies included, as 24% (95% confidence interval of 21 to 28%). The conundrum that this reduction is substantially lower than the decline in caries recorded in many countries will be discussed below. Almost all of the above trials involved permanent teeth: a recent systematic review (22) identified eight trials involving primary teeth and reported caries reductions similar to those recorded for permanent teeth. The ability of fluoride-containing toothpastes to prevent caries in the roots (dentine and cementum) of teeth has been tested in a number of trials and with several compounds, with encouraging results (23).

Concentration of fluoride in toothpastes

There is a dose response for fluoride in many vehicles, including toothpaste. The prevented fractions for various fluoride concentrations in toothpastes, taken from a recent Cochrane review (24) are given in Table 2.

The higher the concentration of fluoride, the greater the effect. There has been considerable discussion about the effectiveness of toothpastes containing less than 1000 ppm. For toothpastes containing about 500 ppm, it can be seen that the 95% confidence interval includes zero, indicating that the prevented fraction (15.4%) is not statistically significantly different from zero at the 5% level of significance. One argument is that such toothpastes cannot be recommended as effective. The counter argument is that

Table 2 Clinical effectiveness of fluoride-containing toothpastes. Prevented fraction (PF), compared with a fluoride-free placebo toothpaste, for toothpastes containing various concentrations of fluoride, obtained by network meta-analysis. Data for caries increment, measured by the D(M)FS index (24)

Fluoride concentration (ppm)	Prevented fraction (%; 5% confidence interval)
250	9.1 (-3.6, 22.0)
440-550	15.4 (-1.9, 32.5)
1000-1250	23.0 (19.3, 26.6)
1450-1500	29.3 (21.2, 37.5)
1700-2200	33.7 (16.5, 50.8)
2400-2800	35.5 (27.2, 43.6)

there is such a clear dose-response for six concentrations from zero to 2800 ppm, that it is disingenuous to conclude that concentrations around 500 ppm are ineffective. Added to that, while there were 54 trials comparing 1000 ppm with 0 ppm (placebo), there were only two comparisons of 500 ppm toothpastes with a 0 ppm placebo: it is very unlikely that any more trials will be undertaken with a 0 ppm placebo, since it would now be ethically unacceptable to withhold benefits of fluoride-containing toothpastes from subjects for at least two years. Thus, the issue is difficult to resolve and opinion on the role of 500 ppm toothpastes is fairly evenly split across the world.

Frequency of brushing, amount of toothpaste, and method of rinsing after brushing

This information is frequently obtained from subjects during clinical trials by questionnaire. Frequency of brushing information has been available for most trials and meta-analyses have indicated that brushing twice a day is more effective than brushing once a day – the prevented fraction is increased by 14% (95% CI 6 to 22%) moving from once to twice a day (21). However, it should be noted that the trials were not set up to investigate this aspect of toothbrushing. There is little clinical data on the variable 'amount of toothpaste', although opinion is that amount is much less important than fluoride concentration and brushing frequency. There is some evidence that the degree of rinsing the mouth after brushing affects the effectiveness of the fluoride toothpaste (25, 26). Rinsing thoroughly (with water from a cup) reduces effectiveness compared with less thorough rinsing with water from the hand.

Recommendations regarding the use of fluoride-containing toothpastes

The first recommendation is that everyone should use a fluoride-containing toothpaste. A comment was made in 2001 (27) that if all dentate people in the UK brushed their teeth twice a day, the amount of toothpaste used would be twice the current amount. Hypersensitivity to fluoride is exceedingly rare: cases reported were generally due to hypersensitivity to other components. Sensitivity to flavouring or preserving agents in toothpastes is reasonably common. The debate about what concentration is appropriate was mentioned above.

Toxicity issues must be considered when making recommendations and these revolve around two issues. First, what concentration is appropriate for use by the general public? And, second, what concentration is appropriate for young children whose teeth are still forming and who could be at risk of developing dental fluorosis? The first question has been answered, certainly in Europe, as there is a ceiling of 1500 ppm for fluoride-containing toothpastes sold 'over the counter'. Toothpastes containing more than this concentration have to be prescribed. This is so in many other countries too. The second question is more difficult. There is no EU recommendation. The UK Department of Health said in 2009, for children up to 3 years: "Use only a smear of toothpaste containing no less than 1000 ppm fluoride" (28). The European Academy of Paediatric Dentistry is more flexible, saying in 2009: "A children's toothpaste with a lower concentration of fluoride may be indicated although the evidence for a caries-preventive effect of formulas with less than 500 ppmF is insufficient" (29). A recent report of a trial in Brazil made the interesting comment that "The anticaries effect of the low-F dentrifice [500 ppm] was similar to the conventional F dentrifice [1100 ppm] when used by caries-inactive children. However, in children with active caries lesions the low-F dentrifice was less effective than the 1100-μgF/g dentrifice in controlling the progression of lesions" (30). This rather reinforces evidence from Australia that there is little penalty of a rise in caries experience, or reduced caries prevention, in recommending use of 500 ppm toothpastes rather than 1000 ppm toothpastes for use by young children (31). Thus, some flexibility in advice may be needed. Toothpastes containing around 500 ppm are on sale and recommended in many areas of the world.

Toothbrushing for children under 7 years should be supervised by an adult, to ensure adequate brushing and prevent unnecessary swallowing of toothpaste. Children up to 3 years of age should have only a smear of toothpaste placed on their toothbrush; children aged 3 to 6 years should use no more than a small pea-sized amount of paste. Thorough rinsing after brushing should be avoided; spitting out excess toothpaste is usually sufficient. Brushing is recommended last thing at night and at least one other occasion during the day.

Effectiveness and recommendations for use of fluoride-containing mouthrinses

There has been less research into fluoride mouthrinses than with fluoride toothpastes. The reasons are likely to be, first, that mouthrinses are easier to formulate and, second, the habit of mouthrinsing at home is much less established than toothbrushing. The market is smaller, although its popularity has increased over the past 20 years. Some issues will now be considered.

Type of fluoride compound, concentration of fluoride and frequency of rinsing

Results of trials indicate that rinses containing acidulated phosphate fluoride, stannous fluoride or ammonium fluoride were not as effective as sodium fluoride rinses at neutral pH. It is also easier to formulate a pleasant-tasting rinse with sodium fluoride. The one trial comparing an amine fluoride rinse with a sodium fluoride rinse showed them to be of equal effectiveness; however, the combination of amine fluoride and stannous fluoride seems to be superior (18). The vast majority of rinses sold now contain sodium fluoride at neutral pH, although the amine fluoride / stannous fluoride formulation has a big market share in Europe.

Concentrations of fluoride in mouthrinses tested have varied between 45 ppm and 3000 ppm. However, unlike toothpastes which are used daily, mouthrinsing may be done daily, once, twice or three times a week, every two weeks, or 3 or 4 times a year. Mouthrinses with higher concentrations of fluoride are usually for infrequent use. Frequency as low as 3 or 4 times a year would not now be recommended. In the textbook mentioned above (8), cross-tabulations were made to examine prevented fractions in relation to fluoride concentration and rinsing frequency. The authors concluded: "... there was only a moderate trend towards increasing effectiveness with increasing fluoride concentration especially within the most popular range 200 to 1000 ppm F." In addition: "In conclusion, rinsing frequency would appear to be important and the concentration of fluoride slightly less important than frequency. Rinsing once a week or more is likely to be more effective than less frequent rinsing." The most common quantity of rinse dispensed is 10ml; with a rinsing duration of one to two minutes. The rinse is then expectorated.

Recommendations regarding the use of fluoride-containing mouthrinses

Almost all the fluoride mouthrinses now on sale contain around 225 ppm F – this is usually on the label as '0.05% sodium fluoride' -- although there are other compounds in mouthrinses (*vide supra*). These are recommended for rinsing once a day, for 1 to 2 minutes, before spitting out. Advice is to avoid eating or drinking for 15 minutes after rinsing, and to rinse at a different time to toothbrushing. This is to increase the frequency of exposure of dental plaque to fluoride: a trial testing the use of a fluoride mouthrinse straight after brushing with a fluoride toothpaste recorded no added benefit over brushing alone (32).

The toxicity of fluoride mouthrinses should be considered, since 10ml of a 225 ppm mouthrinse contains 2.25 mg F Young children swallowing this quantity daily would create an unacceptable risk to forming teeth. Because of this potential risk, the use of fluoride mouthrinses is not recommended (in the UK, at least) by children younger than 8 years. Alcohol has traditionally been included as a solvent for flavouring and other ingredients: during the last 15 years, health professionals have urged that alcohol be removed, with widespread success. While everyone (with teeth) is advised to brush their teeth with a fluoride-containing toothpaste (toothbrushing has benefits other than caries prevention), such a blanket recommendation is not made for fluoride mouthrinses. They can be prescribed or recommended by dental professionals, or they may be purchased because the user likes the idea of using a rinse.

The role of fluoride toothpastes and mouthrinses in the prevention of dental caries

There is no magic bullet for preventing or curing dental caries. An earlier article in this issue of the Journal (1) discussed approaches to preventing caries development and noted that those of greatest practical importance are diet, fluoride and fissure sealing. This article (1) also divided ways of delivering fluoride into Automatic, Home care, and Professional care, with toothpastes and mouthrinses grouped under Home care (Table 4 in reference 1). The above approaches are not alternatives but they should be selected to provide the most appropriate -- including considerations of cost and availability -- combinations at a national level, community level and the level of the individual. It is beyond the scope of this article to consider these preventive strategies in detail, but some comments will be made which relate to the current use of fluoride-containing toothpastes and mouthrinses.

First, diet: use of an effective fluoride toothpaste and mouthrinse is not an excuse for ignoring control of sugar intake – both diet control and fluoride are important. Second, there is substantial evidence that fluoride toothpastes and mouthrinses are effective in areas where the water supply contains an optimum concentration of fluoride (about 0.8 to 1.0 ppm in Europe) (21, 33) – there is added benefit. This applies to other ways of delivering fluoride, too. For example, there is added benefit from using a fluoride-containing toothpaste and a fluoride-containing mouthrinse (34), although the timing of their use should be considered (*vide supra*). Professional application of topical fluorides in the form of varnish, solution or gel will bring benefit in addition to the benefit from regular use of a fluoride toothpaste (34). It has already been stated that everyone should brush their teeth with a fluoride-containing toothpaste, but the decision whether to also use a fluoride mouthrinse or attend a dental professional to receive a topical application of fluoride will depend, primarily, on perception of risk of dental caries development and cost of the mouthrinse or topical application of fluoride. That is a personal decision made, it is likely, with the aid of professional advice.

One of the conundrums, yet unravelled, is why the decline in caries severity recorded in many countries is so much greater than the relatively modest 24% given as the prevented fraction for fluoride toothpastes in general by a Cochrane review (21). Do the results of 3 year trials underestimate the effect of lifelong use of fluoride toothpastes? Has the use of other fluoride therapies increased the benefit? Are we now better at controlling our sugar intake? These are questions which we leave to be answered by others. It should be recognised that caries prevalence has not declined in every country; in some, it has increased (6, 35). We need a better understanding of the factors operating in these countries. To give just one example, it is known that in some countries, so called 'fluoride toothpastes' made locally do not contain fluoride or contain fluoride in an inactive form.

While water fluoridation or the addition of fluoride to salt or milk (see previous articles in this issue of the Journal) are equitable community measures – in that they reduce social inequalities – it has to be accepted that home care products, i.e. toothpastes and mouthrinses, increase social inequalities, since the more affluent can afford to buy them while the less affluent may struggle to do so. It is likely that the widespread use of fluoride toothpastes has increased inequalities in oral health both within countries and between countries. That is definitely not to infer that they are 'bad' but, rather, that specific programmes are needed to ensure that well-formulated, effective toothpastes are available for all. This approach has been strongly supported by WHO (35, 36) in their drive for 'affordable fluoride toothpastes'. There are several examples of successful demonstration programmes of supervised toothbrushing (with fluoride toothpaste) and distribution of toothpaste and brushes to those in greatest need (36-38). On a national scale, to give just one example, all Thai schoolchildren brush their teeth with a fluoride-containing toothpaste every day in school. Although there are inherent advantages for total oral care of toothbrushing over mouthrinsing, mouthrinse programmes are cheaper than brushing programmes and thus favoured in some community programmes (39, 40). There is no doubt that the development, marketing and use of fluoride-containing toothpastes have been a great success story in improving the oral health of thousands of millions of people throughout the world. This would not have occurred without the close collaboration between industry, dental researchers and the dental profession in general – each should be proud of their contribution. The task now is to ensure universal benefit.

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References

1. Rugg-Gunn AJ. Dental caries: strategies to control this preventable disease. Acta Med Acad. 2013;42:117-30.
2. Palmer JD. Dental health of children – an improving picture? Br Dent J. 1980;149:48-50.
3. Anderson RJ. The changes in dental health of 12-year-old schoolchildren in two Somerset schools; a review after an interval of 15 years. Br Dent J. 1981;150:218-21.
4. Glass RL. The first international conference on the declining prevalence of dental caries. J Dent Res. 1982;61:1301-83.

5. WHO/FDI. Report of a working group convened jointly by the FDI and the WHO: changing patterns of oral health and implications for oral health manpower, part1. Int Dent J. 1985;35:235-51.
6. Petersen PE. World Health Organization global policy for improvement of oral health – World Health Assembly 2007. Int Dent J. 2008;58:115-21.
7. Green BN, Johnson CD, Adams A. Writing narrative literature reviews for peer-reviewed journals: secrets of the trade. J Sports Chiropr Rehabil. 2001;15:5-19.
8. Murray JJ, Rugg-Gunn AJ, Jenkins GN. Fluorides in caries prevention. 3rd ed. Oxford: Butterworth-Heinemann; 1991. p. 127-78.
9. Bibby BG. Test of the effect of fluoride-containing dentrifices on dental caries. J Dent Res. 1945;24:297-303.
10. Ericsson Y. Fluorides in dentrifices. Investigations using radioactive fluorine. Acta Odontol Scand. 1961;19:41-77.
11. Torell P, Ericsson Y. Two year clinical tests with different methods of local caries-preventive fluoride application in Swedish schoolchildren. Acta Odontol Scand. 1965;23:287-322.
12. Koch G. Effect of sodium fluoride in dentrifice and mouthwash on the incidence of dental caries in school children. Odontol Revy. 1967;18:48-66.
13. Muhler JC, Radike AW, Nebergall WH, Day HG. Effect of a stannous fluoride-containing dentrifice on caries reduction in children. II. Caries experience after one year. J Am Dent Assoc. 1955;50:163-6.
14. Finn SB, Jamison HC. A comparative study of three dentrifices. J Dent Child. 1963;30:17-25.
15. Mühlemann HR, König KG, Marthaler TM, Schait A, Schmid H. Organische fluoride. Schweiz Mschr Zahnheilk. 1960;70:1037-47.
16. König KG, Mühlemann HR. Caries inhibiting effect of amine fluoride containing dentrifice tested in an animal experiment and a clinical study. In: Mühlemann HR, König KG, editors. The present status of caries prevention by fluoride-containing dentrifices. Berne: Huber; 2002. p. 126-30.
17. Marthaler TM. Caries inhibition after seven years of an amine fluoride dentrifice. Br Dent J. 1968;124:510-5.
18. Madléna M. Experiences with amine fluoride containing products in the management of dental hard tissue lesions focusing on Hungarian studies. A review. Acta Med Acad. 2013;42:189-97.
19. Bánóczy J, Szőke J, Kertész P, Tóth Zs, Zimmermann P, Gintner Z. Effect of amine fluoride/stannous fluoride-containing toothpaste and mouthrinsings on dental plaque, gingivitis, plaque and enamel F accumulation. Caries Res. 1989;23:284-8.
20. Bibby BG, Zander HA, McKelleget M, Labunsky B. Preliminary reports on the effect on dental caries of the use of sodium fluoride in a prophylactic cleaning mixture and in a mouthwash. J Dent Res. 1946;25:207-11.
21. Marinho VCC, Higgins JPT, Sheiham A. Fluoride toothpastes for preventing dental caries in children and adolescents (review). Cochrane database of systematic reviews. 2003;1:CD002278.
22. Santos APP, Nadanovsky P, Oliveira BH. A systematic review and meta-analysis of the effects of fluoride toothpastes on the prevention of dental caries in the primary dentition of preschool children. Community Dent Oral Epidemiol. 2013;41:1-12.
23. Bánóczy J, Nemes J. Effect of amine fluoride (AmF)/stannous fluoride (SnF2) toothpaste and mouthwashes on dental plaque accumulation, gingivitis and root-surface caries. Proc Finn Dent Soc. 1991;87:555-9.
24. Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VCC, Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. Cochrane database of systematic reviews. 2010;1:CD007868.
25. Chesters RK, Huntington E, Burchell CK, Stephen KW. Effect of oral care habits on caries in adolescents. Caries Res. 1992;26:299-304.
26. Twetman S. Caries prevention with fluoride toothpaste in children: an update. Eur Arch Paed Dent. 2009;10:162-7.
27. Rugg-Gunn AJ. Preventing the preventable – the enigma of dental caries. Br Dent J. 2001;191:478-88.
28. Department of Health. Delivering better oral health; an evidence-based toolkit for prevention. 2nd ed. London: Department of Health; 2009. p. 11.
29. Toumba J. Guidelines on the use of fluoride in children: an EAPD policy document. Eur Arch Paed Dent. 2009;10:129-35.
30. Lima TJ, Ribeiro CCC, Tenuta LMA, Cury JA. Low-fluoride dentrifice and caries lesion control in children with different caries experience: a randomized clinical trial. Caries Res. 2008;42:46-50.
31. Do LG, Spencer AJ. Risk-benefit balance in the use of fluoride among young children. J Dent Res. 2007;86:723-8.
32. Blinkhorn AS, Holloway PJ, Davies TGH. Combined effects of a fluoride dentrifice and mouthrinse on the incidence of dental caries. Community Dent Oral Epidemiol. 1983;11:7-11.
33. McDonagh M, Whiting P, Bradley M, Cooper J, Sutton A, Chestnutt I, et al. A systematic review of public water fluoridation. York: NHSCRD, University of York; 2000.

34. Marinho VCC, Higgins JPT, Sheiham A, Logan S. Combinations of topical fluoride (toothpastes, mouthrinses, gels, varnishes) versus single topical fluoride for preventing dental caries in children and adolescents. Cochrane database for systematic reviews. 2004;1:CD002781.
35. Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century; the WHO approach. Community Dent Oral Epidemiol. 2004;32:319-21.
36. Jones S, Burt BA, Petersen PE, Lennon MA. The effective use of fluorides in public health. Bull World Health Org. 2005;83:670-6.
37. Davies GM, Worthington HV, Ellwood RP, Bentley EM, Blinkhorn AS, Taylor GO, et al. A randomised controlled trial of the effectiveness of providing free fluoride toothpaste from the age of 12 months on reducing caries in 5-6-year old children. Community Dent Oral Epidemiol. 2002;19:131-6.
38. Curnow MMT, Pine CM, Burnside G, Nicholson JA, Chesters RK, Huntington E. A randomised controlled trial of the efficacy of supervised toothbrushing in high-caries-risk children. Caries Res. 2002;36:294-300.
39. Twetman S, Petersson LG, Axelsson S, Dahlgren H, Holm A-K, Källestål C, et al. Caries-preventive effect of sodium fluoride mouthrinses: a systematic review of controlled clinical trials. Acta Odontol Scand. 2004;62:223-30.
40. Chen CJ-A, Ling HS, Esa R, Chia JC, Eddy A, Yaw SL. A school-based fluoride mouth rinsing programme in Sarawak: a 3-year field study. Community Dent Oral Epidemiol. 2010;38:310-4.

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The cariostatic mechanisms of fluoride

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This article discusses the possible cariostatic mechanisms of the action of fluoride. In the past, fluoride inhibition of caries was ascribed to reduced solubility of enamel due to incorporation of fluoride (F^-) into the enamel minerals. The present evidence from clinical and laboratory studies suggests that the caries-preventive mode of action of fluoride is mainly topical. There is convincing evidence that fluoride has a major effect on demineralisation and remineralisation of dental hard tissue. The source of this fluoride could either be fluorapatite (formed due to the incorporation of fluoride into enamel) or calcium fluoride (CaF_2)-like precipitates, which are formed on the enamel and in the plaque after application of topical fluoride. Calcium fluoride deposits are protected from rapid dissolution by a phosphate –protein coating of salivary origin. At lower pH, the coating is lost and an increased dissolution rate of calcium fluoride occurs. The CaF_2, therefore, act as an efficient source of free fluoride ions during the cariogenic challenge. The current evidence indicates that fluoride has a direct and indirect effect on bacterial cells, although the *in vivo* implications of this are still not clear. **Conclusion**. A better understanding of the mechanisms of the action of fluoride is very important for caries prevention and control. The effectiveness of fluoride as a cariostatic agent depends on the availability of free fluoride in plaque during cariogenic challenge, i.e. during acid production. Thus, a constant supply of low levels of fluoride in biofilm/saliva/dental interference is considered the most beneficial in preventing dental caries.

Key words: Dental caries, Fluorides, Tooth remineralization.

Introduction

The use of fluoride in dentistry is one of the most successful preventive health measures in the history of dental care. However, the mechanism of fluoride action is still not clearly understood. The cariostatic effect of fluoride was first discovered in relation to the natural fluoride content of drinking water. Later, supplementation of public water supplies with controlled levels of fluoride was the first approach, involving the use of fluoride for caries control. In the middle of the previous century, it was generally believed that fluoride had to be incorporated into dental enamel during development. This would lead to the formation of enamel with reduced solubility. This is usually referred to as the systemic cariostatic effect of fluoride (1). Many clinical trials were designed to prove the systemic mode of action (2-4). Additionally, laboratory analyses revealed that fluoride concentration in surface enamel was higher in teeth that developed under the influence of water fluoridation (5). A hypothesis evolved which suggested that fluoride helped to make the enamel crystal “more perfect“, and therefore less acid soluble. Le Geros et al. (6) performed a physicochemical investigation of enamel from deciduous teeth. They found that enamel from children who had been subjected to prenatal fluoridation exhibited more homogeneous and less extensive patterns of acid-etching, denser crystal populations in intraprismatic regions, larger prism dimensions, greater total mineral density, a higher degree of crystallinity, smaller a-axis dimensions, more fluoride and less carbonate contents. However, in vitro studies revealed that the reduction in enamel solubility by pre-eruptive incorporation of fluoride is minor and therefore it is unlikely that the fluoride incorporated into enamel plays an important role in the observed caries reduction (7, 8). Also, in some studies no significant correlation could be established between dental caries experience and enamel fluoride concentration (9, 10).

The topical effect of fluoride was demonstrated by Bibby et al. (11), who compared the caries-preventing efficacy of fluoride lozenges, intended to be sucked, with coated fluoride pills intended to be swallowed in a group of 5 to 14-year-old children. In the group using lozenges fewer carious lesions developed compared to the group using pills. They concluded that the caries reduction was the result of fluoride acting on the external surface of the teeth, because the lozenges were in contact with teeth much more than pills which were swallowed. This study provided clear evidence that the mechanism of the action of fluoride is mostly post-eruptive. Later, topical fluoride agents were introduced to provide fluoride to individuals in non-fluoridated areas. In a large number of studies, topical fluorides have been shown to be effective in caries prevention (12). In the 1980s, the concept was established that fluoride controls caries lesion development, primarily through its topical effect on de- and remineralisation processes taking place at the interface between the tooth surface and the oral fluids (13, 14). This concept was established after very elegant in situ studies described by Øgaard et al. (15). They placed human and shark enamel (composed almost of pure fluorapatite) in a removable appliance and covered them with orthodontic bands to allow plaque accumulation. Microradiographic analyses revealed that carious lesions were formed in both substrates. However, fluoride supplementation, in the form of mouth rinses, inhibited lesion development. This observation has indicated that structurally bound fluoride is not very effective in inhibiting demineralisation, while fluoride in solution (NaF solution) leads to a high degree of protection. Furthermore, a randomized, double blind, longitudinal study, testing the caries - preventing efficacy of prenatal fluoride supplementation in children up to age 5, failed to support the hypothesis that prenatal fluoride has a strong caries preventive effect (16). When fluoride was first introduced in caries prevention, water fluoridation was followed by a decline in caries, while interruptions in fluoridation were followed by increasing caries levels. However, the latest decades have shown a significant caries decrease despite the fact that there was poor water fluoridation (17, 18). The authors proposed that one of the reasons might be the availability of other fluoride-containing products, e.g. fluoride dentifrices.

The results of more recent epidemiological and laboratory studies can be summarized by stating that post-eruptive (topical) application of fluoride plays the dominant role in caries prevention (1). However, this concept does not invalidate the “systemic” methods of fluoridation. The recent studies have shown a beneficial pre-eruptive effect of water fluoride on caries control. The use of fluoridated water until eruption only has a greater effect than the use only between eruption and age 15 in all categories of surfaces on the first molar, especially in pit and fissure surfaces (19, 20).

The present article discusses the current concept of the mechanisms of the fluoride cariostatic effect, an understanding of which is necessary for promotion of caries control.

The composition of enamel and dentine

The dental hard tissue consists of inorganic and organic materials in different quantities. Highly calcified enamel has approximately 85% mineral by volume, organic material 3% and 12% water by volume (1). Histologically, enamel is composed of so called prisms or rods, each being composed of clusters of small crystallites. The spaces between the prisms and the crystallites are filled with water and organic material (protein and lipids), and form the diffusion pathways for acids, mineral components, and fluoride ions (21). The solid phase of enamel consists mainly of crystallized calcium phosphate, which persists in different forms (mainly as hydroxyapatite and some less stable forms such as dicalcium phosphate dihydrate (DCPD), brushite or octacalcium phosphate (OCP). The mineral component of human dental enamel is basically a calcium-deficit carbonate hydroxyapatite. Carbonated calcium hydroxyapatite is more soluble than calcium hydroxyapatite, particularly in acidic media (14, 22, 23). The pure hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] allows the incorporation of many ions that fit into the crystallite structure and affect its solubility. The substitution in the hydroxyapatite crystal occurs during development with carbonate, magnesium, fluoride, etc. Fluoride improves the quality of mineralized tooth tissues in general, by reducing the relative amounts of carbonated apatite. The reaction between hydroxyapatite and low concentrations of fluoride has been postulated to be an ionic exchange, in which fluoride replaces and assumes the positions of the hydroxyl ions in the crystal lattice structure. The replacement of hydroxyl groups with the smaller fluoride ions should result in a more stable apatitic structure. If the OH^- ion in the pure hydroxyapatite is completely replaced by a fluoride ion (F^-) the resulting mineral is fluorapatite [$Ca_{10}(PO_4)_6F_2$]. However, pure fluorapatite can practically never be found. Only 10% of the hydroxyl groups can be substituted by fluoride in the surface enamel (24).

The main mineral phase of permanent dentin is also hydroxyapatite. Dentine contains (by volume) 47% apatite, 33% organic components and 20% water. The crystallites have much smaller dimensions than those found in enamel, which makes dentine more susceptible to caries attack than enamel. Smaller crystallites dissolve faster when placed in an under-saturated solution. The organic matrix is mainly composed of collagen. It forms the backbone of dentine and serves as a template for the deposition of apatite crystallites within the collagen helix. Dental caries is a biochemical process characterized initially by the dissolution of the mineral, which in turn exposes the organic matrix to breakdown by bacterial-derived enzymes, as well as by the host derived enzymes (e.g. metalloproteinase) present in dentine and saliva (1, 25).

Caries development

Over 100 years ago, dental caries was described as localized, progressive destruction of the tooth initiated by acid dissolution of the outer tooth surface. In the presence of fermentable carbohydrates, organic acids are produced by plaque microorganisms, which colonize the tooth surface. The acids (i.e. lactic, pyruvic, acetic, propionic, butyric) can dissolve the calcium phosphate mineral of the enamel or dentine (demineralisation) (26). This concept evolved as the foundation for our current knowledge of caries aetiology. Dental caries is the net result of consecutive cycles of de- and remineralisation of dental tissues at the interface between the biofilm (plaque) and the tooth surface, with demineralisation being caused by production of acids through oral bacteria after sugar consumption. The acids diffuse through the plaque into the pores of the sound enamel surface, releasing hydrogen ions, which can dissolve the underlying enamel. The dissolved mineral ions, calcium and phosphate will then back-diffuse into the surface layer and induce the precipitation of the mineral phases in this region. At the same time, some of the dissolved mineral ions will diffuse out of the enamel surface into the oral environment. It is known that incipient or small carious lesions ("white spot lesions") in human enamel consist of a subsurface area of demineralisation with an overlying, apparently intact, surface zone. It is considered that the enamel surface layer is a result of reprecipitation of minerals (remineralisation) dissolved from the subsurface. The leaching of calcium and phosphate from enamel can cause collapse of the tooth structure and the formation of a cavity. Demineralisation and remineralisation can be considered a dynamic process, characterized by the flow of calcium and phosphate out of and back into the enamel (27).

The saliva plays an important role, including buffering (neutralizing) the acid and providing minerals that replace those dissolved from the tooth during demineralisation challenge. The enamel surface is in constant contact with saliva, which is considered to be saturated with certain calcium phosphate salts, thereby maintaining the integrity of the enamel surface. It was found that, within physiological pH limits, the salivary content of calcium and inorganic phosphate was sufficient to supersaturate the saliva with respect to hydroxyapatite. The protective factors, which include salivary calcium, phosphate and proteins, salivary flow, and fluoride in saliva, can balance, prevent or reverse dental caries (26).

When a biofilm covers the enamel surface, it reduces the access of saliva to the tooth. The relevant fluid phase in this case is the biofilm fluid, which, under resting conditions, is also supersaturated with respect to the enamel. This would favour remineralisation of previously demineralized enamel or promote the formation of supragingival calculus (1). However, when the oral fluids become unsaturated with respect to the apatites e.g., caused by a pH drop, a change in apatite composition may occur. In the pH range below about 5.5, the oral fluids are unsaturated with respect to hydroxyapatite, which therefore may dissolve. The low fluoride concentrations prevailing in oral fluids under physiological conditions will ensure a concurrent supersaturation with respect to fluorapatite, theoretically in the pH range of about 5.5 - 4.5, so that dissolution of hydroxyapatite competes with simultaneous fluorapatite or mixed fluorohydroxyapatite formation. Consequently, hydroxyapatite dissolves from the subsurface and fluorohydroxyapatite forms in the surface layers (28).

Inhibition of demineralisation

The highest fluoride concentrations in enamel are found in the surface. They are usually around 1,000-2,000 ppm in non-fluoridated areas and 3,000 in fluoridated areas. Subsurface enamel generally contains fluoride at levels of about 20-100 ppm, depending on fluoride ingestion during tooth development. These levels are far below those able to confer expressive reduction on the solubility of hydroxyapatite (26). Based on solubility data, the thermodynamic solubility product constant (Ksp) of fluorapatite is only slightly less than that of hydroxyapatite (28). Concentrations of fluoride found in shark enamel are many times higher than those typically found in human enamel, but even so they were unable to inhibit demineralisation completely (15). On the other hand, it has been observed that low concentrations (up to 1 ppm) of fluoride in a solution can reduce and even inhibit enamel demineralisation (24). It was shown that inhibition of demineralisation is a logarithmic function of the fluoride concentration in a solution (14). These results indicated that if fluoride is present in the solution surrounding the crystals (enamel fluid) it is adsorbed strongly to the surface of carbonated apatite crystals acting as a potent protection mechanism against acid dissolution of the crystal surface. When the entire crystal surface is covered by adsorbed fluoride (F_A), it will not dissolve upon a pH fall caused by bacterial-derived acids. Crommelin et al. (29) observed that fluorapatite-coated hydroxyapatite dissolved largely the same as fluorapatite, although the hydroxyapatite in a hydroxyapatite and fluorapatite mixture dissolved the same as hydroxyapatite. Therefore, significant protection could be obtained if all crystals along the acid ions diffusion pathway are coated with fluorapatite. On the other hand, when the coating of F_A is partial, the uncoated parts of the crystal will undergo dissolution (30). Ten Cate and Duijsters (31) showed that the amount of mineral loss during demineralisation is a function of both pH and fluoride concentration. When the fluoride concentration in the solution is elevated the fluorapatite is correspondingly increased and it appears sufficient to prevent a caries lesion from developing. While fluoride adsorbed to the crystal surface effectively protects the crystal from dissolution, fluoride present in the solution (enamel fluid) is equally important, since the higher the concentration of fluoride in enamel fluid (F_L), the higher the probability that it adsorbs and protects crystal (1). Thus, to interfere in the dynamics of dental caries formation, fluoride must be constantly present in the oral environment. Demineralisation of enamel is inhibited by concentrations of fluoride in the sub-ppm range (21). Frequent low-level applications of fluoride are more effective than high-dose applications a few times a year, because F_L and thus F_A are maintained high with frequent applications (30).

Enhancement of remineralisation

The acids produced by the plaque bacteria diffuse through the plaque into the enamel and dissolve minerals (calcium, phosphate and fluoride) wherever there is a susceptible site. If minerals diffuse out of the tooth and into the oral environment, then demineralisation occurs. If this process is reversed, the mineral is reabsorbed into the tooth and the damaged crystals are rebuilt, we then have remineralisation. The role of fluoride in the remineralisation process was found to be rather complex. Fluoride acts by inhibiting mineral loss at the crystal surface and by enhancing this rebuilding or remineralisation of calcium and phosphate in a form more resistant to subsequent acid attack (32). Over 30 years ago Brown et al. (33) predicted that low concentrations of fluoride would enhance remineralisation. Traces of fluoride in a solution during dissolution of hydroxyapatite will make the solution highly supersaturated with respect to fluorohydroxyapatite. This will speed up the process of remineralisation. Fluoride will adsorb to the surface of partially demineralized crystals and attract calcium ions. Koulourides (34) demonstrated that acid-softened enamel, rehardened by fluoridation, acquired significant secondary resistance to acid attack, developing so-called "acquired resistance". The acquired fluoride enhances both remineralisation and demineralisation resistance.

Role of fluorapatite and calcium fluoride

There are three principle forms of fluoride ion reactivity with apatite:

1) Iso-ionic exchange of F^- for OH^- in apatite:
   $Ca_{10}(PO_4)_6(OH)_2 + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2 + 2OH^-$
2) Crystal growth of fluorapatite from supersaturated solutions:
   $10\ Ca^{2+} + 6PO_4^{3-} + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2$
3) Apatite dissolution with CaF_2 formation:
   $Ca_{10}(PO_4)_6(OH)_2 + 20F^- \rightarrow 10\ CaF_2 + 6PO_4^{3-} + 2OH^-$

The first two reactions may occur during long-term exposure to low fluoride levels in the solution (such as between 0.01 and 10 ppm F) from either systemic or latent topical sources. These reactions result in fluoride incorporation that, in a traditional sense, would be defined as *"firmly" bound fluoride*, since it is part of the apatitic structure. This fluoride present in the solid phase is also known as fluorohydroxyapatite or "systemic" fluoride. With the increasing fluoride concentration, an additional chemical reaction with the formation of significant amounts of calcium fluoride (CaF_2 or "CaF_{2}-like" material) begins to dominate. Fluoride concentrations ranging from 100-10,000 ppm F are required to produce CaF_2 as a reaction product (often called F_{on}). These concentrations are present in topicals, such as professional gels and varnishes, or over the counter toothpastes and mouth rinses (35).

As early as in 1945, Gerould (36) reported that calcium fluoride was a major product on enamel when teeth were exposed to high concentrations of fluoride. It is visible by scanning with an electron microscope (SEM) as small globules on the surface of fluoridated teeth. The globular precipitates on the enamel are more homogeneous when the fluoride concentration of the applied solution is higher (37). The globular structure of the calcium fluoride is thought to be due to the incorporation of phosphate during its formation on the tooth surface (38), since pure calcium fluoride is cubical rather than spherical. For a long period the general view was that the formation of calcium fluoride on enamel is unfavourable, because calcium fluoride is soluble in saliva to the same extent as in water (39). The oral fluids are unsaturated with respect to calcium fluoride, thus this salt dissolves whenever it is exposed to saliva (40). However, several studies have shown that calcium fluoride is quite insoluble in saliva at neutral pH, and that it can persist on the tooth surface for weeks and months after topical application of fluoride (41-43).The resistance of calcium fluoride is presumably caused by adsorption of secondary phosphate (HPO_4^{2-}) to calcium sites in the surface of calcium fluoride crystals and by pellicle proteins at neutral pH. At lower pH, as during a caries attack, primary phosphate will be the dominant phosphate ion species ($H_2PO_4^-$), which is unable to inhibit the dissolution of calcium fluoride. Thus, fluoride ions released during cariogenic challenges are due to the reduced concentration of secondary phosphate ions at acid pH. The released fluoride is subsequently built into hydroxyapatite through dissolution/reprecipitation reactions. After a caries attack, the calcium fluoride globules are again stabilized by adsorption of proteins and secondary phosphate (44). Calcium fluoride thus constitutes a pH-controlled reservoir of fluoride on the enamel. Calcium fluoride is contaminated with phosphate, not only on the surface, but also inside the crystal. This phosphate-contaminated calcium fluoride is more soluble than pure calcium fluoride, and may thus release fluoride at a higher rate than pure calcium fluoride (38). CaF_2 formed at low pH contains less internal phosphate, and has been shown to be less soluble. This may be of clinical significance for fluoride applied topically a few times per year (41). The calcium fluoride formation, its resistance in the oral environment and release of fluoride ions at low pH, explain the long-term effect of topically applied fluoride. It is suggested that the potential for formation of calcium fluoride should probably be increased in topical fluoride agents (45). Increased time of exposure, increased concentration, lowered pH, saliva and calcium pre-treatment have proved to be effective means of increasing calcium fluoride deposition on enamel in vitro (46-49).

The antimicrobial action of fluoride

In spite of extensive literature on the antimicrobial effects of fluoride on oral microflora, today there is very little consensus that the anticaries effect of fluoride is related to inhibition of oral bacteria.

The current evidence indicates that fluoride has a multitude of direct and indirect effects on bacterial cells, some of which may have a significant influence on the acid –producing microorganisms in dental plaque (50). Fluoride exerts its effect on oral bacteria by direct inhibition of cellular enzymes (directly or in combination with metals) or enhancing the proton permeability of cell membranes in the form of hydrogen fluoride (HF) (51, 52).

In order to provoke any antimicrobial effect, fluoride has to enter the bacterial cell. Fluoride diffuses into cariogenic bacteria in the form of HF (a weak acid, pKa 3.15). At lower external pH, more HF is formed and more of it diffuses into the cell. Once inside the cell, the HF dissociates into H^+ and F^-, because of the higher internal pH of cells, such as oral streptococci, than external. This continued diffusion and dissociation leads to the accumulation of fluoride in the cell and the acidification (accumulation of H^+) of the cytoplasm. The result is a reduction in both the proton gradient and the enzyme activity. Current information indicates that fluoride ions within the cell interfere with the glycolitic enzyme (enolase) activity and proton-extruding adenosine triphosphatase (H^+/ATP-ase), which is involved in the generation of proton gradients through the efflux of protons from the cell, at the expense of ATP (53).Thus, fluoride effectively inhibits the carbohydrate metabolism of acidogenic oral bacteria, including the uptake of sugars. In spite of these known effects, there is no general agreement that the antimicrobial effects of F contribute to the anticaries effect of fluoride (53, 54).

Many investigators tend to dismiss the role of fluoride in the metabolic activity of bacteria, on the grounds that only large concentrations are effective, and that there are no differences in the Streptococcus mutans populations in persons residing in fluoridated and in non-fluoridated areas (55). In addition, the widespread use of toothpastes, which have been responsible for the decrease in caries prevalence over the last three decades, has not resulted in a reduction in the number of the mutans streptococci (56). Lynch et al. (57) concluded that low levels of plaque and salivary fluoride, resulting from the use of 1,500 ppm fluoride toothpastes, are insufficient to have a significant antimicrobial effect on plaque bacteria. It seems that this effect is dependent on factors such as fluoride concentration and associated antibacterial components, such as fluoride counter ions (amine, stannous), preservatives, surfactants or antimicrobials added specifically for that purpose (zinc salts, triclosan, essential oil extracts, etc.) (58). A single application of professionally applied topical fluoride at a high concentration, although transient, reduces the plaque's ability to produce acid, but has little clinical significance in controlling dental caries.

A recent review, however, concluded that fluoride concentrations, as found in dental plaque, have a biological action on critical virulence factors of *S. mutans* in vitro, such as acid production and glucan synthesis, but the in vivo implications are still not clear (51).

Conclusion

Understanding the mode of action of fluoride has an essential role in the further development of products and programmes for caries prevention. In the past, the cariostatic effect of fluoride was attributed to the incorporation of fluoride in the hydroxyapatite crystal lattice and the reduced solubility of the so-formed fluoridated hydroxyapatite. Recent findings have altered this view. The current evidence from clinical and laboratory studies suggests that the caries-preventive mode of action of fluoride is mainly topical. Specifically, fluoride continually present in the oral fluids affects the demineralisation and remineralisation processes. Fluoride present in the solution surrounding the crystals (enamel fluid) in a sub-ppm range is able to adsorb to the surface of the carbonated apatite crystals, inhibiting demineralisation. However, in the clinical situation, the optimum fluoride level to prevent caries development is not known. When fluoride ions are present during remineralisation, they become incorporated in the apatite structure, forming fluor-hydroxyapatite mixed crystals, which are more resistant to future acid challenges. The formation of intraoral reservoirs capable of supplying ions for a prolonged period is crucial for the success of topical treatments. Fluoride, which is retained on the teeth after brief exposure to topical fluoride agents or toothpastes, is retained as calcium fluoride. Calcium fluoride is most likely the provider of free ions during cariogenic challenges. Calcium fluoride globules are protected from rapid dissolution by a phosphate -protein coating of salivary origin, which will open at low pH, when, incidentally, the fluoride is most needed. The fluoride present inside the solid enamel is most likely of lesser importance than fluoride in solution. In fact, this fluoride is not effective until exposed, due to crystallite dissolution. Upon post-eruption acidic challenge, "firmly" bound fluoride would be released to the fluid phase, thus inhibiting demineralisation and enhancing remineralisation. Evidence from cohort studies also supports fluoride's systemic mechanism of caries inhibition, especially in the pit and fissure surfaces of permanent first molars. Some of the efficacy of fluoride is attributed to the effect on the plaque volume and metabolic aspects of the plaque bacteria, although the *in vivo* implications of this are still not clear.

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References

1. Buzalaf MA, Pessan JP, Honório HM, ten Cate JM. Mechanisms of action of fluoride for caries control. Monogr Oral Sci. 2011;22:97-114.
2. Backer Dirks O, Künzel W, Carlos JP. Caries preventive water fluoridation. Caries Res. 1978;12(Suppl 1):7-14.
3. Thylstrup A, Bille J, Bruun C. Caries prevalence in Danish children living in areas with low and optimal levels of natural water fluoride. Caries Res. 1982;16(5):413-20.
4. Newbrun E. Effectiveness of water fluoridation. J Public Health Dent. 1989;49(5 Spec No):279-89.
5. Chan JT, Qiu CC, Whitford GM, Weatherred JG, Clardy RK. The distribution of fluoride of prenatal origin in the rat: a pilot study. Arch Oral Biol. 1989;34(11):885-8.
6. LeGeros RZ, Glenn FB, Lee DD, Glenn WD. Some physico-chemical properties of deciduous enamel of children with and without prenatal fluoride supplementation (PNF). J Dent Res. 1985;64(3):465-9.
7. Larsen MJ. Dissolution of enamel. Scand J Dent Res. 1973;81(7):518-22.
8. Fejerskov O, Thylstrup A, Larsen MJ. Rational use of fluorides in caries prevention. A concept based on possible cariostatic mechanisms. Acta Odontol Scand. 1981;39(4):241-9.

9. Nasir HI, Retief DH, Jamison HC. Relationship between enamel fluoride concentration and dental caries in a selected population. Community Dent Oral Epidemiol. 1985;13(2):65-7.
10. Retief DH, Harris BE, Bradley EL. Relationship between enamel fluoride concentration and dental caries experience. Caries Res. 1987;21(1):68-78.
11. Bibby BG, Wilkins E, Witol E. A preliminary study of the effects of fluoride lozenges and pills on dental caries. Oral Surg Oral Med Oral Pathol. 1955;8(2):213-6.
12. Pessan JP, Toumba KJ, Buzalaf MA. Topical use of fluorides for caries control. Monogr Oral Sci. 2011;22:115-32.
13. Ten Cate JM. In vitro studies on the effects of fluoride on de- and remineralisation. J Dent Res. 1990;69 Spec No:614-9; discussion 634-6.
14. Featherstone JD, Glena R, Shariati M, Shields CP. Dependence of in vitro demineralisation of apatite and remineralisation of dental enamel on fluoride concentration. J Dent Res. 1990;69 Spec No:620-5; discussion 634-6.
15. Øgaard B, Rölla G, Dijkman T, Ruben J, Arends J. Effect of fluoride mouthrinsing on caries lesion development in shark enamel: an in situ caries model study. Scand J Dent Res. 1991;99(5):372-7.
16. Leverett DH, Adair SM, Vaughan BW, Proskin HM, Moss ME. Randomized clinical trial of the effect of prenatal fluoride supplements in preventing dental caries. Caries Res. 1997;31(3):174-9.
17. Künzel W, Fischer T. Rise and fall of caries prevalence in German towns with different F concentrations in drinking water. Caries Res. 1997;31(3):166-73.
18. König KG. Reasons for increasing the fluoride content of children’s toothpastes. Oralprophylaxe. 2001;23:27-31.
19. Groeneveld A, Van Eck AA, Backer Dirks O. Fluoride in caries prevention: is the effect pre- or post-eruptive? J Dent Res. 1990;69 Spec No:751-5; discussion 820-3.
20. Singh KA, Spencer AJ. Relative effects of pre- and post-eruption water fluoride on caries experience by surface type of permanent first molars. Community Dent Oral Epidemiol. 2004;32(6):435-46.
21. ten Cate JM, Featherstone JDB. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med. 1991;2(3):283-96.
22. LeGeros RZ, Tung MS. Chemical stability of carbonate- and fluoride-containing apatites. Caries Res. 1983;17(5):419-29.
23. Kautsky MB, Featherstone JD. Effect of salivary components on dissolution rates of carbonated apatites. Caries Res. 1993;27(5):373-7.
24. Margolis HC, Moreno EC. Physicochemical perspectives on the cariostatic mechanisms of systemic and topical fluorides. J Dent Res. 1990;69 Spec No:606-13; discussion 634-6.
25. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. J Dent Res. 2006;85(1):22-32.
26. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol. 1999;27(1):31-40.
27. Fejerskov O, Kidd EA, Nyvad B, Baelum V. Defining the disease: an introduction. In: Fejerskov O, Kidd E, editors. Dental caries. The disease and its clinical managment. 2nd ed. Oxford: Blackwell-Munksgaard; 2008. p. 3-6.
28. ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci. 1997;105(5 Pt 2):461-5.
29. Crommelin DJ, Higuchi WI, Fox JL, Spooner PJ, Katdare AV. Dissolution rate behavior of hydroxyapatite-fluorapatite mixtures. Caries Res. 1983;17(4):289-96.
30. Arends J, Christoffersen J. Nature and role of loosely bound fluoride in dental caries. J Dent Res.1990;69 Spec No:601-5; discussion 634-6.
31. ten Cate JM, Duijsters PP. Influence of fluoride in solution on tooth demineralisation. II. Microradiographic data. Caries Res. 1983;17(6):513-9.
32. ten Cate JM, Featherstone JDB. Physicochemical aspects of fluoride-enamel interactions. In: Fejerskov O, Ekstrand J, Burt BA, editors. Fluoride in dentistry. 2nd ed. Copenhagen: Munksgaard; 1996. p. 252-72.
33. Brown WE, Gregory TM, Chow LC. Effects of fluoride on enamel solubility and cariostasis. Caries Res. 1977;11(Suppl 1):118-41.
34. Koulourides T. Increasing tooth resistance to caries through remineralisation. Foods Nutr Dent Health. 1982;2:193-207.
35. White DJ, Nancollas GH. Physical and chemical considerations of the role of firmly and loosely bound fluoride in caries prevention. J Dent Res. 1990;69 Spec No:587-94; discussion 634-6.
36. Gerould CH. Electron microscope study of the mechanism of fluorine deposition in teeth. J Dent Res. 1945;24:223-33.
37. Rošin-Grget K, Linčir I, Tudja M. Effect of amine fluoride on enamel surface morphology. Coll Antropol. 2000;24(2):501-8.
38. Christoffersen J, Christoffersen MR, Kibalczyc W, Perdok WG. Kinetics of dissolution and growth of calcium fluoride and effects of phosphate. Acta Odontol Scand. 1988;46(6):325-36.

39. McCann HG. The solubility of fluorapatite and its relationship to that of calcium fluoride. Arch Oral Biol. 1968;13(8):987-1001.
40. Fejerskov O, Larsen MJ, Richards A, Baelum V. Dental tissue effects of fluoride. Adv Dent Res. 1994;8(1):15-31.
41. Øgaard B. CaF(2) formation: cariostatic properties and factors of enhancing the effect. Caries Res. 2001;35(Suppl 1):40-4.
42. Dijkman AG, De Boer P, Arends J. In vivo investigations on the fluoride content in and on human enamel after topical applications. Caries Res. 1983;17(5):392-402.
43. Caslavska V, Gron P, Kent RL, Joshipura K, DePaola PF. CaF2 in enamel biopsies 6 weeks and 18 months after fluoride treatment. Caries Res. 1991;25(1):21-6.
44. Rølla G, Saxegaard E. Critical evaluation of the composition and use of topical fluorides, with emphasis on the role of calcium fluoride in caries inhibition. J Dent Res. 1990;69 Spec No:780-5.
45. Øgaard B, Seppä L, Rølla G. Professional topical fluoride applications - clinical efficacy and mechanism of action. Adv Dent Res. 1994;8(8):190-201.
46. Saxegaard E, Rölla G. Fluoride acquisition on and in human enamel during topical application in vitro. Scand J Dent Res. 1988;96(6):523-35.
47. Rošin-Grget K, Linčir I, Andrijanić L. In vitro fluoride uptake by enamel from different amine fluoride concentrations. Caries Res. 2002;36:266-9.
48. Rošin-Grget K, Šutej I, Linčir I. The effect of saliva on the formation of KOH-soluble fluoride after topical application of amine fluoride solutions of varying fluoride concentration and pH. Caries Res. 2007;41(3):235-8.
49. Larsen MJ, Richards A. The influence of saliva on the formation of calcium fluoride-like material on human dental enamel. Caries Res. 2001;35(1):57-60.
50. Hamilton IR, Bowden GHW. Fluoride effects on oral bacteria. In: Fejerskov O, Ekstrand J, Burt BA, editors. Fluoride in dentistry. 2nd ed. Copenhagen: Munksgaard; 1996. p. 230-51.
51. Koo H. Strategies to enhance the biological effects of fluoride on dental biofilms. Adv Dent Res. 2008;20(1):17-21.
52. Marquis RE. Antimicrobial actions of fluoride for oral bacteria. Can J Microbiol. 1995;41(11):955-64.
53. Hamilton IR. Biochemical effects of fluoride on oral bacteria. J Dent Res. 1990;69 Spec No: 660-7; discussion 682-3.
54. ten Cate JM, van Loveren C. Fluoride mechanisms. Dent Clin North Am. 1999;43(4):713-42.
55. Bowen WH. Caries prevention--fluoride: reaction paper. Adv Dent Res. 1991;5:46-9.
56. Van Loveren C. The antimicrobial action of fluoride and its role in caries inhibition. J Dent Res. 1990;69 Spec No:676-81; discussion 682-3.
57. Lynch RJ, Navada R, Walia R. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. Int Dent J. 2004;54(5 Suppl 1):304-9.
58. Marsh PD. Contemporary perspective on plaque control. Br Dent J. 2012;212:601-6.

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Experiences with amine fluoride containing products in the management of dental hard tissue lesions focusing on Hungarian studies: A review

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Fluorides play a significant role in the promotion of oral health, fostering remineralization, inhibiting demineralization processes in the enamel, and having antibacterial activity. The effects of fluorides are mostly exerted by their topical effect. The beneficial effect of amine fluorides (AmF) on caries and dental plaque reduction has been known for a long time. The caries reducing and plaque-inhibiting effect of stannous fluoride (SnF_2) was also reported. However, the combination of amine fluoride/stannous fluoride has shown a much better inhibition of plaque accumulation then these products alone. There have been several clinical studies with AmF or AmF/ SnF_2 products, using toothpaste, gel, combination of toothpaste and gel/fluid, toothpaste and mouth rinse. The aim of this article is to review the clinical experiences with these products based on Hungarian studies. The first Hungarian studies with AmF containing gel were published by Szőke and Kozma (1989) and Dénes and Gábris (1991). Madléna et al. (2002) performed a study with an AmF-containing toothpaste and gel in high risk groups of adolescents. The first Hungarian study with AmF/SnF_2 products was published by Bánóczy et al. (1989). Based on the favourable results of these products used in combination for 12 weeks, other studies (Madléna et al. 2004, 2012) assessed the effects of toothpastes and mouth rinse containing AmF/SnF_2 on plaque accumulation, within a shorter period of time, in young adults and orthodontic patients. **Conclusion.** Regular use of different oral hygienic products containing an AmF and AmF/SnF_2 combination contributes to the prevention of plaque accumulation and consequently to the prevention of dental diseases.

Key words: Amine fluoride, Amine fluoride/Stannous fluoride, Plaque accumulation, Prevention, Oral health.

Introduction

In spite of the caries decline experienced in the industrialised countries in twelve year olds, in Hungary caries has remained a major problem in older age groups, concerning the dental hard tissues (1). This situation is strongly associated with bad oral hygiene, that is improper oral hygiene habits of individuals (2).

Orthodontic treatment mainly with fixed appliances represents a special situation because of the increased retention of plaque, which can cause an increased risk of caries and periodontal diseases (3). For the prevention of carious lesions it is anyhow necessary to perform proper mechanical plaque removal. However, most people have no adequate mechanical cleaning method for various reasons, so it is necessary to improve the removal of plaque with chemical agents (4). The combination of mechanical and chemical methods ensures greater effect as after the mechanical reduction of the amount of plaque only a disorganized thin layer will remain on the tooth surface, which may be further reduced easily with chemical agents (5).

Fluorides play a significant role in the promotion of oral health, fostering remineralization, inhibiting the demineralization processes in the enamel, and having antibacterial activity. These beneficial effects may be improved when fluoride is associated with amin -or stannous ions (6).

Amine fluorides in dentistry

Amine fluoride (AmF) is an organic type of fluoride associated with a reduction in dental plaque adhesiveness, due to the greater affinity of hydrophilic counter-ions to the enamel surface. It spreads over all surfaces in the oral cavity especially quickly (due its tenside character), shows longer clearance in the oral cavity and dental plaque, and has a pronounced activity on plaque. AmF is strongly glycolytic (for 3-6 hours) and develops a highly bacteriostatic and bactericide effect (5).

The use of amine fluorides in dentistry was first recommended by Mühlemann and co-workers in 1960 (7). Based on their favourable characteristics in *in vitro* and *in vivo* studies, amine fluoride products were suggested as alternatives or adjuncts for systemic fluoridation by Mühlemann in 1967, and Schmid in 1983 (8, 9). However according to the opinion of Ahrens from 1983, the results of these studies were influenced by different factors, where age, methods and frequency of application, fluoride-concentration and duration played a role and made comparability difficult (10).

As a result the favourable effects AmFs could be successfully applied in reducing the prevalence of dental diseases. For the general public, AmF is available in the form of dentifrices, gels, mouth rinses, and for healthcare professionals in the form of prophylaxis pastes.

Clinical studies with amine fluoride containing (Elmex®) products

Clinical studies with amine fluorides have been performed using toothpaste, gel, with combined use of toothpaste and gel/fluid, or with combined use of toothpaste and mouth rinse.

In the early period there were more clinical studies published on AmF toothpaste, where the first was published in 1968 by Marthaler (11). Most of them showed considerable reduction in caries prevalence [in DMFT (Decayed, Missing, Filled Teeth)/ DMFS (Decayed, Missing, Filled Surfaces) values between 7.1 and 35%] similar to those

Table 1 Clinical studies with amine fluoride toothpastes

Authors	Year	Duration (year)	Probands (n)	Age (year)	Caries reduction % DMFT-S	p
Marthaler	1968	7	142/118	6-8	23-35	<0.001
Patz and Naujoks	1970	3	?	15-18	7.1	ns
Marthaler	1974	6	50/59	7.5	33	<0.02
Cahen et al.	1982	3	668/708	6-8	21.6; 20.9	<0.001
Leous	1995	3	330/140	18-20	25.6	ns

Tabela 2 Clinical studies with amine fluoride gels

Authors	Year	Duration (ys)	Probands (n)	Age (vs)	Caries reduction% (DMFS)	p
Marthaler	1970	3	?	6-7	45	p<0.05
Shern et al.	1970	2	?	?	31	p<0.05
Franke	1977	7	?	7	45	p<0.05
Obersztyn, Kolwinski	1983	1.5	787/449	19-20	41	Not calc.
Szőke and Kozma	1989	3	134/122	6-7	53	p<0.001

studies performed showing amine fluoride gel caries reductions of 31-53%, or with combined use of AmF containing products (Tables 1, 2). Most of these studies evaluated caries prevalence, but some of them assessed oral hygiene, and also reported a significant improvement in the test groups.

Longitudinal studies

The first Hungarian study with AmF gel was performed by Szőke and Kozma in 1989 (12). In this study, the AmF gel was applied for 3 years in children who were 6 years old. Besides the significant improvement in oral hygiene in the test group compared to the controls, there was a significant difference in the caries increment between the two groups, in both DMFT and DMFS values.

Studies in high risk groups

A study performed in orthodontic patients was published by Dénes and Gábris in 1991 (13). This double blind, randomised study was performed in adolescents treated with fixed orthodontic appliances. During the examination period, the authors compared the effect of Elmex gel and fluid application on caries increment compared to the control group, without intensive fluoridation. Regarding their main results, the Elmex® products reduced the caries increment in orthodontic patients after an average 18 months (average treatment time), the reduction was much higher in both Elmex® groups than in the controls. The reduction in caries increment was the best and highest in the group using gel, with a reduction of 79 %.

Another longitudinal study was performed showing the beneficial effect of toothpaste and gel containing AmF in high risk groups of adolescents (14). The study consisted of two parts. During the baseline examinations the risk of oral diseases (assessing caries prevalence, oral hygiene, salivary characteristics, and socio-economic factors) was determined in groups of adolescents living in different localities of Hungary: in Budapest (the capital of Hungary, and in Debrecen, which is the second largest city in the country in the eastern part of Hungary). Then, on the basis of the baseline findings where high risk was shown in the examined population, during the follow up study, a school based preventive program was established scheduled for two years, with the different use of products containing amine fluoride (toothpaste and gel) in these high risk groups. The participants were stratified according to their school-classes, and divided into two test and one control groups in both cities. Test group „A" used tootpaste and gel containing AmF, Test group „B" used toothpaste and placebo gel containing AmF, and the control („C") group continued their usual oral care habits without AmF containing products.

The DMFT, DMFS and Visible Plaque Index (VPI) values (according to Ainamo and Bay, 1983) (15) were evaluated at baseline and after two years. The DMFT increment was significantly lower in both test groups compared to the control group. Concerning the DMFS values, the caries increment was significantly lower in the group with combined use of toothpaste and gel containing AmF than in the control group (Figures 1 and 2).

The combined use of amine fluoride containing toothpaste and gel provided a significant reduction in DMFS mean values (38% including white spot lesions) and visible plaque index values (18%) ($p<0.05$). The VPI values decreased in all three groups due to regular oral health instruction and motivation, but there was only a significant reduction in the groups where the participants used toothpaste and gel containing AmF together.

In the above-mentioned population the therapeutic value of products containing AmF was assessed statistically, by logistic regression analysis (16). The results showed that the chance of remineralization of buccal incipient lesions was higher in the group using gel and toothpaste together than in the other test group (using only toothpaste) and much higher than in the control group.

Summarizing the results: combined use of products containing amine fluoride (toothpaste and gel) provided more pronounced caries reduction, improvement of oral hygiene and remineralisation effect than use of the toothpaste alone, in adolescents with high risk and without a systemic fluoride background.

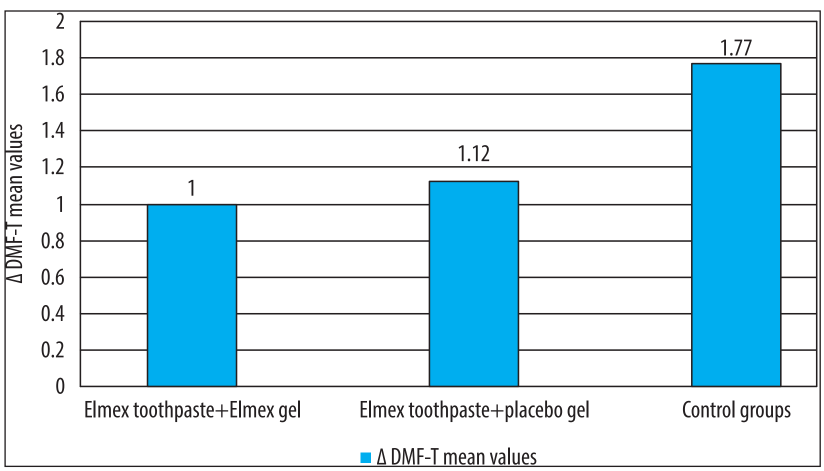

Figure 1 Two-year incremental DMF-T findings for subjects who completed the study (14) ($p<0.05$).

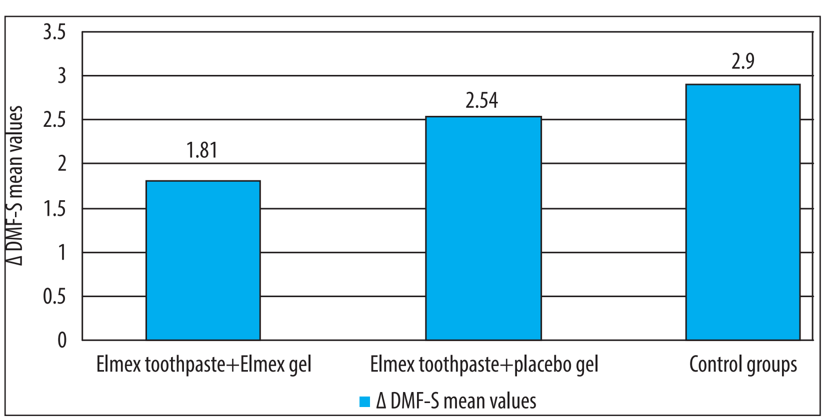

Figure 2 Two-year incremental DMF-S summary for subjects who completed the study (14) ($p<0.05$).

Amine fluorides/stannous fluorides in dentistry

The beneficial effect of amine fluorides on caries and dental plaque reduction has been known for a long time. The caries reducing and plaque inhibiting effect of stannous fluoride (SnF_2) was also reported many years ago. Stannous fluoride releases both F^- and Sn^{2+} ions in the oral cavity, which ensure effectiveness against caries and an antimicrobial characteristic (17). However, the combination of amine fluoride /stannous fluoride (AmF/SnF_2) showed much better inhibition of plaque accumulation then these products alone (18, 19). AmF possesses antibacterial activity, which could be enhanced when amine fluoride is associated with stannous fluoride (6). Oral hygienic products containing amine fluoride/stannous fluoride reduce acid production within the dental plaque (20) and retard supragingival plaque regrowth (21-23), therefore these products could be more effective in the oral cavity. The antibacterial effect of AmF/SnF_2 seems to be more prolonged in patients who are not at high risk, with a smaller amount of plaque (24).

The first summarized results of clinical trials investigated the plaque and gingivitis inhibiting effects of the AmF/SnF_2 combination and were published in 1991 (25). After the mouth rinse a toothpaste containing AmF/SnF_2 also became available and these products could be found on the market as Meridol® mouth rinse and Meridol® toothpaste.

Clinical studies with products containing amine fluoride/stannous fluoride (Meridol®)

Clinical studies with amine fluoride/stannous fluoride were performed using toothpaste, mouth rinse or with combined use of toothpaste and mouth rinse. Relatively few studies have reported the clinical results of the use of AmF/SnF_2 toothpaste and combined use of toothpaste and mouth rinse. Although these studies did not evaluate the caries reducing effect directly, the plaque reduction could be associated with all plaque induced dental diseases (caries and periodontal diseases). The examinations proved the effectiveness of this combination over various periods of time.

Medium term studies

The first study, lasting for 12 weeks, was published by Bánóczy and co-workers in 1989 (26), performed on 92 schoolchildren with a mean age of 12.4 years, showing that use of AmF/SnF_2 toothpaste resulted in significant reduction of plaque accumulation and gingival inflammation, but the combined use of toothpaste and mouth rinse proved to be more effective than the use of toothpaste alone. In this study participants were involved who were randomly distributed in four groups:

- Group 1.: used placebo toothpaste
- Group 2.: used amine fluoride/stannous fluoride toothpaste (Meridol®)
- Group 3.: used placebo toothpaste and amine fluoride/stannous fluoride rinse (Meridol®)
- Group 4.: used Meridol® toothpaste and Meridol® rinse

The examined parameters were: Plaque Index (PI, according to Silness and Löe, 1964) (27) and Sulcus Bleeding Index (SBI, according to Mühleman and Son, 1971) (28). Concerning the results of the Plaque Index values, the greatest decrease was seen in the fourth group (using AmF/SnF_2 containing toothpaste and mouth rinse together) which was statistically significantly higher than in the other groups. Similar results were found with the Sulcus Bleeding Index values (Figure 3). A significant increase in enamel fluoride content was observed after use of the test toothpaste alone or after a combined use of these products. The plaque fluoride con-

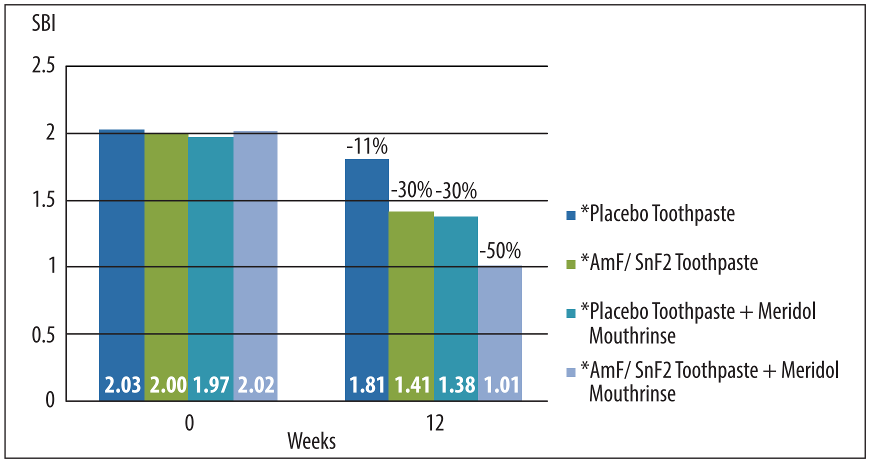

Figure 3 Decrease in the Sulcus Bleeding Index values after 12 weeks' use ($p<0.05$).

tent also showed a statistically significant increase in the fourth group.

Later, a five month, double blind study was performed to evaluate the effectiveness of toothpaste and mouth rinse containing AmF/SnF_2 on the accumulation of dental plaque and gingivitis, and the remineralizing effects of these products, in cases of exposed root surfaces and root surface caries, compared with the sodium fluoride (NaF) system (29). Forty-four adults were randomly divided into two groups: group 1 used toothpaste and mouth rinse containing AmF/SnF_2, group 2 used NaF toothpaste and mouth rinse twice a day. The participants brushed their teeth with standardized „Multi-effect" toothbrushes, mouth rinses were used after each tooth brushing, keeping 10 ml of the solution in the mouth for one minute. By the end of the examinations a significantly higher reduction of Plaque Index (according to Silness and Löe, 1964) (27) and Sulcus Bleeding Index (according to Mühlemann and Son, 1971) (28) was found in both groups. The Root Caries Index (RCI) decreased in both groups, and the reduction in mean RCI values was more pronounced and significantly higher using AmF/SnF_2 products (47.4%) than in the NaF group (10.0%).

On the basis of these two studies, it may be concluded that in both studies clinically and statistically significant improvements were found in relation to plaque and gingiva indices. The improvement by 63.7% in the Plaque Index in the longer study was much better than in the shorter one (33.3%), which showed the contribution of time of application. The results of the first study showed the beneficial effects of AmF/SnF_2 products on F content of plaque and enamel, which can help to prevent carious processes. Further, the results of the second study show the efficacy of products containing AmF/SnF_2 in adults with periodontal disease (exposed root surfaces) and regarding the changes of RCI mean values the superiority was shown of AmF/SnF_2 combination over combinations containing NaF.

Short term studies

Based on the previously experienced favourable results of combined use of products containing AmF/SnF_2 after 12 weeks use (26), another Hungarian study aimed to assess the effects of different use of toothpaste and mouth rinse containing AmF/SnF_2 within a shorter period of time (4 weeks) in young adults (30, 31, 32).

The study population consisted of 42 healthy probands, with a mean age of around 28 years. The criteria of the involvement in

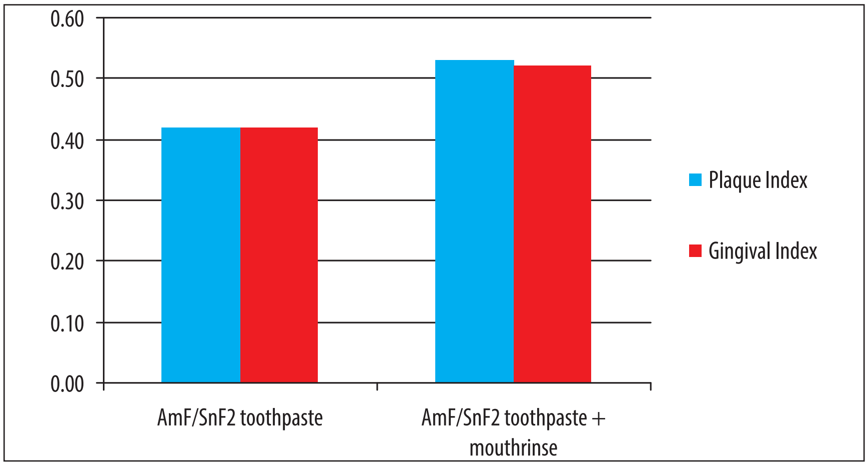

Figure 4 Reduction in Plaque Index and Gingival Index after four weeks' use (30) ($p<0.01$).

this study were: having no antibiotic or systemic therapy, taking no drugs that influence oral flora and gingival health, being without clinical signs of periodontitis [PPD (Periodontal Pocket Depth) <4 mm] and having at least 20 natural teeth. The participants were randomly divided into two groups:

Group 1: used toothpaste containing AmF/SnF_2 twice a day (morning and evening) for tooth brushing with a Meridol® toothbrush for 3 minutes and Group 2: used toothpaste containing AmF/SnF_2 for tooth brushing for 3 minutes with a Meridol® toothbrush and after tooth brushing rinsed with a mouth rinse containing AmF/SnF_2.

The probands exercised no oral hygiene for two days prior to baseline examination. On the days of baseline and final examinations they had no breakfast and exercised no oral hygiene. Plaque accumulation was assessed by their full mouth score on the Plaque Index according to Silness Löe (1964) (27). Gingival status was scored for the whole mouth, following the criteria of the Gingival Index according to Löe and Silness (1963) (33). After four weeks the patients were re-examined without previous oral hygiene and breakfast by the same examiner, and the results were recorded blindly.

Concerning the results in this short term (four weeks) clinical study on young adults, both the regular use of an AmF/SnF_2 toothpaste alone, as well as the combined use of the toothpaste and mouth rinse resulted in a significant reduction in dental plaque (and consequently the development of caries lesions as well) and Gingival Index (PI and GI) values. The reduction of PI and GI values was significantly greater in the combined AmF/SnF_2 group than in the group using toothpaste only (Figure 4).

It was mentioned earlier that orthodontic patients belong to high risk groups because of increased plaque retention and difficulties in performing proper oral hygiene. Few investigations appear to have been undertaken into the effects of AmF or SnF_2 (13, 34, 35). Øgaard et al. (36) examined the effect of combined use of AmF/SnF_2 toothpaste/mouth rinse compared with NaF, in relation to the maxillary anterior teeth, during orthodontic treatment with fixed appliance. They found better oral health in the tested groups using AmF/SnF_2 products daily (36).

Based on the beneficial effects of AmF/SnF_2 products in young adults, a similar study was repeated in high risk groups, namely orthodontic patients treated with a fixed appliance, where the aim was to investigate the influence of toothpaste and mouth rinse containing AmF/SnF_2 on plaque accumulation and gingival health, in a similar short term study (37). The examined persons were 40 (by the end of the study 39) young orthodontic patients with a mean age of around 20 years. The probands were divided into similar two groups, as in the previous study: Group 1: used toothpaste containing AmF/SnF_2 twice a day (morning and evening) for tooth brushing with a Meridol® toothbrush for 3 minutes; Group 2: used toothpaste containing AmF/SnF_2 for tooth brushing for 3 minutes with a Meridol® toothbrush and after tooth brushing rinsed with mouth rinse containing AmF/SnF_2. Besides the PI and GI, the parameters examined were extended to include Bleeding on Probing (BoP) and Periodontal Probing Depth (PPD).

Regarding the results, the use of products containing AmF/SnF_2 resulted in beneficial clinical effects on plaque accumulation (so consequently on the choice of the development of carious lesions) and gingival health, after placement of fixed orthodontic appliances. PI (as well as GI) decreased significantly in both examination groups. The reduction of PI (and GI) values was greater in the combined AMF/SnF_2 group than in the group using toothpaste only, but there were no statistically significant differences between the two groups in this high risk population during the short period of time. The experiences were similar in the values of BoP and PPD (significant differences could be found in the control and test groups, but were not noticed between the two groups). These beneficial effects may be pronounced after long term and combined use of AmF/SnF_2 products in this high risk population treated with fixed appliances.

Conclusion

Most of the Hungarian studies performed related to children and adolescents, and mainly high risk groups. The results, statistically evaluated, seem to be best in the groups with combined use of oral hygiene products containing different amine fluoride or amine fluoride/stannous fluoride (toothpaste and gel or toothpaste and mouth rinse).

Conflict of interest: The author declares that she has no conflict of interest.

References

1. Madléna M, Hermann P, Jáhn M, Fejérdy P. Caries prevalence and tooth loss in Hungarian adult population: results of a national survey. BMC Public Health. 2008;8:364.
2. Madléna M, Hermann P, Tollas O, Gerle J, Fejérdy P. Oral hygienic, nutritional habits and dental surgeon attendance of Hungarian adult population [In Hungarian]. Fogorv Sz. 2007;100(3):91-7.
3. Madléna M. The importance and possibilities of proper oral hygiene in orthodontic patients. In: Bourzgui F, editor. Orthodontic: basical aspects and clinical consideration. Rijeka: INTECH; 2012. p. 69-110.
4. Binney A, Addy M, Newcombe RG. The plaque removal effects of single rinsings and brushings. J Periodontol. 1993;64(3):181-5.
5. Brecx M. Strategies and agents in supragingival chemical plaque control. Periodontol 2000. 1997;15(1):100-8.
6. Van Loveren C. Antimicrobial activity of fluoride and its in vivo importance: identification of research questions. Caries Res. 2001;35(suppl 1):65-70.
7. Mühlemann HR, König KG, Marthaler TM, Schait A, Schmid H. Organic Fluoride [In German].Schweiz Mschr Zahnheilk. 1960;70(12):1037-47.
8. Mühlemann HR. The caries-preventing effect of the amine fluorides. 4. 10 years of experiences [In German]. Quintessenz. 1967;18(8):109-17.
9. Schmid H. Chemistry and surface effects of amine fluoride[In German]. Dtsch Zahnarztl Z. 1983;38 Suppl 1:S9-13.
10. Ahrens G. Efficiency of fluoride dosage forms: solutions and gels[In German]. Dtsch Zahnarztl Z. 1983;38 Suppl 1:S65-9.
11. Marthaler TM. Caries inhibition after seven years of supervised use of an amine fluoride dentifrice. Br Dent J. 1968;124(4):510-5.
12. Szöke J, Kozma M. Results of 3-year study of toothbrushing with a fluoride amine gel [In German]. Oralprophylaxe. 1989;11(4):137-43.
13. Dénes J, Gábris K. Results of a 3-year oral hygiene programme including amine fluoride products treated with fixed orthodontic appliances. Eur J Orthodont. 1991;13(2):129-33.

14. Madléna M, Nagy G, Gábris K, Márton S, Keszthelyi G, Bánóczy J. Effect of Amine Fluoride Toothpaste and Gel in High Risk Groups of Hungarian Adolescents: Results of a Longitudinal Study. Caries Res. 2002;36(2):142-6.
15. Ainamo J, Bay I. Problems and proposals for recording gingivitis. Int Dent J. 1975; 25(4) 229-35.
16. Márton S, Nagy G, Gábris K, Bánóczy J, Madléna M. Logistic regression analysis of oral health data in assessing the therapeutic value of amine fluoride containing products. Oral Health Dent Manag. 2008;7(4):26-9.
17. Lilienthal B. The effect of a stannous fluoride mouthwash on acid formation in the mouth and some observations on the mechanism of inhibition. Aus Dent J. 1956;1(4):221-7.
18. Mühlemann. HR, Saxer UP, Moermann W. Reduction of plaque and gingivitis by stannous fluoride stabilized with amine fluoride. Caries Res. 1981;15(2):186.
19. Renggli HH. Plaquehemmender Effekt kombinierter Fluoridspülungen. Schweiz Monatsschr Zahnheilk. 1983;93(8):581-4.
20. Damen JJ, Buijs MJ, ten Cate JM. Acidogenecity of buccal plaque after a single rinse with amine fluoride-stannous fluoride mouthrinse solutions. Caries Res. 2002;36(1):53-7.
21. Zimmermann A, Flores-de-Jacoby L, Pan P, Pan P. Gingivitis, plaque accumulation and plaque composition under long-term use of Meridol. J Clin Periodontol. 1993;20(5):346-51.
22. Bánóczy J, Gombik A, Szőke J, Nász I. Effect of an antibacterial varnish and amine-fluoride/stannous fluoride (AmF/SnF2) toothpaste on Streptococcus mutans count in saliva and dental plaque of children. J Clin Dent. 1995;6(2):131-4.
23. Arweiler NB, Netuschil L, Reich E. Alcohol-free mouthrinse solutions to reduce supragingival plaque regrowth and vitality. A controlled clinical study. J Clin Periodontol. 2001;28(2):168-74.
24. Weiland B, Netuschil L, Hoffmann T, Lorenz K. Substantivity of amine fluoride/stannous fluoride folloing different modes of application: A randomized, investigator-blind, placebo-controlled trial. Acta Odontol Scand. 2008;66(5):307-13.
25. Flores-Jacoby L. Möglichkeiten der Plaque-und Gingivitis prevention. Berlin: Quintessence-Verlag Gmbh; 1991.
26. Bánóczy J, Szőke J, Kertész P, Tóth Z, Zimmermann P, Gintner Z. Effect of amine fluoride/stannous fluoride-containing toothpaste and mouthrinsings on dental plaque, gingivitis, plaque and enamel F -accumulation. Caries Res. 1989;23(4):284-8.
27. Silness J, Löe H. Periodontal diseases in pregnancy II.Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;(2):121-35.
28. Mühlemann HR, Son S. Gingival sulcus bleeding – leading symptomof inital gingivitis. Helv Odont Acta. 1971;15(2):107-13.
29. Bánóczy J, Nemes J. Effect of amine fluoride (AmF) /stannous fluoride (SnF2) toothpaste and mouthwashes on dental plaque accumulation, gingivitis and root-surface caries. Proc Finn Dent Soc. 1991;87(4):555-9.
30. Madléna M, Dombi C, Gintner Z, Bánóczy J. Effect of amine fluoride/stannous fluoride toothpaste and mouthrinse on dental plaque accumulation and gingival health. Oral Dis. 2004;10(5):294-7.
31. Bánóczy J, Dombi C, Gintner Z, Madléna M. Plaque and gingivitis prevention with amine fluoride/stannous fluoride toothpaste and mouthrinse [In German]. Oralprophylaxe. 2005;27(3):95-8.
32. Madléna M, Gintner Z, Bánóczy J, Dombi C. Evaluation of clinical effects ofamine fluoride/stannous fluoride containing toothpaste and mouthrinse [In Hungarian]. Fogorv Sz. 2005;98(6):229-32.
33. Löe H Silness J. Periodontal disease in pregnancy.I. Prevalence and severity. Acta Odontol Scand. 1963;(12):533-51.
34. Boyd RL, Chun YC. Eighteen-month evaluation of the effects of 0.4% stannous fluorideg el on gingivitis in orthodontic patients. Am J Orthod Dentofac Ortop. 1994;105(1):35-41.
35. Boyd RL, Leggott PJ, Robertson PB. Effects on gingivitis of two different 0.4% SnF2 gels. J Dent Res. 1988;67(2):503-7.
36. Øgaard B, Afzelius AA, Larsson E, Adolfsson U. A prospective randomized clinical study on the effects of amine fluoride/stannous fluoride toothpaste/mouthrinse on plaque, gingivitis and initial caries lesion development in orthodontic patients. Eur J Orthod. 2006;28(1):8-12.
37. Madléna M, Bánóczy J, Götz G, Márton S, Kaán M, Nagy G. Effects of Amine and Stannous Fluorides on Plaque Accumulation and Gingival Health in Orthodontic Patients treated with Fixed Appliances: A Pilot Study. Oral Health Dent Manag. 2012;11(2):57-61.

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Caries risk assessment models in caries prediction

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Objective. The aim of this research was to assess the efficiency of different multifactor models in caries prediction. **Material and methods**. Data from the questionnaire and objective examination of 109 examinees was entered into the Cariogram, Previser and Caries-Risk Assessment Tool (CAT) multifactor risk assessment models. Caries risk was assessed with the help of all three models for each patient, classifying them as low, medium or high-risk patients. The development of new caries lesions over a period of three years [Decay Missing Filled Tooth (DMFT) increment = difference between Decay Missing Filled Tooth Surface (DMFTS) index at baseline and follow up], provided for examination of the predictive capacity concerning different multifactor models. **Results.** The data gathered showed that different multifactor risk assessment models give significantly different results (Friedman test: Chi square = 100.073, p=0.000). Cariogram is the model which identified the majority of examinees as medium risk patients (70%). The other two models were more radical in risk assessment, giving more unfavorable risk –profiles for patients. In only 12% of the patients did the three multifactor models assess the risk in the same way. Previser and CAT gave the same results in 63% of cases – the Wilcoxon test showed that there is no statistically significant difference in caries risk assessment between these two models (Z = -1.805, p=0.071). **Conclusions.** Evaluation of three different multifactor caries risk assessment models (Cariogram, PreViser and CAT) showed that only the Cariogram can successfully predict new caries development in 12-year-old Bosnian children.

Key words: Caries risk assessment, Multifactorial model, Prediction.

Introduction

Caries risk is defined as the probability of an individual developing at least a certain number of caries lesions during specific period of time (1). Risk assessment is important step in decision-making and treatment planning. Routine preventive measures and recommendations are very effective within the general population but they do not target patients that are at greater than average risk (2). Caries risk assessment is a very complex issue, due to its multicausal etiology, the numerous and complex relations and interactions between caries risk factors, and the dependency of each factor on dose, frequency and duration. Many models for caries risk assessment (CRA) had been suggested, including different numbers and combinations of caries risk factors as caries predictors, different modes of evaluation and interpretation of risk assessment results. Models that contain only one or two risk factors, cannot predict future caries development successfully (3, 4, 5). In assessing the risk, more complex models, that operate with several risk factors, should be used. Nowadays, there are few risk models based on a multifactor concept (6, 7, 8). Caries risk models should also evaluate risk factors together and simultaneously, so some of the recent CRA models are based on information technology (9).

Most research has analyzed the role and power of single or mulitple risk factors as predictors of future caries development, but very little research has evaluated the success of multifactor models suggested for caries risk assessment, as shown in Siyama and al.'s review of literature (10). Accuracy of prediction models must be determined in longitudinal studies, but most research has a cross-sectional study design (11).

The aim of this study was to assess the efficiency of three different, multifactor caries risk assessment models [Cariogram, Previser and Caries-Risk Assessment Tool (CAT)], by comparing caries risk assessment with the actual caries increment in 12-year-old children, over a 3-year period.

Subjects and methods

Subjects

The study population consisted of 109 schoolchildren who were 12-years old at the start of the study. All the children live in Sarajevo, the capital of Bosnia and Herzegovina (the total population of Sarajevo is nearly 450,000 inhabitants). Three elementary schools were selected – a private school where parents pay full school fees, a private school where underprivileged children have free education, and one state-run school from the Novo Sarajevo municipality. 40 pupils were selected randomly from each school. Eleven children did not participate in the study (5 children chose not to participate in the study, 6 children did not come to examinations). This resulted in a study population of 109 children at baseline, 60 boys and 49 girls. The follow-up study was conducted 3 years later. The children were re-examined by the same examiner, using the same procedure as that practiced at baseline. The follow-up sample included 70 children (64.2% of the initial study population), 38 boys and 32 girls. School principals, teachers and children were given written information about the study. The parents signed informed consent forms for participation in the study. The Ethical Committee of the University of Sarajevo, Bosnia and Herzegovina approved the study.

In Bosnia and Herzegovina, all children up to age of 18 have free access to regular dental care at public dental clinics. During the period of three years, the participants in the study received regular dental care.

Study design

At baseline, the study consisted of the following steps: the questionnaire and an interview, clinical examination, saliva sampling, and caries risk assessment using CRA models. Children completed questionnaires in the school classrooms, prior to examinations. The questionnaire contained questions about: general data, questions about socioeconomic background, diet, fluoride program, tooth brushing habits, medical history and dental care. Clinical examinations consisted of: scoring of oral hygiene using the Silness - Löe plaque index, determination of dental status [Decay Missing Filling Tooth (DMFT) and Decay Missing Filled Tooth Surface (DMFS) indexes], gingival check-up (changes in colour, shape, structure and consistency of gingiva). Clinical examination was carried out by the same examiner following WHO criteria (12). After the questionnaire, interview and clinical examination, saliva sampling was performed for each child. Saliva sampling consisted of:

measurements of mutans streptococci in the saliva using a Dentocult® SM Strip mutans test, measurements of lactobacilli in the saliva using a Dentocult® LB test, measurements of saliva buffer capacity using a Dentobuff® Strip test, measurement of the saliva secretion rate (paraffin stimulated saliva for 5 minutes). All tests were from Orion Diagnostica, Espoo, Finland.

Caries risk assessment, using different multifactor caries risk assessment models, consisted of the following steps. All data gained from the questionnaire and interview, clinical examination and saliva sampling are actually caries related factors/parameters needed for creation of risk profiles, based on three different caries risk assessment models. The various parameters are given a score according to predetermined scales for each CRA model (Table 1). Different combinations of scored factors/parameters are entered into each of the CRA models – Cariogram, PreViser and CAT model and caries risk assessment is performed for each examinee.

Table 1 Caries-related factors and scores used for Cariogram, PreViser and CAT models

Diet, contents (based on the lactobacillus test counts)	
Score 0	Very low fermentable carbohydrate intake (<1.000 CFU/ml)
Score 1	Low fermentable carbohydrate intake (10.000 CFU/ml)
Score 2	Moderate fermentable carbohydrate intake(100.000 CFU/ml)
Score 3	High fermentable carbohydrate intake (>1.000.000 CFU/ml)
Diet, frequency (estimation of the number of meals and snacs per day)	
Score 0	Maximum 3 meals per day
Score 1	4-5 meals per day
Score 2	6-7 meals per day
Score 3	>7 meals per day
Oral hygiene (based on the plaque amount)	
Score 0	Very good oral hygiene (Plaque index <0.4)
Score 1	Good oral hygiene (Plaque index =0.4 -1.0)
Score 2	Poor oral hygiene (Plaque index =1.1- 2.0)
Score 3	Very poor oral hygiene (Plaque index >2.0)
Mutans streptococcus (estimation of the level of mutans streptococcus in saliva)	
Score 0	Very low or zero amounts (<20.000 CFU/ml)
Score 1	Low level (20.000 – 100.000 CFU/ml)
Score 2	High amounts (100.000 – 1.000.000 CFU/ml)
Score 3	Very high amounts (>1.000.000 CFU/ml)
Fluoride program (estimation of the extent of fluoride available in the oral cavity)	
Score 0	Maximum fluoride program
Score 1	Fluoride supplements, irregularly
Score 2	Fluoride toothpaste only
Score 3	No fluoride
Saliva secretion (estimation of the flow rate of stimulated saliva)	
Score 0	Normal saliva secretion (≥0.7 ml/min)
Score 1	Low saliva secretion (0.3-0.7 ml/min)
Score 2	Very low saliva secretion (≤ 0.3 ml/min)
Saliva buffering capacity (estimation of saliva to buffer acids)	
Score 0	Adequate buffer capacity (pH≥ 6.0)
Score 1	Reduced buffer capacity (pH 4.5-5.5)
Score 2	Low buffer capacity (pH≤4.0)

Continuation of Table 1 Caries-related factors and scores used for Cariogram, PreViser and CAT models

Socioeconomic status (estimation of socioeconomic status of child/child's parent)	
Score 0	High
Score 1	Mid-level
Score 2	Low
Visit to dentist	
Score 0	Regular use of dental care
Score 1	Irregular use of dental care
Score 2	No usual source of dental care
Child has decay?	
Score 0	No
Score 1	Yes
Times per day that child teeth are brushed	
Score 0	2-3 times per day
Score 1	1 per day
Score 2	<1 times per day
Gingivitis (red, puffy gums)	
Score 0	Absent
Score 1	Present
Dental floss	
Score 0	Regular use of dental floss
Score 1	Irregular use of dental floss
Time lapsed since child's last cavity	
Score 0	24-35 months
Score 1	12-23 months
Score 2	<12 months
Sealants	
Score 0	Yes
Score 1	No
Medical history (general diseases or conditions associated to dental caries)	
Score 0	No disease, healthy
Score 1	A general disease that can influence the caries process to a mild degree
Score 2	A general disease that can influence the caries process to a high degree
Caries experience (DMFT index at baseline)	
Score 0	DMFT = 0
Score 1	DMFT = 1
Score 2	DMFT = 2
Child wears braces or orthodontic/oral appliances?	
Score 0	No
Score 1	Yes
Child has decay?	
Score 0	Yes
Score 1	No

CAT=Caries-Risk Assessment Tool; CFU=Colony Forming Units; DMFT=Decay Missing Filling Tooth.

Cariogram

Cariogram is an interactive computer program which assesses risk of new caries lesion development. It presents the caries risk profile of an individual graphically, simultaneously taking into account the interaction of different causative factors of caries. It creates an individual future „risk scenario", based on the given scores and interactions of 9 factors/parameters of direct relevance to caries, entered in the Cardiogram. The factors are: caries experience, related diseases, diet content and frequency, plaque amount, mutans streptococci level, fluoride program, saliva secretion and buffering capacity (Table 1). The Cariogram contains many „if" conditions – it can operate with 5 million combinations of caries related factors (13). According to the weighted formula, after all data of relevance for caries are collected from the individuals, scored and entered in the Cariogram, the program presents a pie diagram with the following sectors: bacteria, diet, susceptibility and circumstances. The caries risk is express in the sector „chance of avoiding caries". When the chance of avoiding caries is high, the caries risk is small and vice versa. The chance varies on a scale from 0 to 100% - chance from 0 to 20% means that the individual has high caries risk, from 21 to 80% medium risk and from 81-100% low risk for future caries development.

PreViser – oral health risk assessment software

PreViser, Inc. is an evidence-based online risk prediction system that uses software technology to predict common oral diseases, specifically periodontitis, caries and oral cancer. PreViser is the part of OHIS™ (Oral Health Information System) and contains three different parts: a Periodontal Assessment Tool (PAT), a Caries Risk Tool and an Oral Cancer Risk Tool. The "Caries (tooth decay), Root, and Fracture Risk Assessment" input form for patient age 9-18, consists of questions about patient history and clinical data (general information about the patient, data about the tooth most recently exposed to saliva for the last 12 months and months that patient has been caries free, data about oral hygiene, diet content and frequency, fluoride program and orthodontic appliances). PreViser calculates risk online, in a central software unit, and expresses it as low/moderate/high risk. High risk means that the patient is very likely to have a cavity within the next 3 years.

Caries-Risk Assessment Tool

The Caries-Risk Assessment Tool (CAT) is the tool for risk assessment of infants, children and adolescents, supported by the American Academy of Pediatric Dentistry. This tool is based on a set of physical, environmental and general health factors. Factors evaluated in the CAT, determined by interviewing the parent/primary caregiver are: exposure to fluoride, dietary and oral hygiene habits, socio-economical status, dental care, medical conditions that impact motor coordination/cooperation or impair saliva, orthodontic/oral appliances, the time lapsed since child's last cavity. Clinical evaluation connotes visible plaque, gingivitis, presence of enamel demineralization, enamel defects, deep pits and fissures, and previous caries experience. Radiography and saliva tests (levels of mutans streptococci and lactobacilli) are not essential for using CAT (supplemental professional assessment). Depending on the score/value given to the parameters, individual overall caries risk assessment is based on the highest level of any aforesaid risk indicator (low/medium/high risk).

The caries increment was estimated after 3 years. At follow up, all the children were examined by the same examiner. Following the same diagnostic criteria and procedure as done at baseline, the DMFT and DMFS index was computed for each examinee.

Statistical methods

Results are shown using descriptive statistics (frequency distributions, mean±SD) and presented in tables and figures. The Kappa coefficient was used to evaluate the extent of agreement between risk profiles assessed by two different CRA models. To test the differences in caries risks, assessed with three different CRA models (Cariogram, PreViser and CAT), a non-parametric Friedman test was used. The Wicoxon signed rank test was used to test the differences between two risk profiles. The level of significance was set at p<0.05. Logistic regression analyses were carried out to test CRA models to predict the development of new caries lesions. The response variable was DMFS increment (difference between DMFS at baseline and follow-up), presented as no new caries/new caries over a period of 3 years. For statistical analyses, SPSS 15.0 (SPSS, Inc, Chicago, Il) version 9 software was used.

Results

The figure presents caries risk assessment results at baseline for 109 examinees with three different multifactor models. According to the Cariogram, 70.9% of children were in the medium risk group and only 7.3% in the high risk group. According to PreViser and CAT the majority of children were in the high risk group.

Risk assessed with Cariogram and Previser was identical in only 30.02% of individuals. In the group of 30.02% identical risk profiles, 10.1% of the examinees showed assessed risk as low, 12.8% as medium and 7.3% as high. Cariogram and CAT created identical risk profiles in only 19.2% cases (6.4% low, 5.5% medium and 7.3% high caries risk). Agreement of risk profiles between Previser and CAT was found in 62.4% examinees (3.7% low risk, 1.8% medium risk and 56.9% high risk).

To evaluate the extent of agreement between risk assessed by different models, the Kappa coefficient was used. There was poor agreement between Cariogram and PreViser (kappa coefficient=0.139, p=0.000), Cariogram and the CAT model (Kappa coefficient=0.053, p=0.049) and between PreViser and CAT (Kappa coefficient=0.103, p=0.143).

To test the differences between caries risk profiles assessed with Cariogram, PreViser and CAT, the non-parametric Friedeman test was used. Mean ranks were: Cariogram 1.36,

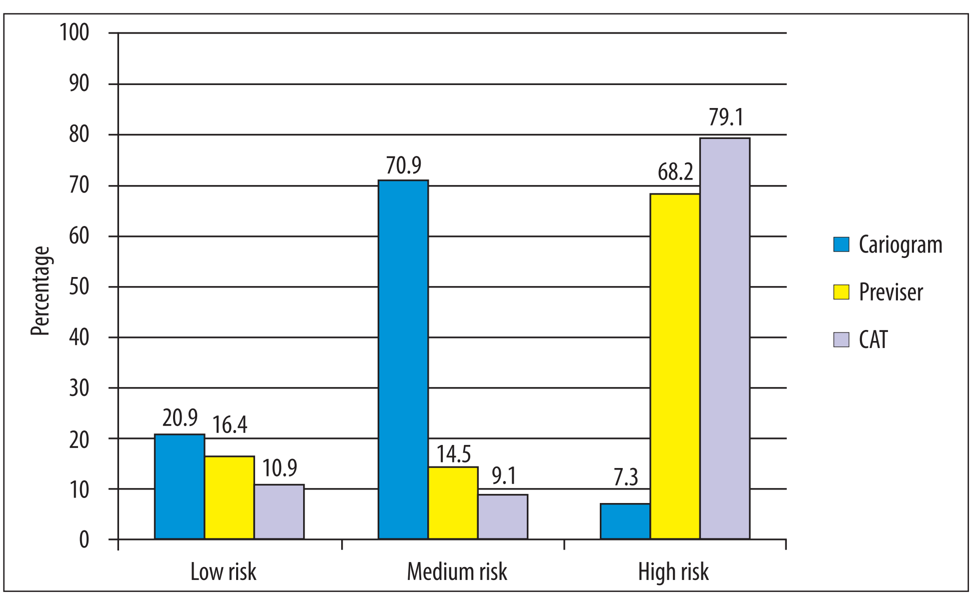

Figure1 Caries risk assessment at baseline using Cariogram, Previser and CAT models.

Table 2 Cross-tabulation between individual risk profiles assessed with Cariogram, PreViser and CAT

Cross-tabulation		PreViser			
		Low risk	Medium risk	High risk	Total
		n (%)	n (%)	n (%)	n (%)
Cariogram	Low risk	11 (10.1)	2 (1.8)	10 (9.2)	23 (21.1
	Medium risk	7 (6.4)	14 (12.8)	57 (52.3)	78 (71.6)
	High risk	0 (0)	0 (0)	8 (7.3)	8 (7.3)
	Total	18 (16.5)	16 (14.7)	75 (68.8)	109 (100)
		CAT			
Cariogram	Low risk	7 (6.4)	4 (3.7)	12 (11.0)	23 (21.1)
	Medium risk	5 (4.6)	6 (5.5)	67 (61.5)	78 (71.6)
	High risk	0 (0.0)	0 (0.0)	8 (7.3)	8 (7.3)
	Total	12 (11.0)	10 (9.2)	87 (79.8)	109 (100.0)
		CAT			
PreViser	Low risk	4 (3.7)	1 (0.9)	13 (11.9)	18 (16.5)
	Medium risk	2 (1.8)	2 (1.8)	12 (11.0)	16 (14.7)
	High risk	6 (5.5)	7 (6.4)	62 (56.9)	75 (68.8)
	Total	12 (11.0)	10 (9.2)	87 (79.8)	109 (100.0)

CAT= Caries-Risk Assessment Tool.

Previser 2.23, CAT 2.41. The test showed that there are significant differences between risks assessed with three different CRA models (Chi square = 100.073, p = 0.000).

The Wilcoxon signed rank test was performed to test the differences in risk profiles between the two related groups. The results showed that there are significant differences between caries risk assessed with the Cariogram and the PreViser (Z=-6.944, p=0.000) models and between risk assessed with Cariogram and CAT (Z=-8.010, p=0.000). There was no significant difference between individual risk assessed with PreViser and CAT (Z=-1.805, p=0.071).

Table 3 presents the Cariogram, PreViser and CAT risk profiles (assessed as low/medium/high) related to DMFT and DMFS indexes means at baseline and follow-up and actual caries increment of DMFT and DMFS indexes over the period of three years.

Omnibus tests of model coefficients showed Chi – square equal to 6.172 on 2 degrees of freedom (p=0.046), indicating that at least one of the covariates is significantly associated with caries increment over 3 years. Based on pseudo R square values (Cox&Snell R square = 0.084, Nagelkerke R Square = 0.141), it can be concluded that between 8.4 and 14.1 per cent of variability is explained by this set of variables. The Hosmer-Lemesh test (Chi square = 0.000, p=1.000) supports our prediction model (binary logistic model with forward LR method), as being worthwhile.

Table 4 presents the results of binary logistic regression for all three CRA models. The dependent variable was the DMFS increment (difference between DMFS at baseline and follow-up), presented as dichotomous variables (no new caries/new caries, coded 0/1) over a period of 3 years. As independent variables, the value (low/medium/high risk) of risk profiles for each of the model (Cariogram, PreViser, CAT) was entered in the binary logistic model separately. Table shows that only the Cariogram can predict the development of new caries lesions in the future ($p<0.05$). The lowest risk group (Cariogram 0) was chosen as the

Table 3 Caries risk assessment made by Cariogram, Previser and CAT and DMFT and DMF(S) increment over a period of 3 years

Caries risk assessment	Risk		
	Low risk	Medium risk	High risk
Individuals at baseline		n (%)	
Cariogram	23 (21.10)	78 (71.56)	8 (7.34)
Previser	18 (16.51)	16 (14.68)	75 (68.81)
CAT	12 (11.01)	10 (9.17)	87 (79.82)
Individuals at follow-up		n (%)	
Cariogram	13 (18.57)	52 (74.29)	5 (7.14)
Previser	11 (15.71)	13 (18.57)	46 (65.71)
CAT	8 (11.43)	5 (7.14)	57 (81.43)
DMFT at baseline		(mean±SD)	
Cariogram	2.13±2.67	5.50±3.12	5.75±1.58
Previser	0.67±0.97	3.69±2.12	6.04±2.86
CAT	2.42±2.50	4.10±2.42	5.22±3.27
DMFT at follow-up		(mean±SD)	
Cariogram	4.15±3.31	8.02±4.03	8.00±3.08
Previser	2.91±2.43	5.54±3.62	8.85±3.58
CAT	4.75±3.58	8.40±2.70	7.56±4.17
DMFT increment		(mean±SD)	
Cariogram	1.77±1.88	2.40±2.36	1.80±1.79
Previser	2.18±2.32	1.92±2.18	2.35±2.27
CAT	2.38±1.92	2.60±1.82	2.19±2.33
DMF(S) increment		(mean±SD)	
Cariogram	2.54±2.44	4.71±4.34	5.00±7.07
Previser	2.82±3.19	3.08±2.87	5.04±4.75
CAT	3.13±2.53	3.80±5.81	4.54±4.41

CAT=Caries-Risk Assessment Tool; DMFT=Decay Missing Filling Tooth.

Table 4 Logistic regression model for Cariogram, Previser and CAT

Covariates	B	S.E.	Wald	df	p	Exp (B)	95.0% CI for Exp (B)	
Cariogram 0	-	-	6.08	2	0.048	-	Lower	Upper
Cariogram 1	1.609	1.12	2.04	1	0.153	5.00	0.551	45.39
Cariogram 2	2.442	1.01	5.83	1	0.016	11.50	1.58	83.38
Constant	-0.405	0.913	0.197	1	0.657	0.667	-	-

CAT=Caries-Risk Assessment Tool.

reference value. Individuals with high caries risk (assessed with the Cariogram) have 11.5 times more chance of developing new caries lesions in the future, compared to low caries risk individuals (Odds ratio = 11.5, p=0.016, CI=1.58-83.3).

Discussion

Caries risk assessment (CRA) is an important step in dental treatment based on the concept of minimally invasive therapy, where therapeutic and prophylactic measures are planned, based on the results of caries risk assessment. The use of numerous and non-standardized CRA protocols can lead to bias in caries risk assessment. The Moss study showed that equable criteria are essential for determination and assessment of high-risk patients (14), and these are provided by applying standardized multifactor CRA models. There is no recommendation for dental practitioners in Bosnia and Herzegovina as to which protocol or CRA model to use in caries prediction. Bader's research showed that even if a certain protocol for CRA was recommended, it remained unclear how it was used in dental practice (15) and even more, what was the reason for using a certain type of CRA protocol (16).

CRA models vary, from simple ones that operate with only one caries risk factor to complex, multifactor models that include more sophisticated methods, like microbial tests, saliva analyses and so on. Models that include only one caries – related factor or a combination of two risk factors, cannot predict the risk of future caries development successfully (3, 4, 5). In our study, we used three different, standardized, multifactor CRA models (Cariogram, PreViser and CAT) to assess the risk in 12 year old children. The caries risk was categorized as low, medium or high, using all three models for each individual. The results showed that very often, CRA models assessed the risk differently – with one model, the child was assessed as a low risk but with another model as a medium, or high-risk patient. PreViser and CAT were more radical in CRA, giving more unfavorable risk profiles, compared to the Cariogram. According to the CAT model, almost 80% of children were in the high-risk group. The reason for this is probably the way of categorization of risk groups, where individual overall caries risk assessment is based on the highest level of any aforesaid risk indicator (low/medium/high risk). That way, the same importance was given to each of the caries risk factors included in the CAT model. What this means is that a patient with beneficial (categorized as low risk) values/scores of all factors and only one unfavorable factor (categorized as high risk) will be categorized as a "high risk patient". The PreViser model gave more favorable overall risk profiles for examinees than the CAT model, where about 70% children were in the high-risk group. The Cariogram model assessed less than 10 % children as high risk. Its categorization into risk groups is more moderate compared with the other two models. All three models assess the risk identically for only 12 % of examinees. The greatest agreement in results was shown by PreViser and CAT (63% of cases), where the majority of children were in the high-risk group (57%).

There is not much scientific evidence of the efficiency of multifactor risk models. Evaluation of the Cariogram model through longitudinal research (examinees being school children aged 10-11 and older examinees aged 55, 65, 75) has showed that it can predict caries efficiently, better than any other model that includes single risk factors (13, 17, 18). Based on the results of Hänsel-Petersson et al. from 2002 and 2003, Brathall D. concluded that the Cariogram is capable of sorting out individuals into risk-groups that have an actual chance of developing new caries lesions in the future (19). On the other hand, Holgerson et al. showed that a modified Cariogram applied to preschool children was not particularly useful in identifying high caries risk patients in a low-caries community (20). In the study of Utreja et al., conducted to evaluate the accuracy of the Cariogram in predicting the occurrence of caries in first permanent molars in 30 children aged 8, the results revealed that the Cariogram had a diagnostic accuracy of 63.33%, thus emphasizing the need for better prediction models (21). In our study, the Cariogram was used on the basis of the original manual, without any modification of the program or risk factors entered into the Cariogram (22). The study results showed that the Cariogram can predict risk well in our examinees. Up until now, there has only been research proving PreViser efficacy in risk prediction, and that was only for the PAT module (23, 24, 25, 26). There has been no research to evaluate PreViser efficiency in caries risk assessment. The Caries risk Assessment Tool (CAT) helps in identification of caries risk factors, identification of the high-risk children and better caries prediction (27). Based on our results, PreViser and CAT cannot predict caries successfully in our examinees.

The analyses of the advisability and adequacy of risk factors entered into a certain multifactor risk model, the mode of registration and scoring of risk factors, are beyond the scope of this research. However, it should be noticed that there is still no international consensus about which factors in risk assessment should be considered and how to evaluate them (28). Zero et al. evaluated predictive power of multifactor CRA models in patients of various age. They showed that the predictive powers of different CRA models depend on the characteristics of the population for which the models are designed, and that any combination used for CRA is not constantly good when applied to different groups of examinees (e.g. different age groups) (29). Powel concludes that the CRA model must be adjusted to the patient's age as well as to caries prevalence inside the population (30). Each specific target population group (preschool children, teenagers, adults, and older patients) should have their own set of caries-risk variables to be considered during CRA. Taking all of the above into consideration, it should be noted that the Cariogram, the PreViser and the CAT were created and developed for populations of relatively low caries prevalence (USA, Sweden). It is somewhat expected that these models would not show equal efficiency when applied to 12-year-old Bosnian children (within a population of high caries prevalence and a completely different model of dental care compared to Sweden and the USA) (31). For these reasons, before we reject the models that did not show good efficiency in caries prediction (PreViser and CAT), the same models should be tested on a larger number of examinees from various populations and different age groups.

Conclusion

Within all the limitations of this study, it can be concluded that our results suggest that of the three different multifactor CRA models (Cariogram, PreViser and CAT) only the Cariogram can successfully predict new caries development in Bosnian 12-year-old children (a high risk community).

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References

1. Kalwitzki M, Weiger R, Axmann - Krcmar D, Rosendahl R. Caries risk analysis: considering caries as an individual time - dependent process. Int J Paediatr Dent. 2002;12(2):132-42.
2. Berg JH. Dental caries detection and risk management by risk assessment. Journal Compilation. 2007;1(7):49-55.
3. Bratthall D, Ericsson D. Test for assessment of caries risk. In: Thylstrup A, Fejerskov O, editors.

Textbook of Clinical cariology. 2nd ed. Copenhagen: Munksgaard; 1994. p. 333-53.

4. Reich E, Lussi A, Newbrun E. Caries - risk assessment. Int Dent J. 1999;49:15-26.
5. Steiner M, Helfenstein U, Marthaler TM. Dental predictors of high caries increment in children. J Dent Res. 1992;71:1926-33.
6. Russell JI, MacFarlane TW, Atchison TC, Stephen KW, Burchell CK. Prediction of caries increment in Scottish adolescent. Community Dent Oral Epidemiol. 1991;19:74-7.
7. Demers M, Broader JM, Mouton C, Simard PL, Trahan L, Veilleux G. A multivariate model to predict caries increment in Montreal children aged 5 years. Community Dent Health. 1992;9:273-81.
8. Van Houte J. Microbiological predictors of caries risk. Adv Dent Res. 1993;7(2):87-96.
9. Fors GH, Sandberg CH. Computer - aided risk management - A software tool for the Hidep model. Quintessence Int. 2001;32:309-20.
10. University of Toronto (homepage on Internet). Siyam A, Zangooei B, Sourial M, Sokary M, Soliman M, Shetty S, et al. Predictors of new caries in children and adolescents. – An evidence based report. (cited 2013 Mar 13) Avaible from: http://www.utoronto.ca/dentistry/newsresources/evidence_based/PredictorsNewCaries.pdf.
11. The Swedish Council on Technology Assessment in Health Care. Caries – diagnosis, risk assessment and non-invasive treatment. A systematic review. Summary and conclusions. Report No 188. Stockholm: SBU; 2007.
12. Oral Health Surveys: Basic methods. 4th ed. Geneva: WHO; 1997.
13. Hänsel Petersson G, Twetman S, Bratthall D. Evaluation of a computer program for caries risk assessment in schoolchildren. Caries Res. 2002;36:327-40.
14. Moss EM. Efficacy not clearly established for professional caries prevention methods in high - risk patients. J Evid Base Dent Pract. 2002;2:199-201.
15. Bader JD, Perrin NA, Maupomé G, Rindal BD, Rush W. Validation of a simple approach to caries risk assessment. J Public Health Dent. 2005;25(2):76-81.
16. Samardi R, Gabre P, Gahnberg L. Strategies for caries risk assessment in children and adolescent at public dental clinics in a Swedish country. Int J Paediatr Dent. 2009;19(2):135-40.
17. Hänsel-Petersson G, Fure S, Bratthall D. Evaluation of a computer based caries risk assessment program in an elderly group of individuals. Acta Odont Scand. 2003;61(3):164-71.
18. Hänsel-Petersson G, Isberg PE, Twetman S. Caries risk profiles in schoolchildren over 2 years assessed by Cariogram. Int J Paediatr Dent. 2010;20(5):341-6.
19. Bratthall D, Hänsel Petersson G. Cariogram – a multifactorial risk assessment model for a multifactorial disease. Community Dent Oral Epidemiol. 2005;33:256-64.
20. Holgerson PL, Twetman S, Stecksèn-Blicks C. Validation of an age-modified caries risk assessment program (Cariogram) in preschool children. Acta Odontol Scand. 2009;67(2):106-12.
21. Utreja D, Simratvir M, Kaur A, Kwatra KS, Singh P, Dua V. An evaluation of the Cariogram as a predictor model. Int Dent J. 2010;60(4):282-4.
22. Hänsel Petersson G. Assessing caries risk - using the Cariogram model. Swed Dent J Suppl. 2003;(158):16.
23. Page RC, Martin A, Loeb CF. The Oral health Information Suite (OHIS): Its use in the management of periodontal disease. J Dent Educ. 2005;69(5):509-20.
24. Page RC, Martin J, Krall EA, Mancl L, Garcia R. Longitudinal validation of a risk calculator for periodontal disease. J Clin Periodontol. 2003;30:819-27.
25. Persson Rutger G, Mancl L, Martin J, Page RC. Assessing periodontal disease risk – a comparison of clinicians assessment versus a computerized tool. J Am Dent Assoc. 2003;134(5):575-82.
26. Page RC, Krall EA, Martin J, Mancl L, Garcia R. Validity and accuracy of a risk calculator in predicting periodontal disease. J Am Dent Assoc. 2002;133(5):569-76.
27. American Academy of Pediatric Dentistry (homepage on the Internet). Policy on use of a Caries risk Assessment Tool (CAT) for infants, children and adolescents. Oral Health Policies. (cited 2013 Mar 13) Avaible from: http://www.aapd.org/members/referencemanual/pdfs/02-03/P_ CariesRiskAssess.pdf.
28. Horowitz A. A Report on the NIH Consensus Development Conference on Diagnosis and Management of Dental Caries Throughout Life. J Dent Res. 2004;83(Spec Iss C):C15-C17.
29. Zero D, Fontana M, Lennon M, Dent B. Clinical applications and outcomes of using indicators of risk in caries management. J Dent Educ. 2001;65(10):1126-32.
30. Powell VL. Caries risk assessment: relevance to the practitioner. JADA. 1998;129:349-53.
31. Zukanović A, Kobašlija S, Ganibegović M. Caries risk assessment in Bosnian children using cariogram computer model. Int Dent J. 2007;57(3):177-83.

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Oral health attitudes and caries-preventive behaviour of Czech parents of preschool children*

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Objective. To characterize the oral health-related attitudes and behaviour of Czech parents of preschool children. **Materials and methods**. A representative sample of 796 parents was recruited for the cross-sectional questionnaire survey. Study data were collected using a validated questionnaire with 44 attitudinal items related to different aspects of caries prevention. The data were analyzed by explorative factor analysis, extracted factors were subjected to reliability analysis and Kruskal–Wallis ANOVA was used to test differences in the factor scores in respondents with different levels of education and self-perceived SES. **Results.** The factor analysis extracted 3 factors, labelled "Toothbrushing – perceived significance and parental efficacy"; "External caries control" and "Internal caries control". They explained 28.9% of the data variability. The comparison of the factor scores in groups with different SES and education of mothers showed highly significant differences. For all three factors, median values of the aggregated Likert scale increased with increasing SES and education of the mother. **Conclusion**. The parents report that they are aware of their responsibility for the prevention of tooth decay in their children. In caries prevention they concentrate on toothbrushing. Dietary measures do not seem to be of similar importance to them. The increasing self-perceived SES of the family and the education level of the mother have a significantly positive effect on the caries-preventive attitudes of the parents. Based on the study results, the message to the public-health sector in the Czech Republic should include the need to highlight the importance of a non-cariogenic diet and the role of fluorides in caries prevention.

Key words: Childhood caries, Oral health-related attitudes, Questionnaire survey, Socio-economic status.

Introduction

The main aetiological factors of dental caries include cariogenic bacteria, frequent intake of fermentable carbohydrates, disorders of salivary production and the composition and poor mineralization of hard dental tissues (1). The above-mentioned major risk factors are modified by socioeconomic, psychological and behavioural risk factors, which act as indirect causal agents (2) and

*The article is dedicated to professor Zdenek Broukal on the occasion of his 70th birthday, with gratitude and appreciation, professionally and personally.

help explain the distribution of dental caries within the population. The Czech Republic is a country with relatively high caries prevalence in preschool children. In 2010, the proportion of 5yr-old children with intact teeth was 45 percent (3).

Thus, the aim of this cross-sectional questionnaire survey was to characterize oral health-related attitudes and the caries-preventive behaviour of Czech parents of preschool children in a representative national cohort of parents of preschool children. A similar study performed in the Czech Rep. in 2008 by Lencova et al. investigated the relationship between the parental locus of control and caries experience in preschool children, and concluded that higher internal parental locus of control is associated with better control of caries experience in their preschool children (4). The present study focused on a broader description of parental attitudes to dental caries prevention in association with their self-perceived socioeconomic status and the education level of the parents.

Materials and methods

The study was performed in 31 sites from different regions of the country. The study sites were selected, for the purposes of representative nationwide oral health surveys, by the Czech Institute of Health Information and Statistics, in compliance with the World Heath Organization recommendations (5). At each site, 1-2 nurseries were randomly selected from the national registry of preschool establishments. Subsequently, the principals of the selected nurseries were invited to join the survey. Two of the nurseries refused cooperation; therefore, alternative nurseries were selected. After obtaining the consent of the head teachers, in each nursery, the parents of 30 children aged 3-5 years were invited to participate in the questionnaire survey. At the nurseries with a lower number of eligible children, parents of all the children were invited. The study was approved by the competent Ethics Committee of the General University Hospital in Prague.

The standardized and validated questionnaire used in this study was employed in an international study on childhood caries and was based on psychological theoretical models (Theory of planned behaviour, Aizen 2002, Health belief model, Rosenstock 1952 and Locus of control, Walston 1976) presenting personal attitudes and beliefs as behavioural predictors (2). The parental questionnaire contained 44 attitudinal items associated with parental oral health beliefs related to dental decay, and information on the parents' education and self-perceived socio-economical status. Each item was measured on a five-point Likert scale, ranging from strongly disagree [1] to strongly agree [5]. The coding for the negatively formulated items was reversed so that for all items higher scores reflected more positive/ correct attitudes. Missing data was imputed, allowing for up to 3 missing values. Parental education was recorded in three categories: "primary school or no formal education", "secondary school" and "college/ university". Self-perceived socio-economical status (SES) was recorded on a scale/ ladder ranging from 1-10 as "low SES" (1-3), "middle SES" (4-7) or "high SES" (8-10).

The anonymous questionnaire forms were delivered to the nurseries and, after being distributed to the parents by the school principals and filled-out, they were returned to the investigators by regular post. The data were then transfered from paper to electronic form. Out of 1248 families with preschool children invited to participate in the study, i.e. to complete the parental questionnaires, 796 questionnaires were completed (response rate: 63.8%).

Statistical analysis

The data were analyzed by factor analysis, which made it possible to find latent associations among a large number of variables with a similar variability, and group them into a smaller set of newly formed factors. The method of factor extraction was Principal Components Analysis (PCA) with rotation (Varimax). Factor loadings (FL), i.e. correlation coefficients between individual questionnaire variables and the factors, were calculated. Only variables with FL higher than 0.4 were included in the factors. Attitudinal items in each factor were subjected to reliability analysis to test the internal consistency of the data. From the attitudinal items of individual factors, aggregated Likert-scale scores were calculated for each individual respondent, and median values were determined in groups of respondents differing according to the level of education and self-perceived SES. Factor scores of all extracted factors were tested using Kruskal–Wallis one-way analysis of variance in groups of respondents differing according to the level of education and self-perceived SES. A probability level of 95 percent was used. The statistical analyses were performed using STATISTICA 10 (StatSoft Inc.).

Results

The factor analysis extracted 3 factors, which explained 28.9% of the questionnaire data variability. The extracted factors including factor loadings of the variables, modus values and interquartile ranges are presented in Tables 1-3. Reliability analysis of each factor revealed the high internal consistency of the data - Cronbach´s alpha values above 0.70.

The factor 1 was labelled Toothbrushing – perceived significance and parental efficacy (Table 1). As is obvious from Table 1, it was related to the parents´ willingness or determination to encourage and supervise their children during toothbrushing, or the positive attitudes of the "people around" towards toothbrushing. This factor explained 18% of the data variance with Cronbach´s alpha value 0.82.

Factor 2 was labelled External caries control (Table 2). This factor explained 6.3% of data variance with Cronbach´s alpha value 0.75. It included mostly health-external attitudes to caries control, related to the

Table 1 Factor 1: Toothbrushing – perceived significance and parental efficacy

Items of questionnaire	Factor loading	Modus	Interquartile range (Q75-Q25)
We feel it is important that we check our child's teeth for decay.	0.42	4	1
As a family we intend brushing our child's teeth for him/her daily.	0.64	4	1
The people in my family would feel it was important to help brush our child's teeth daily.	0.64	4	1
The people we know well would feel it was important to brush our child's teeth daily.	0.53	4	0
We feel able to brush our child's teeth for him/her.	0.65	4	0
I don't know how to brush my child's teeth properly.*	0.50	4	0
We feel it is important to check if our child has brushed his/her teeth	0.62	4	1
We don't have time to help brush our child's teeth daily.*	0.58	4	1
We cannot make our child brush his/her teeth daily.*	0.56	4	1
My child's teeth are brushed as part of my child's daily washing routine (washing hands and face).	0.59	4	1

* Items were reversed before the analysis.

parent´s belief that the occurence of dental caries in their children depends on chance, luck, or the dentist.

Factor 3 was labelled "Internal caries control" (Table 3). This factor explained 4.6% of data variance with Cronbach´s alpha value 0.78. It included items related to parental awareness that it is their responsibility to control caries risk in their child, the perceived seriousness of caries occurrence in their child, and the perceived significance of cariogenic nutrition control, use of fluoride toothpastes and toothbrushing routine in the prevention of dental caries.

Table 2 Factor 2: External caries control

Items of questionnaire*	Factor loading	Modus	Interquartile range (Q_{75}-Q_{25})
It is the responsibility of the dentist to prevent our child getting tooth decay.	0.49	4	1
No matter what we do, our child is likely to get tooth decay.	0.56	4	1
It just looks bad if our child has tooth decay.	0.50	4	1
If our child gets tooth decay, it is by chance.	0.40	4	0
It would not make any difference to our child getting tooth decay, if we helped him/her brush every day.	0.52	4	1
It is worthwhile to give our child sweets/biscuits to behave well.	0.43	4	0
In our family, it would be unfair not to give sweets to our child every day.	0.60	4	0
It is not worth it to battle with our child to brush his/her teeth twice a day.	0.42	4	1
It is just bad luck if our child gets tooth decay.	0.52	4	1
The dentist is the best person to prevent tooth decay in our child.	0.54	4	1

*Items were reversed before the analysis.

Table 3 Factor 3: Internal caries control

Items of questionnaire	Factor loading	Modus	Interquartile range (Q_{75}-Q_{25})
As a family, we are confident that we can reduce the chances of our child getting tooth decay	0.47	4	0
Tooth decay would have major consequences on our child's general health	0.48	4	1
Tooth decay is a serious problem in baby teeth	0.51	4	0
As parents, it is our responsibility to prevent our child getting tooth decay	0.49	4	1
We can prevent tooth decay in our child by reducing sugary foods and drinks between meals	0.41	4	1
If we brush our child's teeth daily, we can prevent our child getting tooth decay in the future	0.52	4	0
If our child uses a fluoride toothpaste, it will prevent tooth decay	0.46	4	1
We can prevent tooth decay in our child by helping him/her with brushing daily	0.54	4	0
It would not make any difference to our child getting tooth decay, if we helped him/her brush every day*	0.42	4	1
Our child eating sugary foods and drinks in between meals would cause tooth decay	0.48	4	1

*Item was reversed before the analysis.

From the 10 attitudinal items (each scored from 1 to 5 on a Likert scale by the respondents) in each of the 3 factors, aggregated Likert-scale scores were calculated for each individual respondent, ranging from 10 to 50. These scores reflected the strength of parental attitudes within each factor from negative / incorrect (lower scores) to positive / correct (higher scores). Additionally, median values of aggregated Likert-scale scores were determined in groups of respondents with different self-perceived SES and education levels. In the above groups of respondents, the factor scores of all three factors were tested using Kruskal–Wallis one-way analysis of variance. The results are presented in Tables 4-6.

The comparison of the factor scores in groups with different self-perceived SES and the education level of the mothers showed highly significant differences ($p \leq 0.005$). For all three factors, median values of the aggregated Likert scale increased with increasing SES and education of the mother. The fathers´ education had no significant effect on toothbrushing behaviour ($p=0.11$), but significantly influenced external caries control (factor 2, $p=0.00$) and internal caries control (factor 3, $p=0.01$).

Table 4 Factor scores in parents with different self-perceived socio-economical status

Variables	SES[1]			Statistics		
	Low (n=61)	Middle (n=596)	High (n=31)	K-Wχ^2 value	D. f.	p
Factor 1 (Factor scores)[2]	Toothbrushing – perceived significance and parental efficacy			8.15	2	0.00
Aggregated Likert scale (median)	40	41	43			
Factor 2 (Factor scores)[2]	External caries control			14.30	2	0.00
Aggregated Likert scale (Median)	35	38	39			
Factor 3 (Factor scores)[2]	Internal caries control			10.06	2	0.00
Aggregated Likert scale (Median)	30	31	32			

[1]Grouping variable; [2]Dependent variable.

Table 5 Factor scores in groups with different maternal education level

Variables	Maternal education level[1]			Statistics		
	Prim. school/ no formal education (n=36)	Secondary school (n=473)	College/ university (n=254)	K-Wχ^2 value	D. f.	p
Factor 1 (Factor scores)[2]	Toothbrushing – perceived significance and parental efficacy			22.41	2	0.00
Aggregated Likert scale (median)	40	40	42			
Factor 2 (Factor scores)[2]	External caries control			32.20	2	0.00
Aggregated Likert scale (median)	36	38	40			
Factor 3 (Factor scores)[2]	Internal caries control			31.16	2	0.00
Aggregated Likert scale (median)	31	31	32			

[1]Grouping variable; [2]Dependent variable.

Table 6 Factor scores in groups with different father's education level

Variables	Father's education level[1]			Statistics		
	Prim. school/ no formal education (n=49)	Secondary school (n=440)	College/ university (n=249)	K-Wχ2 value	D. f.	p
Factor 1 (Factor scores)[2]	Toothbrushing – perceived significance and parental efficacy			3.65	2	0.11
Aggregated Likert scale (median)	41	40	41			
Factor 2 (Factor scores)[2]	External caries control			12.18	2	0.00
Aggregated Likert scale (median)	36	38	39			
Factor 3 (Factor scores)[2]	Internal caries control			9.03	2	0.01
Aggregated Likert scale (median)	31	31	32			

[1]Grouping variable; [2]Dependent variable.

Discussion

Despite the fact that the three extracted factors explained only one third of the data variability, they had high internal consistency, showing high reliability of the scales. The factors reflected the perceived relevance of caries-preventive measures used in preschool children by Czech parents. High priority was given to toothbrushing. Its perceived significance and parental efficacy accounted for the first factor, which itself explained 18% of the data variance. The second factor reflected a perceived external locus of caries control and explained 6 percent of the data variance. The last factor was labelled "Internal caries control" and explained nearly 5% of the data variance. It included miscellaneous items related to caries prevention, including the perceived seriousness of the disease, nutritional factors and the preventive action of fluoride.

The extracted factors involved 30 out of 44 attitudinal items of the original questionnaire. This outcome is related to the exploratory factor analysis, which is a method facilitating interpretation of multiple data. Despite the fact that the original questionnaire contained attitudinal items related to nutrition or the use of fluoride toothpastes, only two nutritional items were included in Factor 2, and two nutritional items and one item related to fluoride toothpastes were included in Factor 3. This could reflect parental beliefs that prevention of the tooth decay is mainly about toothbrushing and to a lesser extent about cariogenic nutrition control or the use of fluoride.

As is obvious from Tables 1-3, parental attitudes to caries prevention were generally correct, i.e. positively oriented. Nevertheless, comparison of their attitudes according to self-perceived SES and the education level of the parents showed some significant differences. For all three factors, the median values of the aggregated Likert scale increased with increasing SES and the differences in the factor scores among the groups were statistically highly significant. The situation was similar, i.e. the differences in attitudes to caries prevention were highly significant among the groups with different levels of maternal education. The father's education had no significant effect on toothbrushing behaviour, but significantly influenced external caries control (factor 2) and internal caries control (factor 3). This could have been influenced by the fact that most of the questionnaires (92%) were completed by the mothers.

Assumed shortcomings of the study

Based on the official data of the Czech statistical office, in 2011 there were 350,029 children aged 3-5 years in the Czech Republic (6). Given the 5 percent margin of error and 95 percent desired confidence level, a minimum of 384 subjects was needed. Even though the number of subjects and stratification of the study sites in this survey was determined in accordance with the Czech Institute of Health Information and Statistics, it was not possible to recruit a completely random sample, as the participation of the subjects in the study was voluntary, based on informed consent. Based on experience from previous studies, the parents with the most negative attitudes towards the prevention of dental caries, whose children have the highest levels of caries, tend to refuse to participate.

In general, questionnaire data are self-reported, which means that respondents could have the tendency to report what they assume is expected rather than what they really believe. In addition, questionnaire items could reflect the knowledge of the respondents rather than their real behaviour. However, the acceptable response rate and stratified national sample size used in this survey increase the validity of the findings. Moreover, the results of the survey confirm the literature findings that caries-preventive behaviour in families with children is positively related to the higher SES of the family and the education level of the parents, especially the education of the mother.

Conclusion

The positive finding of this study is that Czech parents in general report that they are aware of their own responsibility for the prevention of tooth decay in their children. However, this internal control becomes more pronounced with the rising self-perceived SES of the family. A less favourable finding is that in the prevention of dental caries parents concentrate on toothbrushing, and dietary measures and fluoride do not seem to be so important to them. Increasing self-perceived SES of the family and the education level of the mother have a significantly positive effect on caries-preventive attitudes. Based on the findings of this study, the message to the public-health sector in the Czech Republic should include the need to highlight the importance of non-cariogenic diet and the role of fluorides in caries-preventive education. School-based preventive programs would probably be the most appropriate way adequately to target risk groups with low SES and low education of the parents.

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References

1. Featherstone JD, Adair SM, Anderson MH, Berkowitz RJ, Bird WF, Crall JJ, et al. Caries management by risk assessment: consensus statement. J Calif Dent Assoc. 2003;31(3):257-69
2. Pine CM, Adair PM, Petersen PE, Douglass C, Burnside G, Nicoll AD, et al. Developing explanatory models of health inequalities in childhood dental caries. Community Dent Health. 2004;21:86-95.
3. Lenčová E, Pikhart H, Broukal Z. Early childhood caries trends and surveillance shortcomings in the Czech Republic. BMC Public Health. 2012;12:547.
4. Lenčová E, Pikhart H, Broukal Z, Tsakos G. Relationship between parental locus of control and caries experience in preschool children - cross-sectional survey. BMC Public Health. 2008;8:208.
5. World Helath Organization. Oral health surveys: Basic methods. 4th ed. Geneva: World Health Organization; 1997.
6. Czech statistical office. Population structure according to age in 2001 [cited 2013 May 22]. Available from: http:// http://www.czso.cz/cz/pram_dila/401912o.pdf.

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Current possibilities in occlusal caries management

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Dental caries is a multifactorial disease that affects most populations throughout the world and it is still the primary cause of oral pain and tooth loss. The occlusal surfaces of posterior teeth are the most vulnerable sites for dental caries due to their anatomy. Therefore, the aim of the following article is to summarize current knowledge on occlusal caries development and the possibilities of its prevention. Although the overall caries rate today has fallen for populations in industrialized countries, the rate of occlusal surface caries has not decreased. This may be explained with fact that topically applied fluorides and their mode of action prevent caries better on smooth than on occlusal surfaces. As we know, tooth decay of first permanent molars causes a great deal of different short and long term difficulties for patients. Therefore, there is a continuous need for implementation of programs for caries prevention in permanent teeth. Nowadays, we like to treat our patients by minimally invasive methods. A very important step in our effective preventive treatment is sealing pits and fissures as a cornerstone of occlusal caries management. Reliable assessment of caries activity is also very important for defining treatment needs and plans. A very important decision, which should be made during occlusal caries management, is the selection of restorative material according to the treatment plan. **Conclusion.** Current possibilities in occlusal caries prevention and management are very effective. Therefore, dentists today do not have any excuse for avoiding the philosophy of Minimally Invasive Dentistry, especially when we talk about caries management of occlusal surfaces in permanent molars.

Key words: Occlusal caries, Prevention, Pit and fissure sealing.

Introduction

Dental caries is a multifactorial disease that affects most populations throughout the world and it is still the primary cause of oral pain and tooth loss (1). From different epidemiological studies we know that 60-90% of the total population suffers from dental caries and problems with caries start in very young patients with primary teeth (2, 3).

On the other hand, over past decades the common consensus from many reports worldwide was that dental caries had declined significantly and was continuing to decline in the population. The dental community has prided itself on its efforts that have reduced dental caries, including use of systemic and topical fluorides, toothpastes, sealants, improvements in diet, oral health education and dental care (4, 5). There are, however, some recent studies that report alarming increases in caries incidence. These increases are in children and adults, primary and permanent teeth, and include coronal and root surfaces (6-8). The emerging public health issues are related to disparities in the prevalence and treatment of dental caries. An important facet is the social impact of differences in dental caries for specific groups of individuals throughout the world. The increases in caries appear to occur in lower socioeconomic groups, new immigrants and children. While the causes of these increases in caries are unclear, it is possible that the benefits of prevention are not reaching these groups.

The dental hard tissues – enamel, dentin and cementum – form relevant solid surfaces which are coated by a pellicle, to which microbial cells attach (9). Saliva modifies the complex interplay between the teeth and the surrounding biofilm: the secretion, flow rate and composition of saliva are dynamic parameters that are controlled by the physiological and pathological conditions of the host (10). The bacteria in the biofilm are metabolically active and they cause fluctuations in the pH of saliva. These fluctuations induce the loss of mineral from the tooth, when the pH decreases, or gain of minerals when the pH increases. The localized destruction of the hard tissues, the caries lesion, is the sign or symptom of the disease (1). Occlusal surfaces of posterior teeth are the most vulnerable sites for dental caries due to their anatomy, favoring plaque retention and maturation (11). Although the overall caries rate has fallen for populations in industrialized countries, the rate of caries lesions in pits and fissures has not decreased at the same time (12). As we see, young permanent teeth are especially vulnerable, and early progression of occlusal caries lesions in first permanent molars causes a great deal of different short and long term difficulties for patients (13, 14). One of the main reasons for the less effective occlusal caries prevention today, in the so-called "post-fluoride" generation, is the fact that topically applied fluorides prevent caries better on smooth surfaces than in pits and fissures. That makes changes in current epidemiologic situation and it is recognized like increase of new primary lesions on occlusal surface (15). Therefore, there is a continuous need for implementation of effective programs to prevent caries in the pits and fissures of the permanent molars of schoolchildren.

Occlusal caries lesions

The term fissure caries was earlier used to describe the caries lesions found in pits and fissures. This definition was based on the assumption that the high incidence of caries lesions in molar pits and fissures was directly related to poor cleaning accessibility to these surfaces. Occlusal pits and fissures vary in shape but are generally narrow (about 0.1 mm wide), with invaginations or irregularities where bacteria and food are mechanically retained. Saliva cannot reach the base of the fissures and those areas cannot be properly cleaned mechanically. Toothbrush bristle is too large (diameter 0.2 mm) to penetrate most fissures. The thickness of enamel at the base of deep fissures is minimal and in many cases the fissures extend practically to the dentinal surface (16).

Present knowledge indicates that the narrow fissures in young permanent molars are not the focus for the caries initiation *per se*. Two factors have been considered of importance for plaque accumulation and caries initiation on occlusal surfaces: the stage of eruption or functional usage of teeth and surface specific anatomy (17, 18). A carious lesion initiates as a local process at the entrance, along to deep fissures as plaque accumulates within the slopes of the cusps on occlusal surfaces. These sites offer protection against physical wear, and favor the formation of micro-cavities that further improve local conditions for oral bacteria, whereas the deepest part of the fissure usually harbors non-vital bacteria or calculus (19). The growth and proliferation of bacteria accelerate demineralization and destruction of occlusal surfaces (1). These areas often become stagnated due to the demineralization/remineralization process and can be clinically identified. Therefore, for conventional pit and fissure caries, the use of the more accurate term “occlusal caries” is suggested (20).

Development of occlusal caries management

We can provide for prevention of occlusal caries on different levels and using different approaches. At the very beginning of occlusal caries prevention Hyatt suggested the prophylactic odontotomy (21). During this procedure small superficial occlusal cavities were made in pits and fissures and restored with amalgam. After that, Boedecker’s first suggestion was that for preventive reasons we can undertake prophylactic restoration with oxyphosphate cements on occlusal surface. Finally, for the same preventive reasons, he suggested “eradication” of occlusal enamel fissures. Using this technique, the slopes of the cusps were reduced in order to ensure adequate accessibility of the pit and fissure system during teeth brushing and successful plaque removal from the occlusal surface (22). When we look back, we realize that those technique had two main disadvantages. Both techniques in the end sacrifice sound enamel and their preventive effectiveness has never been proven by any clinical study. Nowadays, we like to treat our patients using minimally invasive methods or if possible, non-invasively. Therefore, those old techniques for prevention of occlusal caries have only historical value.

What is the scientific and professional background of the modern “Minimally Invasive Dentistry” (MID) philosophy? The goal of MID is to preserve healthy tooth structure as much as possible. It focuses on prevention, remineralization, and minimal restorative intervention. Using scientific advances, MID allows dentists to perform the least amount of dentistry needed while never removing more of the tooth structure than is required to restore teeth to their normal condition. The development of adhesive dentistry and scientific progress in understanding the nature of caries has enabled dentists to do more than simply remove and replace diseased tissue. The new paradigm of MID can be emphasized of following the concept presented by Tyas and coworkers (23): early caries diagnosis; the classification of caries depth and progression; the assessment of individual caries risk; the reduction of cariogenic bacteria; the arresting of active lesions; the remineralization and monitoring of non-cavitated arrested lesions; the placement of restorations in teeth with cavitated lesions (minimal cavity design); the repair rather than the replacement of defective restorations; assessing disease management outcomes at pre-established intervals.

Current approach in occlusal caries prevention

Sealing of sound occlusal enamel

A non-invasive approach is always imperative when we talk about prevention in dentistry. Therefore, professional teeth cleaning with fluoridated paste and application of fluoride varnish on the occlusal surface or sealing of the pits and fissures system are techniques which should be recommended for everyday practice. When we talk about sealing of sound pits and fissures, we can actually distinguish two different levels of intervention and we can recognize preventive and therapeutic sealant application.

When we do preventive sealant application we treat completely sound occlusal enamel without any clinical signs of demineralization. The therapeutically oriented approach of sealant application means that we have the clinically visible first signs of enamel demineralization ("white spots") and that without any invasive intervention we seal that enamel (24). One of the main problems is that pit and fissure sealing must be applied very early, immediately after tooth eruption, when the cooperative ability of children could be very low. In this case, some anatomical factors and lack of proper isolation may reduce the retention of sealants and that decreases efficacy of occlusal caries prevention.

Enamel/dentin lesions sealing

In recent years we have increased therapeutic sealant application over incipient caries to prevent progression of the lesion (25, 26). In this case, the most important fact is how many viable bacteria are left behind that can induce further progression of caries lesion. The second important fact is the adequate adherence of sealing material. Theoretically, good adhesion of sealing material cuts off any possible influence from the oral cavity, the metabolic activity of cariogenic microorganisms is reduced and caries progress stops. Research shows that the application of sealants on fissures with questionably cavitated lesion ("sticky fissures on probing") resulted in the reduction of viable bacteria, compared with unsealed fissures (27). Use of acid etching as a pretreatment also killed between 75-95% of all bacteria in the pits and fissures (28, 29). Analyzing those data, and according to the modern philosophy of MID, it is very obvious that cutting away enamel caries lesions is not necessary.

Sealing of cavitated pits and fissure lesions in dentin is still a procedure which should be better investigated. A few reports show that arresting of dentin lesion is possible, but we believe that the clinical outcome of the treatment is still unpredictable (30). On the other hand, another study shows that sealed caries fissures showed significantly more microleakage and insufficient sealant penetration depth than sound fissures. Neither the use of an adhesive nor its intermediate curing influenced the microleakage score and the penetration ability of sealants (31). Therefore, we still wait for more *in vitro* and *in vivo* studies which would confirm the efficacy and reliability of dentin lesion sealing. In the meantime, when we want to deal with pit and fissure dentin lesions we should stick to procedures such as "preventive resin restorations" (PRR). This restorative approach for occlusal caries was introduced by Simonsen and Stallard and was indicated for small cavitated lesions of occlusal surfaces extending up to the dentin level (32, 33). In this method cavity preparation is minimal and limited to the removal of the local caries lesion. The cavity is then restored with composite resin or glass ionomer cement, before the sealant is applied over the edges of the filled cavity, also covering the remaining pits and fissures.

Simonsen classified preventive resin restorations into 3 categories: type 1 does not penetrate the enamel. Type 2 involves a restorative procedure in the enamel and replacement of lost tooth structure with sealing of the adjacent unprepared pits and fissures accomplished using a flowable resin composite. Type 3 is suggested if the lesion has emerged up to the dentin level. Two materials are used – one to restore and one to prevent future caries attack (34). For the underlying filling material we can use composite resin (flowable or standard) or glass ionomer cement (35). The second material seals the complete fissure and it should be made with composite resin.

Diagnostics of occlusal caries and decision making

Caries diagnosis is a process which can be considered as a three-step procedure: detection of the lesion, assessment of the severity of the lesion and assessment of the lesion activity (36). Accurate and reliable assessment of caries activity is important for determining appropriate treatment needs and establishing an adequate treatment plan (37). From a clinical point of view, caries lesions can be classified as progressive (active) or non-progressive (inactive). If the progression of the lesion is somehow limited, the terms "arrested" or "chronic" can be used. Assessment of the activity of lesions was introduced in a set of clinical caries diagnostic criteria (38). "Nyvad criteria" are based on the physical properties of the surface reflection and texture of early lesions, with chalky and rough lesions being active, and smooth, shiny surfaces being inactive or arrested. The color of the lesion can also be used to make the distinction between arrested and active, as the surface enamel of arrested lesions takes minerals from surrounding fluids, while active lesions retain their white appearance. Active non-cavitated lesions have a higher risk of progressing to a cavity than the same inactive lesions. According to this diagnostic criteria, decision making and establishing of treatment plan is easier and the clinical outcome more predictable. The second diagnostic criteria used very often are the International Caries Detection and Assessment System (ICDAS). This system was developed to create an integrated definition of dental caries and a uniform system to measure the caries process (39). Today we also have ICDAS II, the updated modification of the original criteria (40). The criteria for the ICDAS classification were and have features associated with the "Nyvad criteria" and we can say that the assessment of caries activity is today the cornerstone for decision-making in occlusal caries management.

Materials for pit and fissure sealing

A very important decision during occlusal caries management is the selection of restorative material for pit and fissure sealing. Many different *in vitro* and *in vivo* studies have been undertaking and analyzing those results, it may be concluded that resin-based sealants are still the gold standard and best choice for everyday clinical practice (41, 42). Other materials used for pit and fissure sealing are less effective than resin-based sealants (43). However, some other studies confirm that "alternative" materials, such as glass ionomer cements, can also be very effective for pit and fissure sealing in situations when we need to seal partially erupted molars and have difficulties in proper tooth isolation (44).

Conclusion

Current possibilities in occlusal caries management, as we mentioned in the text above, are great and very effective in preventive and therapeutic approaches, as many different studies confirm (45), especially when we stress the fact that the incidence of caries on occlusal surfaces has increased last two decades in comparison of caries on smooth surfaces. Therefore, dentists today do not have any good excuse for avoiding the philosophy of MID, especially when we talk about prevention and restorative intervention on occlusal surfaces of permanent premolars and molars. From fluorides to resin-based sealants and glass ionomer cements, we have a large number of different possibilities, techniques and materials, to prevent the loss of hard dental tissue. This way of thinking is easy to understand when we consider the fact that any cavitation is the irreversible loss of hard dental tissue which must be replaced with biological inferior material, as all dental restorative materials available today.

Conflict of interest: The author declares that he has no conflict of interest.

References

1. Fejerskov O, Kidd EAM, Nyvad B, Baelum V. Defining the disease: an introduction. In: Fejerskov

O, Kidd EAM, editors. Dental Caries The Disease and its Clinical Management. 2nd ed. Oxford: Blackwell Munksgaard Ltd; 2008. p. 3-6.
2. Yabao RN, Duante CA, Velandria FV, Lucas M, Kassu A, Nakamori M, et al. Prevalence of dental caries and sugar consumption among 6-12 year old schoolchildren in La Trinidad, Benquet, Philippines. Eur J Clin Nutr. 2005;59(12):1429-38.
3. Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. Am J Dent. 2009 Feb;22(1):3-8.
4. Lauris JR, da Silva Bastos R, de Magalhaes Bastos JR. Decline in dental caries among 12-year-old children in Brazil, 1980-2005. Int Dent J. 2012;62(6):308-14.
5. Lussi A, Hellwig E, Klimek J.Fluorides - mode of action and recommendations for use. Schweiz Monatsschr Zahnmed. 2012;122(11):1030-42.
6. Prakash P, Subramaniam P, Durgesh BH, Konde S. Prevalence of early childhood caries and associated risk factors in preschool children of urban Bangalore, India: A cross-sectional study. Eur J Dent. 2012;6(2):141-52.
7. Tubert-Jeannin S, Leger S, Manevy R. Addressing children's oral health inequalities: caries experience before and after the implementation of an oral health promotion program. Acta Odontol Scand. 2012;70(3):255-64.
8. Mamai-Homata E, Topitsoglou V, Oulis C, Margaritis V, Polychronopoulou A. Risk indicators of coronal and root caries in Greek middle aged adults and senior citizens. BMC Public Health. 2012;12:484.
9. Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res. 2004;83 Spec No C:C35-8.
10. Bardow A, Lagerlöf B, Nauntofte B, Tenovuo J. The role of saliva. In: Fejerskov O, Kidd EAM, editors. Dental Caries The Disease and its Clinical Management. 2nd ed. Oxford: Blackwell Munksgaard; 2008. p.189-207.
11. Ripa LW. Sealants revisited: an update of the effectiveness of pit-and-fissure sealants. Caries Res. 1993;27(Suppl 1):77-82.
12. Marthaler TM. Changes in dental caries 1953-2003. Caries Res. 2004;38:173-81.
13. Batchelor PA, Sheiham A. Grouping of tooth surfaces by susceptibility to caries: a study in 5-16 year-old children. BMC Oral Health. 2004;4:2.
14. Sheiham A, Sabbah W. Using universal patterns of caries for planning and evaluating dental caries. Caries Res. 2010;44:141-50.
15. McComb D, Tam LE. Diagnosis of occlusal caries: Part I. Conventional methods. J Can Dent Assoc. 2001;67(8):454-7.
16. Newbrun E. Cariology. 3rd ed. Chicago, Illinois: Quintessence Publishing Co, Inc; 1989.
17. Carvalho JC, Ekstrand KR, Thylstrup A. Dental plaque and caries on occlusal surfaces of first permanent molars in relation to stage of eruption. J Dent Res. 1989;68(5):773-9.
18. Carvalho JC, Thylstrup A, Ekstrand KR. Results after 3 years of non-operative occlusal caries treatment of erupting permanent first molars. Community Dent Oral Epidemiol. 1992;20(4):187-92.
19. Ekstrand KR, Bjørndal L. Structural analyses of plaque and caries in relation to the morphology of the groove-fossa system on erupting mandibular third molars. Caries Res. 1997;31(5):336-48.
20. Carvalho JC, Ekstrand KR, Thylstrup A. Results after 1 year of non-operative occlusal caries treatment of erupting permanent first molars. Community Dent Oral Epidemiol. 1991;19(1):23-8.
21. Hyatt TP. Prophylactic odontotomy. The cutting into the tooth for prevention of disease. Dental Cosmos. 1923;65:234-41.
22. Boedecker CF. Enamel fissure eradication. NY State Dent J. 1964;30:149-54.
23. Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ. Minimal intervention dentistry: a review. FDI Commission Project 1-97. Int Dent J. 2000;50(1):1-12
24. Courson F, Velly AM, Droz D, Lupi-Pégurier L, Muller-Bolla M. Clinical decision on pit and fissure sealing according to the occlusal morphology. A descriptive study. Eur J Paediatr Dent. 2011;12(1):43-9.
25. Welbury R, Raadal M, Lygidakis NA; European Academy of Paediatric Dentistry. EAPD guidelines for the use of pit and fissure sealants. Eur J Paediatr Dent. 2004;5(3):179-84.
26. Feigal RJ, Donly KJ. The use of pit and fissure sealants. Pediatr Dent. 2006;28(2):143-50.
27. Going RE, Loesche WJ, Grainger DA, Syed SA. The viability of microorganisms in carious lesions five years after covering with a fissure sealant. J Am Dent Assoc. 1978;97(3):455-62.
28. Jensen OE, Handelman SL. Effect of an autopolymerizing sealant on viability of microflora in occlusal dental caries. Scand J Dent Res. 1980;88(5):382-8.
29. Kramer PF, Zelante F, Simionato MR. The immediate and long-term effects of invasive and noninvasive pit and fissure sealing techniques on the microflora in occlusal fissures of human teeth. Pediatr Dent. 1993;15(2):108-12.

30. Mertz-Fairhurst EJ, Schuster GS, Fairhurst CW. Arresting caries by sealants: results of a clinical study. J Am Dent Assoc. 1986;112(2):194-7.
31. Hevinga MA, Opdam NJ, Frencken JE, Bronkhorst EM, Truin GJ. Can caries fissures be sealed as adequately as sound fissures? J Dent Res. 2008;87(5):495-8.
32. Simonsen RJ, Stallard RE. Sealant-restorations utilizing a diluted filled composite resin: one year results. Quintessence Int Dent Dig. 1977;8(6):77-84.
33. Ripa LW, Wolff MS. Preventive resin restorations: indications, technique, and success. Quintessence Int. 1992;23(5):307-15.
34. Simonsen RJ. Preventive resin restorations and sealants in light of current evidence. Dent Clin North Am. 2005;49(4):815-23.
35. Garcia-Godoy F. The preventive glass ionomer restoration. Quintessence Int. 1986;17(10):617-9.
36. Ekstrand KR, Ricketts DN, Kidd EA. Occlusal caries: pathology, diagnosis and logical management. Dent Update. 2001;28:380-7.
37. Ekstrand KR, Ricketts DN, Longbottom C, Pitts NB. Visual and tactile assessment of arrested initial enamel carious lesions: an in vivo pilot study. Caries Res. 2005;39(3):173-7.
38. Nyvad B, Machiulskiene V, Baelum V. Reliability of a new caries diagnostic system differentiating between active and inactive caries lesions. Caries Res. 1999;33(4):252-60.
39. Pitts NB, Stamm J. International Consensus Workshop on Caries Clinical Trials (ICW-CCT) Final Consensus Statements: Agreeing Where the Evidence Leads. J Dent Res. 2004;83:125-8.
40. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. Community Dent Oral Epidemiol. 2007;35(3):170-8.
41. Eliades A, Birpou E, Eliades T, Eliades G. Self-adhesive restoratives as pit and fissure sealants: A comparative laboratory study. Dent Mater. 2013 May 10. doi:pii: S0109-5641(13)00089-4. 10.1016/j.dental.2013.04.005. [Epub ahead of print]
42. Souza-Junior EJ, Borges BC, Montes MA, Alonso RC, Ambrosano GM, Sinhoreti MA. Influence of etching time and bonding strategies on the microshear bond strength of self- and light-cured pit-and-fissure sealants. Braz Dent J. 2012;23(5):477-83.
43. Kühnisch J, Mansmann U, Heinrich-Weltzien R, Hickel R. Longevity of materials for pit and fissure sealing--results from a meta-analysis. Dent Mater. 2012;28(3):298-303.
44. Antonson SA, Antonson DE, Brener S, Crutchfield J, Larumbe J, Michaud C, et al. Twenty-four month clinical evaluation of fissure sealants on partially erupted permanent first molars: glass ionomer versus resin-based sealant. J Am Dent Assoc. 2012;143(2):115-22.
45. Simonsen RJ, Neal RC. A review of the clinical application and performance of pit and fissure sealants. Aust Dent J. 2011;56:(1 Suppl):45-58.

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The effect of different enamel surface treatments on microleakage of fissure sealants

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Objective. The aim of this study was to evaluate the effects of different techniques of surface treatment on the microleakage of fissure sealants in permanent molar teeth in vitro. **Materials and methods.** 96 extracted impacted human third molars were randomly divided into 8 surface treatment groups (n=12/group) as 1. Er: YAG laser; (Fidelis II, Fotona, Ljubljana, Slovenia) (125 mj, 20Hz). 2. Er: YAG laser + 37% H_3PO_4 (15s); 3. ER: AG laser + 37% H_3PO_4+Prime&Bond NT; 4. Er: YAG laser + G Bond; 5. Er: YAG laser + Prime&Bond NT; 6. 37% H_3PO_4; 7. 37% H_3PO_4 + Prime &Bond NT; 8. G Bond. Sealant material (Clinpro, 3M ESPE, Seefeld, Germany), was applied into the fissures and light-cured for 20s with LED (Bluephase C5, Ivoclar-Vivadent, Schaan, Liechtenstein). Specimens were subjected to thermocycling (1000×, 5-55°C, dwell time: 15s) and immersed in 0.5% basic fuchsin solution for 24h at 37°C. The samples were sectioned and scored on a 3 point rating scale using a light microscope with a magnification of ×20. One-way analysis of variance was used to analyze data. Multiple comparisons were analyzed using Bonferroni test (p=0.05). **Results.** Er:YAG laser showed the highest microleakage scores whereas Er YAG laser + 37% H_3PO_4 showed the lowest. Although 37% H_3PO_4 group showed higher scores than Er:YAG laser + 37% H_3PO_4, the difference was not statistically significant. **Conclusion.** Etching fissures with phosphoric acid is sufficient prior to fissure sealant application

Key words: Fissure sealant, Laser, Preventive dentistry.

Introduction

Pit and fissure sealants constitute one of the preventive interventions in paediatric dentistry. Their effectiveness for caries management on occlusal surfaces has been documented in numerous clinical studies (1-3). However, their preventive benefits rely directly upon the ability of the sealing material to thoroughly fill pits and fissures so that it remains completely intact. Subsequently, caries development underneath the sealant restoration is avoided (4). However, undetectable caries in fissures and saliva contamination prior to sealant application are two main problems that can lead to poor bonding to enamel. An invasive approach of widening the fissures before sealing application helps the sealant retention (5). Yet, purposeful removal of enamel in a sound tooth may disturb the equilibrium of the fissure system and expose the patient to drilling (6, 7). The latter problem, saliva contamination, is frequently faced after the pretreatment of enamel with phosphoric acid to create microporosites for retention. This could occur at the very critical step, when the cotton rolls are changed after rinsing off the etchant. When the microporosites are coated with saliva, the retention and effectiveness of the fissure sealants are jeopardized (8). Several studies have shown the benefits of adding a bonding agent layer between the etched enamel and the sealant to increase the bond strength in the face of moisture and salivary contamination (9, 10).

Currently, various enamel treatment procedures are still under discussion for the optimization of fissure sealant penetration. In recent years there has been significant progress in the use of lasers in dentistry. Lasers can be a useful device for dental care in children, particularly for those with dental fear by eliminating stressors such as the sight and sensation of a drill (11). Laser etching does not require tooth isolation. The lasered enamel surface becomes fractured and uneven, which helps the adhesion of resin based materials. Furthermore, laser produces an acid resistant surface, which could avoid secondary caries formation (11-13). With all these beneficial features, this technique seems to be promising in overcoming the problems faced during fissure sealant application. To date, laser etching of fissures prior to fissure sealant application has been investigated in various studies (14-19). However, it is still questionable whether laser etching alone eliminates the need for acid etching of the enamel surface prior to placement of fissure sealants. There is scarce information about the use of laser pretreatment in combination with acid etching and bonding agents in dental literature. Microleakage under fissure sealants is only one of the several ways of assessing the success or failure.

Therefore, the aim of the present study was to assess the effectiveness of different enamel surface treatments on microleakage of fissure sealants applied to sound permanent molars in vitro. The hypothesis of the study was that lasered and/or bonded fissure sealants showed less microleakage than sealants prepared with the acid etching technique.

Materials and methods

Recently extracted sound impacted third molars were kept in 0.2% sodium azide solution at a temperature of 4°C prior to the study. The fissures were cleaned with a pumice, using a soft brush and air-water jet. Subsequently, fissures were examined at 20× under a dissecting microscope to exclude ones with cracks, structural defects or incipient caries lesion. A total of 96 teeth were assigned randomly to eight groups (n=12/each) (Table 1), in which different pretreatments were carried out according to the manufacture's instructions (Table 2).

In the laser groups, occlusal fissures were irradiated with an Er: YAG laser (Fidelis II, Fotona, Ljubljana, Slovenia). Before operation the power output was set at 2.5 W. The pulse energy was set at 125 mj and the repetition rate was 20 Hz. The laser beam was delivered in non-contact mode with the hand piece positioned perpendicularly to the fissures.

After each of the pretreatments, the sealant (Clinpro, 3M ESPE, Seefeld, Ger-

Table1 Different pretreatment of fissures prior to sealant application

Group	Surface Pretreatment
1	Er: YAG laser irridation
2	Er: YAG laser irridation + 37% orthophoshoric acid etching
3	Er: YAG laser irridation + 37% orthophoshoric acid etching + Prime &Bond NT
4	Er: YAG laser irridation + G Bond
5	Er: YAG laser irridation + Prime &Bond NT
6	37% orthophoshoric acid etching
7	37% orthophoshoric acid etching + Prime &Bond NT
8	Self etching adhesive (G Bond)

Table 2 Composition and application procedures of etchants and adhesive materials prior to sealing

Product	Composition	Application
Scotchbond etching gel (3MESPE; St Paul, MN, USA)	37% phosphoric acid gel	Apply and leave for 30 s, rinse 15 s, air dry for 10 s.
Prime & Bond NT (Dentsply De Trey; Konstanz, Germany)	Di-and trimethacrylate resins, PENTA, nanofillers, amorphous silicone dioxide, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone	Apply and leave for 20 s, gently air dry, light cure for 20 s.
G Bond	Acetone, distilled water, 4-methacryloxyethyltrimellitate anhydride, urethane dimethacrylate, triethyleneglycol dimethacrylate	Apply and leave for 10 s, dry thoroughly under air pressure for 5 s and light cure for 10 s.

many) was applied into the fissures with a tip syringe and spread with a dental probe to prevent air entrapment. The sealant was light-cured for 20 s using LED (Bluephase C5, Ivoclar Vivadent) with an output of 500 mw/cm^2. All teeth were treated by the same operator. After light curing, the specimens were subjected to thermocycling. The teeth were subjected to a thermocycling regimen of 1000 cycles between 5°C and 55°C water baths. Dwell time was 15 seconds with a 10 second transfer time between baths. Thereafter, microleakage was assessed by the conventional dye-penetration method. The apices of the teeth were covered with composite to avoid dye penetration, and after that, the whole tooth surface, apart from the area within 2 mm of the sealant varnish interface. Specimens were then immersed in 0.5% basic fuchsine solution (Wako Pure Chemical Industry; Osaka, Japan) for 24 h at 37°C. Afterwards the specimens were rinsed thoroughly with water and had their roots cut out using a diamond bur. The sealed crowns were embedded in self-curing acrylic resin and sectioned along the bucco-lingual direction through the mesial, central and distal fissures, resulting in four tooth fragments with six section sides available for inspection. Microleakage was scored by two blinded independent observers, using a light microscope with magnification of × 20 (Leicamicrosystems stereo microscope, Ltd. Stereo and microscope systems; Heerburg, Switzerland). Microleakage per section side was scored on a 3 point rating scale. Score 0 indicated no microleakage visible, score 1 revealed microleakage in up to half of the fissure, while score 2 meant microleakage reaching more than half of the fissure. In case of disagreement between the observers, a third independent observer was consulted to make the final decision. To determine the intra-examination reliability, 10 randomly selected molar fragments were re-evaluated for microleakage. The intra kappa value was found to be 1 by both of the examiners.

Statistical analysis

Statistical analysis was carried out by using Statistical Packages for Social Sciences (SPSS) 15.0 for Windows. One-way analysis of variance was used to compare the microleakage measures of different groups. Multiple comparisons between groups were analyzed using the Bonferroni test. Statistical analysis was conducted at a significance level of $p<0.05$.

Results

The mean microleakage scores and standard deviations of all groups are given in Table 3.

No tooth section was lost during the preparation of the specimens.

All the groups demonstrated microleakage regardless of the surface pretreatments. The lowest microleakage values were obtained in group 2 (laser etching in combi-

Table 3 Mean microleakage scores and standard deviations (SD) of different enamel pretreatment groups

Enamel pretreatment Groups (n=12 for each group)	Mean microleakage scores ± SD (12 teeth × 6 sides)
Laser (n=72)	1.30±1.24[b]
Laser + Acid etching (n=72)	0.23±0.59[a]
Laser + Acid Etching + Prime & Bond NT (n=72)	0.43±0.81[a]
Laser + G Bond (n=72)	0.27±0.61[a]
Laser + Prime & Bond NT (n=72)	1.12±1.25[b]
Acid etching (n=72)	0.45±0.78[a]
Acid + Prime&Bond NT (n=72)	0.80±1.09[b]
G Bond (n=72)	0.83±1.03[b]

[a,b]p <0,001

nation with acid etching). The highest microleakage scoring was observed in group 1 (laser etching). Based on the levels of statistical significance, the following ranking was achieved in terms of lowest to highest microleakage values: Laser + acid etching = laser + GBond = laser + acid etching + Prime&Bond NT = acid etching <acid etching + Prime&Bond NT = GBond = laser + Prime&Bond NT = laser ("<" denotes significantly lower value at $p<0.001$; and "=" denotes no significant difference at $p>0.05$).

Discussion

Microleakage in fissure sealants is often faced, unless the material is handled properly and saliva contamination is controlled (20). The latter problem derives from the sensitivity of the technique of placement of fissure sealants (21). The multi-step requirements such as drying, acid etching, rinsing and drying again increase the saliva contamination risk. Many pretreatment protocols have been developed to overcome this problem. One of them was the bonded sealant technique which supported the idea of using an adhesive system prior to sealant applications (22, 23). Both *in vitro* and *in vivo* studies substantiated the benefits of use of adhesive materials as an intermediate layer under the sealant materials (9, 10). With the introduction of self-etching adhesives, this technique has become even more attractive due to the elimination of the separate etching, rinsing and drying steps. Moreover, a reduction in chair time is another advantage for dentists, especially in treating uncooperative children (24).

Laser etching is another alternative to be used with fissure sealant application. It leads to the formation of more stable and less acid soluble compounds, thus reducing susceptibility to secondary caries (25, 26). In addition, enamel treatment with laser irradiation is claimed to facilitate the receptiveness to adhesive procedures (27, 28). However, in the dental literature there is scarce information about the combined use of laser etching and adhesive systems prior to the placement of resin based sealant material. Microleakage tests are useful methods to evaluate the sealing performance of adhesive systems (29). Dye penetration measurements are the most commonly used techniques. However, in the dental literature, microleakage studies are often quite incomparable due to the different study designs and dye materials used. Various particle sizes of dye materials can affect the dye penetration between the enamel and the resin material, leading to different microleakage results (17). Moshonov et al. (30) reported no microleakage in both laser etched and acid etched samples. They concluded that 1% methylene blue dye solution might have produced these results because the particle sizes were bigger than other solutions, such as 0.2% rhodamine, 50% silver nitrate or 0.5% basic fuchsine solutions. In the present study, 0.5% basic fuchsine solution was used to overcome the dye penetration problem. Three parallel cuts were made in the bucco-lingual direction through the mesial, central and distal fissures, resulting in four molar fragments with six section sides available for inspection, to increase the reliability of measurements. To date, comparative studies regarding laser etching versus acid etching have yielded conflicting results (30-33). Our results are in line with studies reporting that laser etching does not eliminate the necessity for additional acid etching (33, 34), in contrast to those reporting similar etching performance (30). Within the limitations of these in vitro conditions, the current study showed that laser etching alone performed the worst, whereas laser etching in combination with acid etching performed the best among all groups.

However, conventional phosphoric acid was found sufficient to etch the enamel surface to create the required retention of the resin based fissure sealant material.

Based on our results, lasered v. non-lasered fissures prior to application of a total etch adhesive system did not make a significant difference in terms of microleakage. Our finding was supported by Cehreli et al. (35). They reported that the use of Er, Cr: YSGG laser prior to bonded fissure sealant application did not improve microleakage resistance (35). However, laser etching in combination with self-etching adhesive provided less microleakage compared to self etching alone. This may be explained by previous studies, stating the lower bond strengths, greater microleakage and shallow etching patterns of self-etching adhesives (20, 22, 36, 37). It may be suggested that laser etching might promote stronger resistance to microleakage of self etching adhesives.

Conclusion

Within the limitations of the present study, etching fissures with phosphoric acid was sufficient prior to fissure sealant application. Further studies are required to test the effectiveness of laser pretreatments in preventing microleakage and secondary caries formation under resin based sealant material.

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References

1. Weintraub JA. The effectiveness of pit and fissure sealants. J Public Health Dent. 1989;49(5 Spec No):317-30.
2. Hicks MJ, Flaitz C. The acid etch technique in caries prevention: pit and fissure sealants and preventive restorations. In: Pinkham JR, editor. Pediatric Dentistry Infancy Through Adolescence, 3rd edition. Philadelphia: W. B. Saunders; 1999. p. 481-521.
3. Koch G, Poulsen S, Twetman S. Caries prevention in child dental care. In: Koch G, Poulsen S, editors. Pediatric Dentistry: A Clinical Approach. Copenhagen: Munksgaard; 2001. p. 119-45.
4. Simonsen RJ. Retention and effectiveness of a single application of white sealant after 10 years. J Am Dent Assoc. 1987;115(1):31-6.
5. Kramer N, Garcia-Godoy F, Lohbauer U, Schneider K, Assmann I, Frankenberger R. Preparation for invasive pit and fissure sealing: air-abrasion or bur? Am J Dent. 2008;21(6):383-7.
6. Clinical guideline on periodicity of examination, preventive dental services, anticipatory guidance, and oral treatment for children. Pediatr Dent. 2004;26(7 Suppl): 81-3.
7. Welbury, R., M. Raadal, and N.A. Lygidakis, EAPD guidelines for the use of pit and fissure sealants. Eur J Paediatr Dent. 2004;5(3):179-84.
8. Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surfaces: an SEM study. J Am Dent Assoc. 1985;110(3):329-32.
9. Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, Quelhas I, Hebling J. Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. J Dent Res. 2000;79(11):1850-6.
10. Hitt JC, Feigal RJ. Use of a bonding agent to reduce sealant sensitivity to moisture contamination: an in vitro study. Pediatr Dent. 1992;14(1):41-6.
11. Sungurtekin E, Oztas N. The effect of erbium, chromium:yttrium-scandium-gallium-garnet laser etching on marginal integrity of a resin-based fissure sealant in primary teeth. Lasers Med Sci. 2010;25(6):841-7.

12. Oho T, Morioka T. A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation. Caries Res. 1990;24(2):86-92.
13. Usumez S, Orhan M, Usumez A. Laser etching of enamel for direct bonding with an Er,Cr:YSGG hydrokinetic laser system. Am J Orthod Dentofacial Orthop. 2002;122(6):649-56.
14. Lepri TP, Souza-Gabriel AE, Atoui JA, Palma-Dibb RG, Pecora JD, Milori Corona SA. Shear bond strength of a sealant to contaminated-enamel surface: influence of erbium : yttrium-aluminum-garnet laser pretreatment. J Esthet Restor Dent. 2008;20(6):386-92; discussion 393-4.
15. Lupi-Pegurier L, Bertrand MF, Muller-Bolla M, Rocca JP, Bolla M. Comparative study of microleakage of a pit and fissure sealant placed after preparation by Er: YAG laser in permanent molars. J Dent Child (Chic). 2003;70(2):134-8.
16. Hossain M, Yamada Y, Masuda-Murakami Y, Nakamura Y. Removal of organic debris with Er:YAG laser irradiation and microleakage of fissures sealants in vitro. Lasers Med Sci. 2012;27(5):895-902.
17. Youssef MN, Youssef FA, Souza-Zaroni WC, Turbino ML, Vieira MM. Effect of enamel preparation method on in vitro marginal microleakage of a flowable composite used as pit and fissure sealant. Int J Paediatr Dent. 2006;16(5):342-7.
18. Baygin O, Korkmaz FM, Tuzuner T, Tanriver M. The effect of different enamel surface treatments on the microleakage of fissure sealants. Lasers Med Sci. 2012;27(1):153-60.
19. Sancakli HS, Erdemir U, Yildiz E. Effects of Er:YAG laser and air abrasion on the microleakage of a resin-based fissure sealant material. Photomed Laser Surg. 2011;29(7):485-92.
20. Celiberti P, Lussi A. Use of a self-etching adhesive on previously etched intact enamel and its effect on sealant microleakage and tag formation. J Dent. 2005;33(2):163-71.
21. Ganesh M, Shobha T. Comparative evaluation of the marginal sealing ability of Fuji VII and Concise as pit and fissure sealants. J Contemp Dent Pract. 2007;8(4):10-8.
22. Perry AO, Rueggeberg FA. The effect of acid primer or conventional acid etching on microleakage in a photoactivated sealant. Pediatr Dent. 2003;25(2):127-31.
23. Hannig M, Grafe A, Atalay S, Bott B. Microleakage and SEM evaluation of fissure sealants placed by use of self-etching priming agents. J Dent. 2004;32(1):75-81.
24. Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dent Mater. 2001;17(5):430-44.
25. Hicks MJ, Flaitz CM. Epidemiology of dental caries in the pediatric and adolescent population: a review of past and current trends. J Clin Pediatr Dent. 1993;18(1):43-9.
26. Tagomori S, Morioka T. Combined effects of laser and fluoride on acid resistance of human dental enamel. Caries Res. 1989;23(4):225-31.
27. De Munck J, Van Meerbeek B, Yudhira R, Lambrechts P, Vanherle G. Micro-tensile bond strength of two adhesives to Erbium:YAG-lased vs. bur-cut enamel and dentin. Eur J Oral Sci. 2002;110(4):322-9.
28. Eversole LR, Rizoiu I, Kimmel AI. Pulpal response to cavity preparation by an erbium, chromium:YSGG laser-powered hydrokinetic system. J Am Dent Assoc. 1997;128(8):1099-106.
29. Crim GA, Garcia-Godoy F. Microleakage: the effect of storage and cycling duration. J Prosthet Dent. 1987;57(5):574-6.
30. Moshonov J, Stabholz A, Zyskind D, Sharlin E, Peretz B. Acid-etched and erbium:yttrium aluminium garnet laser-treated enamel for fissure sealants: a comparison of microleakage. Int J Paediatr Dent. 2005;15(3):205-9.
31. Gutknecht N, Apel C, Schafer C, Lampert F. Microleakage of composite fillings in Er,Cr:YSGG laser-prepared class II cavities. Lasers Surg Med. 2001;28(4):371-4.
32. Hossain M, Nakamura Y, Yamada Y, Murakami Y, Matsumoto K. Microleakage of composite resin restoration in cavities prepared by Er,Cr:YSGG laser irradiation and etched bur cavities in primary teeth. J Clin Pediatr Dent. 2002;26(3):263-8.
33. Borsatto MC, Corona SA, Ramos RP, Liporaci JL, Pecora JD, Palma-Dibb RG. Microleakage at sealant/enamel interface of primary teeth: effect of Er:YAG laser ablation of pits and fissures. J Dent Child (Chic). 2004;71(2):143-7.
34. Lupi-Pegurier L, Bertrand MF, Genovese O, Rocca JP, Muller-Bolla M. Microleakage of resin-based sealants after Er:YAG laser conditioning. Lasers Med Sci. 2007;22(3):183-8.
35. Cehreli SB, Gungor HC, Karabulut E. Er,Cr:YSGG laser pretreatment of primary teeth for bonded fissure sealant application: a quantitative microleakage study. J Adhes Dent. 2006;8(6):381-6.
36. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005;84(2):118-32.
37. Kanemura N, Sano H, Tagami J. Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. J Dent. 1999;27(7):523-30.

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Demography and market impacts on dental practices' development in Bulgaria during the period of transition 1990-2010

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Objective. The purpose of this study was to present the impact of demography and market dynamics on the organizational and technological development of dental practices in Bulgaria during the period of socio-economic transition from 1990 to 2010. **Material and methods.** Relevant data were gathered from the official registers of the Bulgarian Dental Association, National Health Insurance Fund, National Health Information Institute and research studies, published during the period 1990-2010. They were analysed and are discussed in this paper. **Results.** During the period there was a decrease in population of Bulgaria, from 8,149,468 in 2000 to 7,563,000 in 2010 and a movement from rural areas to cities. There was an aging population with an increase in oral health care needs at a time of decrease in the size of the national labour force and economic growth. Paradoxically, over the last 20 years, the number of dentists in Bulgaria increased by more than 50% from 1997 to 2008. The average age of dentists changed from 39.7 years in 1997 to 41.7 years in 2008. The number of group dental practices increased and the number of individual practices and group practices for specialized dental care and dental centres decreased. The utilization of dental services is currently "about half" that of the mean for all European Union Member States, which is over 80% per year. **Conclusions.** The growing number of elderly people who have retained their teeth suggests an increase in treatment needs. However, the decline in their purchasing power limits the demand for oral health care services. In order to meet this and other challenges Bulgarian dentists have started changing their traditional solo-practice to group-practice.

Key words: Dental demography, Dental service, Utilization of dental services.

Introduction

During the last two decades, dentistry in Bulgaria has faced radical transformation (1). Oral health care evolved from 100% public funding to almost 100% private funding. In the early 1990's dentists lost state control and gained the status of liberal practitioners. As a result, access to oral health care for the population as a whole was affected and the utilization of health services declined. New opportunities in organizing practices but challenges from the new social and economic environment and the demographic dynamics in the country arose (2).

Demographic data for Bulgaria for this period showed a steady trend of a decrease in the population, an aging population, and an uneven population distribution, with movement from rural areas to cities all over the country. In contrast to the decrease in the number of inhabitants, the number of dentists increased rapidly (3). On one hand, the oral health care market was stimulated, on the other hand, the demographic and economic changes depressed dentists' incomes and their potential to invest in new technology and innovative management (4).

This paper tries to open a discussion on a range of market and professional issues and to define long-term trends in oral health care in Bulgaria which have resulted from the radical social transformations, experienced by the population and the dentists.

The aim of this paper was to present the impact of the demographic changes and the commercialization of dental services on the organizational and technological development of dental practices during the period of socio-economic transition in Bulgaria. In particular, the following areas were investigated: 1. Population demography, 2. Dental demography, 3. Dental practices' development, and 4. Utilization of the oral health care services.

Material and methods

The study was based on an analysis of secondary data, which were accessed from the official registers of the NCHI[1] the NSI[2], Bg. D A[3] and research studies, published during the last 20 years.

The analysis of the literature comprised two periods: (1990-2000)[4] and (2000-2010)[5] and covered issues relevant to: health reforms (5-7), the professional status of dentists (8, 9), trends in dental demography (10, 11), the number of dentists and their mobility (12), other dental personnel (13), oral health care service (14), oral treatments' needs (15), utilization of oral health care services (16).

Results

Population demography

Based on the official sources, the population of Bulgaria decreased by 8.2% during the period 2000-2010 (from 8,149,468 in 2000 to 7,563,000 in 2010) (17).

The age and sex distribution of the population showed a slow but stable trend towards a decrease in the number of younger people and a considerable increase in the proportion of over 65 years olds, usually in rural areas. The number of women remained constant. In the younger age groups there was a slight increase in the male population from 49.5% to 50.1%. The larger proportion of elderly women is generally due to the higher life expectation of the female population in Bulgaria (Figure 1).

The population is unevenly distributed over the country, in a small number of major cities (18) such as the capital city of Sofia and some large cities of the Black Sea area (Varna and Bourgas). The population there was either relatively constant or growing. Over the years, the size of the urban population increased at the expense of the rural areas (Figure 2).

The aging of the population occurred at a time of decreasing numbers in the labour force. The percentage of the working population was significantly higher in cities, while the villages are more or less depopulated of people of working age. The number of the working population gradually decreased from 2000 to 2005, then slowly increased and has remained stable in recent years (about 65.8% by the end of the year 2010). This par-

[1] National Center for Health Information
[2] National Statistical Institute
[3] Bulgarian Dental Association
[4] The period between 1990 and 2000 is marked by the change of legislation relative to private practice protection (liberalization and privatization).
[5] The second period is characterized by the introduction of the third party payment (regulation).

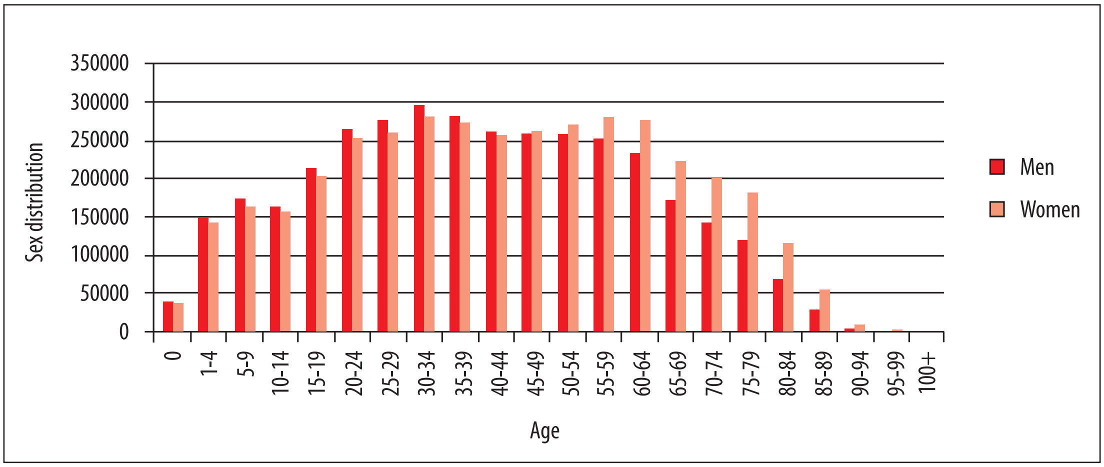

Figure 1 Population of Bulgaria by age and sex distribution (13).

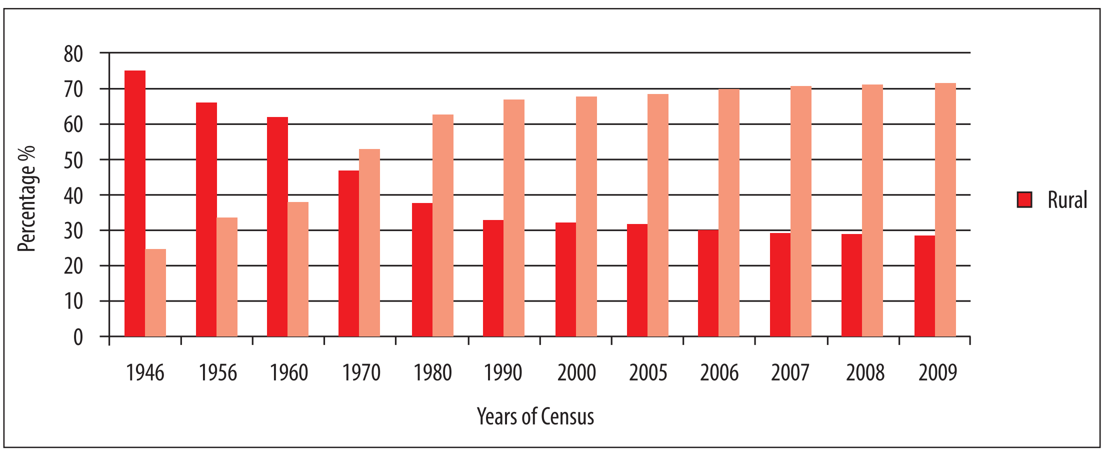

Figure 2 Dynamics of the urban population rate in Bulgaria (11, 17).

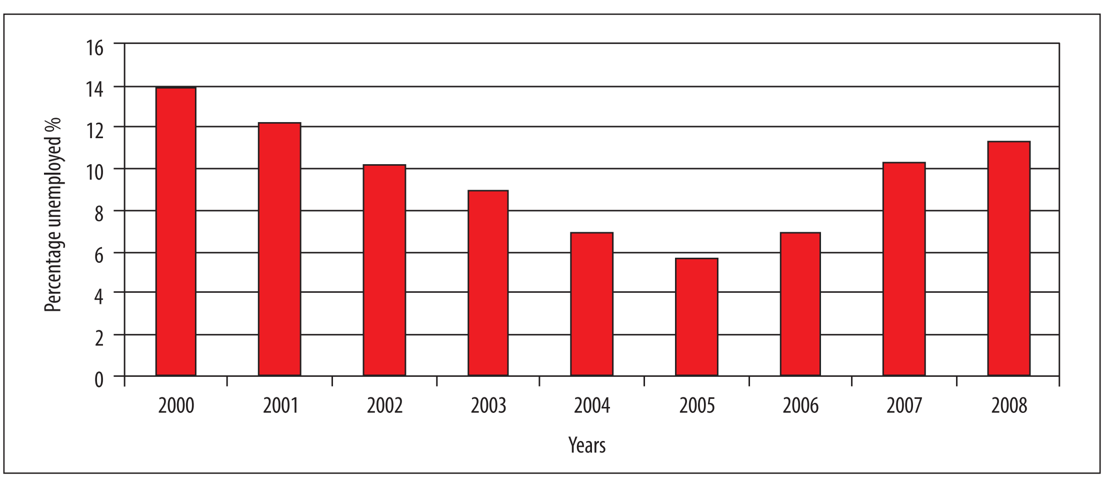

Figure 3 Unemployment rates of population aged between 15 and 64 (19).

adox is due to an increase in the retirement age from 55.0 years for women and 60.0 for men before 1990, to 62.5 for women and 65.0 for men in 2010. Underemployment and unemployment particularly affect young and poorly educated people (19). The number of long-term unemployed (two or more years) is increasing gradually (Figure 3) (19).

Dental Demography

The number of dentists in Bulgaria increased considerably during the studied period. After a sharp increase from 1980 to 2000 (from 4,839 to 6,778) the speed of increase slowed down in the period 2001-2010. The number of dentists started decreasing in 2011, due to the movement of dentists abroad.

By 2010 there were 8,400 registered dentists in Bulgaria (Figure 4), of whom about 66% were women, while in 1990 female dentists represented 75% of the total number of active dentists. A process of defeminization of the dental profession has been observed (20, 21).

Two opposing trends could be seen when observing the average age and male: female ratio for the country as a whole and for the capital city of Sofia (22).

Before the changes, the only opportunity for career development resulted from obtaining a position in the boards of district dental departments or regional dental clinics. Usually these positions were occupied by male dentists (20). Now the only regulator of the number of dentists is the market and no significant difference was found between male and female dentists in their preferences to practice in the capital city or in the countryside (Figure 5a).

The average age of dentists in the country increased from 39.7 years in 1997 to 41.7 years in 2008, while the average age of dentists in Sofia declined from 40.7 years in 1997 to 39.0 years in 2008. Sofia is the preferred place of work for newly graduate dentists (Figure 5b).

The distribution of dentists over the country was uneven. The areas of considerable concentration of population were and still are attractive to dentists. The number of dentists in urban centres grew as follows: in Sofia-city (from 1,017 in 2000 to 1,479 in 2009) in Varna (from 381 dentists in 2000 to 426 dentists in 2009), in Plovdiv (from 785 dentists in 2000 to 936 dentists in 2009), in Pernik (from 112 dentists in 2000 to 136 dentists in 2009). The concentration of dentists in particular parts of the country caused a considerable decrease in the dentists/patients ratio, (Figure 6) (23).

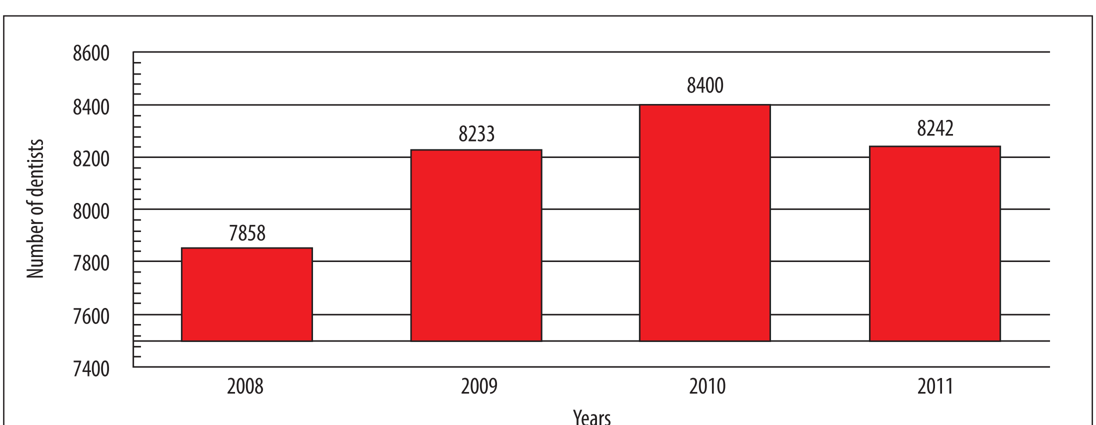

Figure 4 Number of dentists according Bulgarian Dental Association Registers (10).

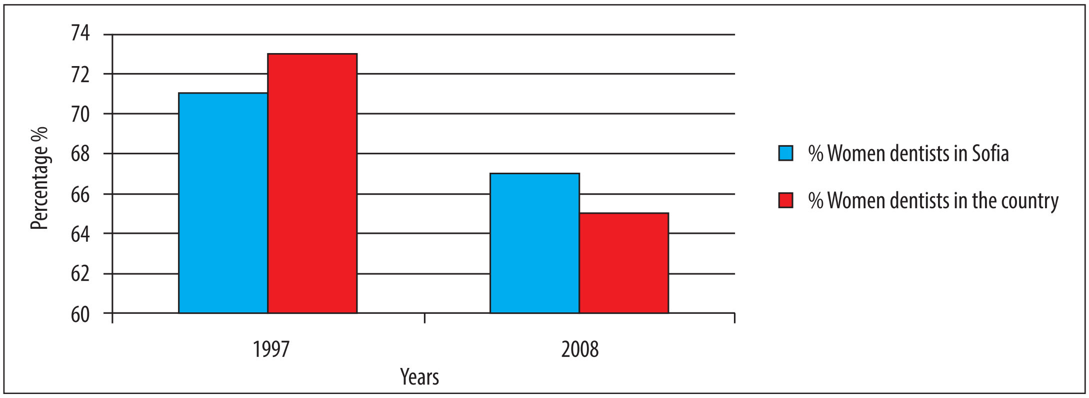

Figure 5a Trends of distribution of women dentists in Sofia and the country.

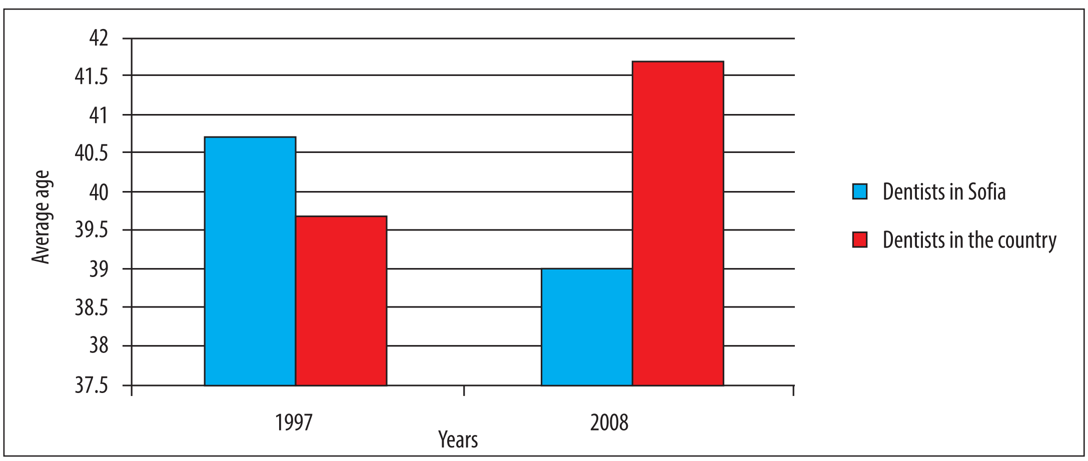

Figure 5b Trends of average age of dentists' dynamics in Sofia and the country.

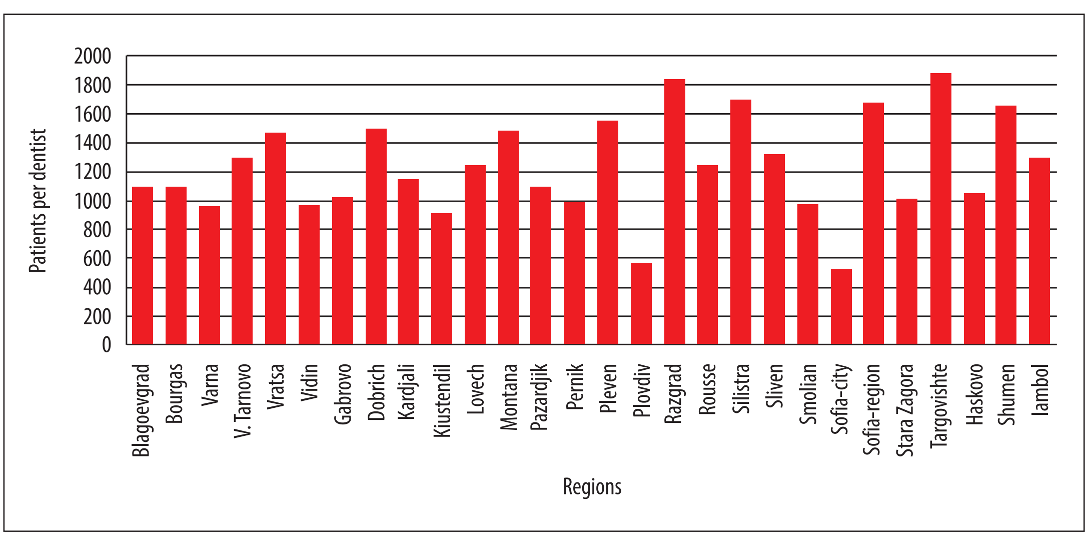

Figure 6 Number of patients per dentist in different regions of the country (13).

Dental practices' development

The change in the structure of dental practices was assessed by studying the following: status of ownership and type of practice, professional development, auxiliary staff development, improvement of equipment.

According to data for the period 1991-2001 the status of dentists was irreversibly transformed from employees in the health care system to freelance practitioners. In the next decade (2000-2010) private practitioners started contracting with the national Health Insurance Fund (24).

The Dental Health Service consists of individual and group practices for general and specialized dentistry and dental and medico-dental centres. A review of official data on dental practices showed that initially (2000-2007) the number of individual general dental practices increased, followed by a reduction in the number between 2007 and 2009. There was growth in the number of general dentistry group practices (2000-2009). The number of individual and group practices for specialized dental care was reduced during the same period. The number of dental centres declined, while the number of medico-dental centres remained relatively constant. The reduction in the number of dental centres (from 82 in 2000 to 49 in 2009) was in parallel with no reduction in the number of beds in medico-dental centres (11).

The percentage of general practitioners increased at the expense of the number of specialists. The number of specialists and dentists specializing declined. For the 20 year period only 371 dentists achieved the status of specialist. At the same time, three times as many specialists retired (13). Also, the system for continuous postgraduate education was under the guidance of the BgDA and private developed educational centres (22).

Only a small percentage of dentists worked with support (auxiliary) staff. The data showed that after an increase in the proportion of dental practices working with auxiliary staff during the period 1996-2000, a period of stagnation followed (2000- 2005) with auxiliary staff present in 40% of practices, followed by a sharp reduction in auxiliary staff utilization for the period 2005-2009 (Figure 7) (13, 22). The training of nurses is developing accordingly to the assistance utilization.

There was a gradual renewal of dental equipment at the expense of imported

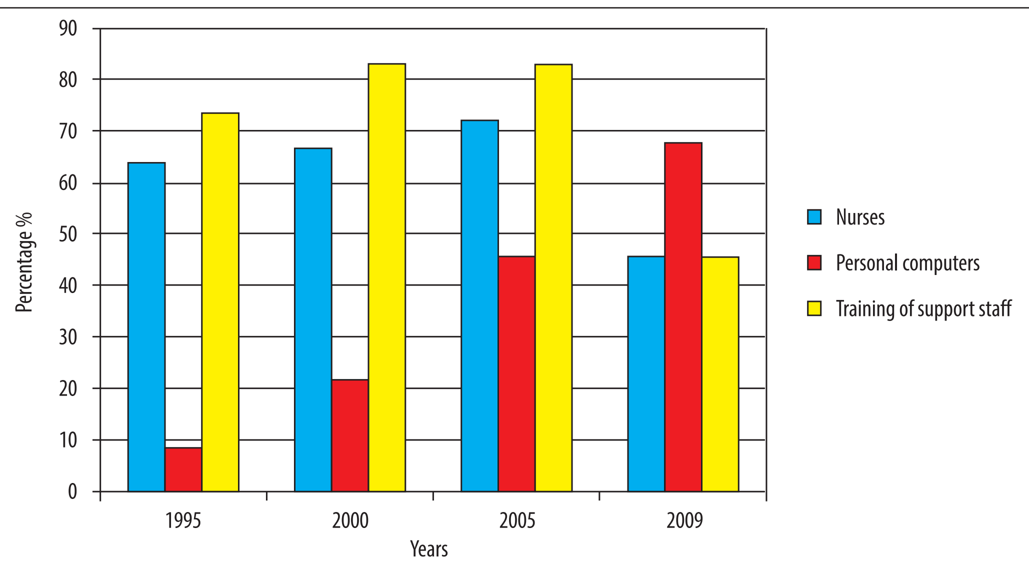

Figure 7 Dental auxiliary staff utilization in dental practices (13).

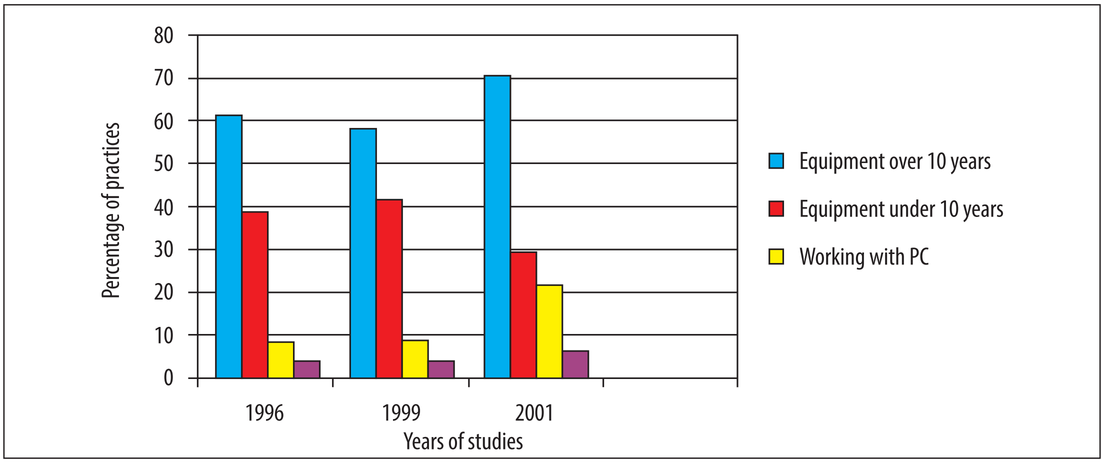

Figure 8 Technological development of dental practices (11).

equipment (from 16.6% for 1996, to 17.7% for 1999 and 24.0% for 2001). At the same time the tendency of using dental equipment over 10 years old grew (1996 - 61.2%, 1999 - 58.2%, 2001 - 70.6%). Slowly but steadily the percentage of practices providing dental implants grew (1996 - 4.1%, 1999 - 4.2%, 2001 - 6.4%). The greatest increase was in the number of practices using personal computers (Figure 8) (11)

Utilization of oral health care services

The percentage of the population aged less than 65 years decreased while the number of dentists markedly increased. The regions of Sofia-city, Plovdiv, Varna, have a constant number of dentists and an unfavourable dentist/patient ratio. The population: dentist ratio permanently decreased (Figure 9).

Utilization of dental services is about 50% of the mean for the North Western European Union (EU). According to BgDA only 45% of

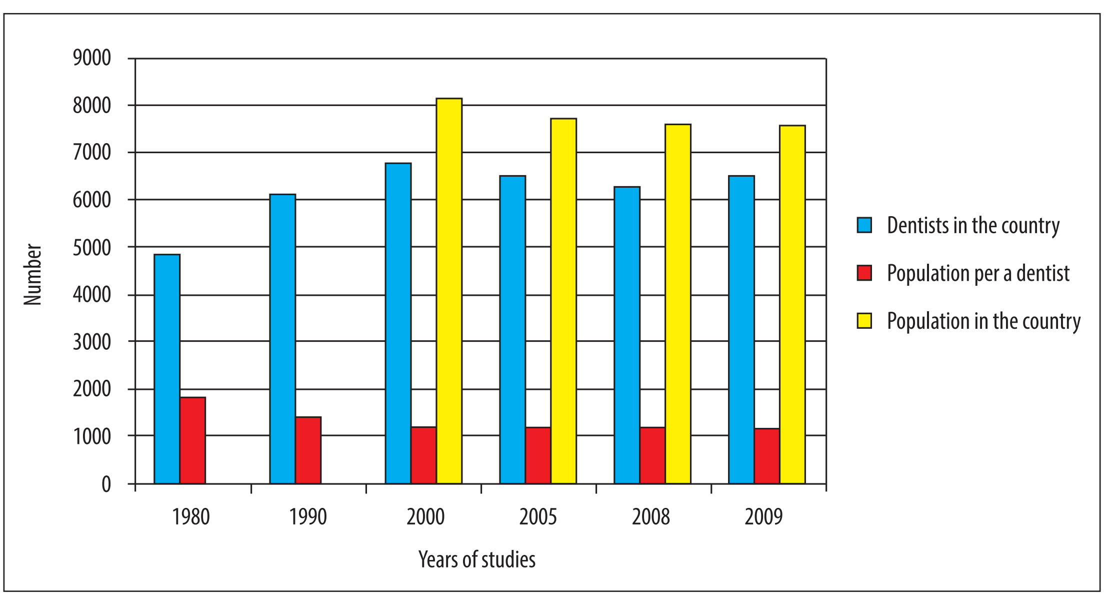

Figure 9 Impact of the demographic changes of the population on patient to dentist ratio for the period (1980-2009), (11, 27).

patients had visited a dentist during the previous year, while in some EU Member States the percentage varied from 83% to 71% (25).

Discussion

During the 1991-2001 period the Health Care System in Bulgaria changed from a monopolistic state-organized and state-regulated model to a pluralistic decentralized model, based on the coexistence of market and community-oriented programs, financed by comprehensive national insurance plans. The health reforms aimed at sustainable changes in regulations, financing and resource allocation, similar to the processes in the national economy, which passed through the stages of liberalization, privatization, and stabilization. The perception of the health system as a public resources consumer had to give way to the perception of an ongoing sector of the national and global economies (23). This general frame of reforms was just one factor in the real professional and technological development in dentistry. The lack of public resources and interest in dental health issues of the Government resulted in limitation of access to dental care and a potential decrease in the level of utilization of dental services. The unemployment rate for Bulgaria is relevant to the population's purchasing power, and in particular, its ability to pay for oral health care services and the impact on the rate of utilization of dental services (19, 25).

The reduction in the size of the population and its uneven distribution over the country was due to social factors rather than demographic conditions. The most attractive areas for the population are those with lower unemployment rates and better conditions for living, such as: Sofia city, Varna, Bourgas, Plovdiv, as well as destinations abroad. Internal and external migration contributed to significant depopulation of other regions, such as Vidin and Targovishte.

In more densely populated regions, characterized by a relatively highly developed economy, the number of oral health care practices is higher and more dentists are registered, while in less developed regions the number of practices and practitioners is lower (27). Overall, the number of dentists is increasing, but the demand for oral health care is not increasing automatically due to financial restraints on consumers. As a result, the migration of the population is followed by migration of dentists in the same direction - from small villages to large agglomerations and / or outside the country. De-feminization of the profession is a lasting trend (22).

Dental practices are characterized by a clear trend towards technological innovation, while retaining the gap between the technology and team development. A surplus of technological innovation, training and equipment, without taking account of the available specialists and their implementation, and ignoring the problems of dental teams, will create new challenges for dentists to develop their practices (13).

Conclusions

The increase in the proportion of Bulgarians over the age of 65 years implies an increase in treatment needs. The decline of purchasing power of the aging population limits the demand for oral health care services. In order to meet the market expectations for technological development, dentists have changed their traditional solo-practice orientation towards group-practice organization. Further research should be focused on oral health care service utilization and the dental practices' preparedness to respond to the real demand for oral health care.

Authors' contributions: Conception and design: LK, KT; Acquisition of data: KT, LK. Analysis and interpretation of data: LK, KT; Drafting the article: KT and LK; Revising it critically for important intellectual content: LK, KT.

Conflict of interest: The authors declare that they have no conflict of interest.

References

1. Widström E, Eaton KA, Borutta A, Dybizbánska E, Broukal Z. Oral Healthcare in Transition in Eastern Europe. Brit Dent J. 2001;190(11):580-4.
2. Koulaksazov S, Todorova S, Tragakes E, Hristova S. In: Tragakes E, editor. Health Care Systems in Transition: Bulgaria. Copenhagen: European Observatory on Health Care Systems; 2003.
3. Katrova L. Systems for the provision of oral health care in the black sea countries. Part 2: Bulgaria. Oral Health and Dental Management in the Black sea countries. 2009;8(2):3-7.
4. Katrova LG. Dental public health, dental profession, dental practice. 1st ed. Sofia: "WINI 1893"; 2011.
5. Katrova L, Katrova T. Politique Sociale en Europe de l'Est: le cas de la sante. In: Krasteva A, Todorov A, editors. La mondialization et les nouvelles limites du politique. Sofia: EOH-2000; 2004. p. 209-19.
6. Katrova L. Health reforms during the transition period and Bulgarian dental policy reform of 1991-2001 and their impact on dental education. SocMed (Sofia). 2009;17(1/2):20-3.
7. Katrova L, Bojinov P, Mihailova I. Oral health care reforms in Bulgaria during the period of transition. Oral health and Dental Management in the Black Sea Countries. 2007;6(4):3-8.
8. Katrova L, Tzokov K, Katrova T. The health reforms and the change of the socio-professional status of dentists in Bulgaria. Problems of Dentistry. 2003;29(5):46-50.
9. Katrova L. Ethical, legal, and professional foundations of the autonomous regulation of the dental profession, the case of Bulgaria. JofIMAB. 2010;16(4):70-6.
10. Bulgarian Dental Association. Registers 2011 [cited 2011 Sep 18]. Available from: http://www.bzs.bg/site/index.php.
11. Tzokov K, Katrova L. Demographic process and structure of dental practices as prerequisites for market development of the dental services in Bulgaria. Problems of Dental Medicine. 2011;37(2):61-5.
12. Katrova L. Generational mobility within dental profession, succession in career choice and perspectives for professional realization in Bulgaria. SocMed (Sofia). 2009;17(3):29-32.
13. Katrova L, Tzokov K. Current status of the dental practice in Bulgaria. Meta-analysis. J of IMAB. [DOI:105272/jimab.2012/182.181]. 2012;18(2):181-7.
14. WHO. The World Health Report 2000: Health Systems: Improving Performance. Geneva: WHO; 2000.
15. Katrova L, Papanchev G, Borissova T, Benkin N. Meeting the needs for caries and caries complication treatment by dentists working under contract with the national health insurance fund (under progress investigation). Problems of Dentistry. 2004;30(6):63-8.
16. Katrova L, Grashkina M. Utilization of the oral health service during the period of transition. J of IMAB. 2000;6(2):246-50.
17. National Statistical Institute. Census 2011 [cited 2012 Feb 28]. Available from: http://www.nsi.bg/census2011/index.php.
18. World Bank. World Development Report 1998-1999: Knowledge for Development. New York: Oxford University Press; 1998.
19. National Statistical Institute. Labour Market [cited 2012 Feb 20]. Available from: http://www.nsi.bg/otrasal.php?otr=26&a1=735&a2=741&a3=745#cont.
20. Katrova L. Gender impact on the socioprofessional identification of dentists in Bulgaria. J Dent Educ. 2004;68 (Suppl 7):S19-22.
21. Katrova L, Surcheva Zh. Career path dynamics of female dentists: actual status and trends. Med Pregled. 2004;40(4):84-9.
22. Katrova L, Tzokov K. Women dental practitioners – the organization of their dental practice particularity. SocMed (Sofia). 2012;20(2/3):59-61.
23. World Bank. Assessing Globalization: World Bank Briefing Papers. Washington, DC: PREM Economic Policy Group and Development Economics Group; 2000.
24. WHO. In: Anna Ritsatakis, Ruth Barnes, Evert Dekker, Patsy Harrington, Simo Kokko, Peter Makara. Exploring Health Policy Development in Europe. Copenhagen: World Health Organization: Regional Office for Europe; 2000.
25. Special Eurobarometer 330. Report Oral Health. 2010. Conducted by TNS Opinion & Social at the request of Directorate General Health and Consumers Survey co-ordinated by Directorate General Communication; 2010. p. 112 [cited 2012 Dec 16]. Available from: http://ec.europa.eu/public_opinion/archives/ebs/ebs_330_en.pdf.
26. Hobdell M, Sinkford J, Alexander C, Alexander D, Corbet E, Douglas C et al. 5.2. Ethics, equity and global responsibilities in oral health and disease. Eur J Dent Educ. 2002;6(Suppl 3):S167-78.
27. National Centre for Health Information. Brief Statistical Book: 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010 [cited 2012 Dec 26]. Available from: http://www.nchi.government.bg.

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International publications of authors from Bosnia and Herzegovina in Current Contents indexed publications in the first half of 2013*

Alečković-Halilović M, Mešić E, Sinanović O, Zukić S, Mustedanagić J. Carnitine palmitoyl transferase deficiency--unrecognized cause of recurrent acute kidney injury. Ren Fail. 2013;35(5):732-4. doi: 10.3109/0886022X.2013.780979. Epub 2013 Apr 8.

Nephrology, Dialysis and Kidney Transplantation Department, University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina.

Metabolic myopathies represent a small percentage of rhabdomyolysis causes that could lead to acute kidney injury (AKI). This could be prevented if this condition is suspected and timely treated. Carnitine palmityl transferase (CPT) deficiency is the most frequent metabolic myopathy and should be considered whenever recurrent myoglobinuria is suspected, and distinguished from the second frequent one, McArdle disease. We present a case of a patient with two medically misinterpreted episodes of AKI in whom the subsequent diagnosis of CPT deficiency was established based on high index of clinical suspicion and correlation of clinical manifestations to specific metabolic defects. Application of simple measures and lifestyle changes improved our patient's life quality and prevented potential new life-threatening complications.

Alić A, Prašović S, Hodžić A, Beširović H, Rešidbegović E, Omeragić J. Fatal verminous pharyngitis and esophagitis caused by Streptocara incognita in mute swans (Cygnus olor). Avian Dis. 2013 Mar;57(1):147-51.

Department of Pathology, Faculty of Veterinary Medicine, University of Sarajevo, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina.

Streptocara spp. infections are reported to cause gastritis, proventriculitis, esophagitis, and pharyngitis in various waterfowls, especially diving ducks. In the present paper, we describe severe fatal diphtheritic pharyngitis and esophagitis caused by Streptocara incognita in three female mute swans (Cygnus olor) in Bosnia and Herzegovina. Prior to death, the swans were showing signs of lethargy, anorexia, and reluctance to move. At necropsy, in all swans severe diphtheritic pharyngitis and esophagitis with deep, dark red hemorrhagic ulcerations were observed. Numerous thin, white, up to 1-cm-long nematodes, identified as S. incognita, were observed embedded in the pharyngeal and esophageal mucosa under the diphtheritic membranes. Histopathology revealed severe fibrinonecrotic inflammation with numerous cross-sections of the parasites. To the authors' knowledge, this is the first report of severe, fatal streptocariasis in mute swans.

Begić H, Tahirović H. The impact of delayed cardiac surgery on the postnatal growth of children with congenital heart disease in Bosnia and Herzegovina. Coll Antropol. 2013 Jun;37(2):507-13.

University of Tuzla, University Clinical Centre, Department of Paediatrics, Tuzla, Bosnia and Herzegovina.

*Data for this survey were collected from PubMed database using the keywords Bosnia and Herzegovina and 2013.

The aim of this study was to evaluate preoperative and postoperative growth in children with congenital heart disease (CHD) when cardio-surgical treatment is delayed. Growth data were analysed on 116 children with various types of CHD (cyanotic lesions (Group 1), left to right shunt (Group 2) and obstructive lesions (Group 3)), who underwent cardiac surgery after a certain period of waiting. Preoperatively, during the time (median 1.13 (0.55-2.39)) years of waiting for surgery, their mean weight z-score decreased from -1.38 (+/- 1.19) to -1.41 (+/- 1.28), and their mean height z-score from -0.65 (+/- 1.41) to -0.81 (+/- 1.36). Children in Group 1 developed a significant linear growth deficit, in Group 2 weight was more affected than height, while in Group 3 both growth parameters were gradually slowly, but not significantly reduced. Postoperatively weight and height z scores, although they showed a linear trend of improvement for all three groups, remained significantly reduced for two years after surgery. At the time of the last examination at the age 9.11 (5.66-13.10) years, the mean height z score -0.16 (+/- 1.28), was significantly reduced $p < 0.0001$, than predicted height 0.23 (+/- 0.82). Growth catch-up was related to age at surgery and preoperative growth deficit. Delayed cardiac surgery in children with CHD aggravated growth deficit and caused slow and incomplete postoperative growth catch-up.

Ćosić A, Ferhatović L, Banožić A, Kraljević S, Marić A, Sapunar D, Puljak L. Pain catastrophizing changes during the menstrual cycle. Psychol Health Med. 2013 Feb 26. [Epub ahead of print]

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Pain catastrophizing is an important predictor of pain intensity and pain-related outcomes. Many studies have shown that the level of this phenomenon is higher in women compared to men. The aim of this study was to investigate whether there is a difference in pain catastrophizing in women during the different phases of their menstrual cycle and whether there is a difference in pain catastrophizing depending on the history of childbirth and dysmenorrhea. A prospective study was conducted among 149 healthy women aged 18-35, with a regular menstrual cycle, 80 of which were nulliparous. The participants filled a sociodemographic questionnaire at the enrollment and the Pain Catastrophizing Scale on the 1st, 12th, and 20th day of the menstrual cycle. Pain catastrophizing scores, including all the subscales, significantly varied throughout the menstrual cycle, being highest on the first day of menstrual cycle and declining subsequently. Pain catastrophizing scores were higher in nulliparous than in parous women. Higher pain catastrophizing scores on the first day of the menstrual cycle were found in dysmehorrhoic women and women who regularly use analgesics for dysmenorrhea. Knowing that pain catastrophizing varies throughout the menstrual cycle may help in creating interventions for pain prevention and treatment in cycling women.

Duranović M, Šehić S. The speed of articulatory movements involved in speech production in children with dyslexia. J Learn Disabil. 2013 May-Jun;46(3):278-86. doi: 10.1177/0022219411419014. Epub 2011 Nov 4.

Faculty of Education and Rehabilitation, Tuzla University, Tuzla, Bosnia and Herzegovina.

A group of children with dyslexia (mean ages 9 and 14 years) was studied, together with group of children without dyslexia matched for age. Participants were monolingual native speakers of the Bosnian language with transparent orthography. In total, the diagnostic tests were performed with 41 children with dyslexia and 41 nondyslexic children. The participants were asked to produce monosyllables, /pa/, /ta/, and /ka/, and the trisyllable /pataka/, as fast as possible. Analysis was undertaken in four ways: (1) time of occlusion duration for plosives (duration of stop), (2) voice onset time for plosives, (3) diadochokinetic rate--articulators rate measured by pronunciation of monosyllables and the trisyllable, and (4) time of moving articulators from one gesture to another-time of interval length (from the explosion of one plosive to the start of the explosion of another plosive). The results suggest that children with dyslexia have significant problems with the speed of articulatory movements involved in speech production.

Gegić M, Numanović F, Delibegović Z, Tihić N, Nurkić M, Hukić M. The importance of serological tests implementation in disseminated candidiasis diagnose. Coll Antropol. 2013 Mar;37(1):157-63.

Tuzla University Clinic Centre, Clinic for Laboratory Diagnosis, Department of Microbiology, Tuzla, Bosnia and Herzegovina.

Candidiasis is defined as an infection or disease caused by a fungus of the genus Candida. Rate of disseminated candidiasis increases with the growth of the number of immunocompromised patients. In the the last few decades the incidence of disseminated candidiasis is in growth as well as the mortality rate. The aim of this survey is to show the importance of serological tests implementation in disseminated candidiasis diagnose. This is a prospective study involving 60 patients with malign diseases with and without clinical signs of disseminated candidiasis and 30 healthy people who represent the control group. Apart from hemoculture, detection of circulating mannan antigen and adequate antibodies of Candida species applying comercial ELISA test was determined in each patient. This survey deals with relevant factors causing disseminated candidiasis. This survey showed that the group of patients with clinical signs of disseminated candidiasis had more patients with positive hemoculture to Candida species, then the group of patients without clinical signs of disseminated candidiasis. The number of patients being examined and positive to antigens and antibodies was higher ($p < 0.01$) in the group of patients with clinical signs of disseminated candidiasis (7/30; 23.3%), then in the group of patients without clinical signs of disseminated candidiasis (0/30; 0%): Average value of titra antigen was statistically higher ($p < 0.001$) in patients with Candida spp. positive hemocultures rather then in patients with Candida spp. negative hemocultures. In the group of patients with clinical signs of disseminated candidiasis 6/30 (20%) of patients had Candida spp.positive hemocultures while in the group of patients without clinical signs of disseminated candidiasis 1/30 (3.3%) of patients had Candida spp. positive hemocultures, which was considerably higher ($p < 0.05$). Correlation of results of hemoculture and mannan antigens and antibodies in patients with disseminated candidiasis were statistically significant, while correlation of results of hemoculture and antibodies was insignificant. Because of low sensitivity of hemoculture and time needed for isolation of Candida spp., introducing serological tests in regular procedures would speed disseminated candidiasis diagnose.

Jegdić V, Rončević Ž, Škrabić V. Physical fitness in children with type 1 diabetes measured with six-minute walk test. Int J Endocrinol. 2013;2013:190454. doi: 10.1155/2013/190454. Epub 2013 Jun 27.

Department of Pediatrics, University Hospital, 88000 Mostar, Bosnia and Herzegovina.

Aim/Hypothesis. To examine whether children with DMT1 are less physically fit than healthy children and to assess whether an elevated level of HbA1c was associated with decreased physical fitness among children with diabetes. Methods. The study was conducted using case-control methodology. The cases were 100 children with T1DM, 7-17,9 years. Study subjects underwent a 6MWT, where distance measured, heart rate, and oxygen saturation was recorded. Results. Results of the 6MWT for children with T1DM and controls were 601.3 ± 86.1 meters versus 672.1 ± 60.6 meters, respectively ($P < 0.001$). The cases were divided into two subgroups, one with HbA1c levels >8% and one with HbA1c <8%. Results for both groups were inferior to the controls ($P < 0.001$). The posttest pulse rate in all subjects was higher than the pretest pulse rate ($P < 0.001$). Pulse oxygen levels were lower than controls at the pretest measurement ($P < 0.001$), and for both cases and controls, pulse oxygen levels decreased after test ($P = 0.004$). However, the change in oxygen saturation did not differ between the groups ($P = 0.332$). Conclusions. Children with T1D are less fit than matched controls. The level of HbA1c did not affect the physical fitness of children with T1D.

Kovačević L, Fatur-Cerić V, Hadžić N, Čakar J, Primorac D, Marjanović D. Haplotype data for 23 Y-chromosome markers in a reference sample from Bosnia and Herzegovina. Croat Med J. 2013 Jun;54(3):286-90.

University in Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina.

AIM: To detect polymorphisms of 23 Y-chromosomal short tandem repeat (STR) loci, including 6 new loci, in a reference database of male population of Bosnia and Herzegovina, as well as to assess the importance of increasing the number of Y-STR loci utilized in forensic DNA analysis. METHODS: The reference sample consisted of 100 healthy, unrelated men originating from Bosnia and Herzegovina. Sample collection using buccal swabs was performed in all geographical regions of Bosnia and Herzegovina in the period from 2010 to 2011. DNA samples were typed for 23 Y STR loci, including 6 new loci: DYS576, DYS481, DYS549, DYS533, DYS570, and DYS643, which are included in the new PowerPlex® Y 23 amplification kit. RESULTS: The absolute frequency of generated haplotypes was calculated and results showed that 98 samples had unique Y 23 haplotypes, and that only two samples shared the same haplotype. The most polymorphic locus was DYS418, with 14 detected alleles and the least polymorphic loci were DYS389I, DYS391, DYS437, and DYS393. CONCLUSION: This study showed that by increasing the number of highly polymorphic Y STR markers, to include those tested in our analysis, leads to a reduction of repeating haplotypes, which is very important in the application of forensic DNA analysis.

Kravić N, Pajević I, Hasanović M. Surviving genocide in Srebrenica during the early childhood and adolescent personality. Croat Med J. 2013 Feb;54(1):55-64.

Department of Psychiatry, Tuzla University Clinical Center, Trnovac bb, 75 000 Tuzla, Bosnia and Herzegovina.

AIM: To examine how the experience of genocide in Srebrenica in the early childhood (ages 1-5) influences the psychological health in adolescence. METHODS:

This study included 100 school-attending adolescents, age 15-16 (born in 1990-91) who were divided in two groups according to the place of residence from 1992-1995: the Srebrenica group - adolescents who lived in Srebrenica during the siege and the non-Srebrenica group who lived in the "free territory," were not wounded, and experienced no losses. We used the socio-demographic questionnaire created for the purposes of our study and the War Trauma Questionnaire, Posttraumatic Stress Reactions Questionnaire, Self-report Depressive Scale (Zung), Freiburg Personality Inventory, and the Lifestyle Questionnaire. RESULTS: Srebrenica adolescents experienced significantly more traumatic experiences (14.26 ± 3.11 vs 4.86 ± 3.16, P<0.001). Although there was no signifcant difference in the total score of posttraumatic stress reactions and intensity of depression between the two groups, significantly higher scores of posttraumatic stress reaction were noticed for several specific questions. The most prominent defense mechanisms in both groups were projection, intellectualization, and reactive formation. Srebrenica adolescents had higher sociability levels (34.7% vs 16.0%, $\chi(2)=7.231$, P=0.020). CONCLUSION: Srebrenica adolescents reported significantly more severe PTSD symptoms and significantly greater sociability. Our findings could be used for planning treatment and improving communication and overcoming traumas in war-affected areas.

Markota D, Markota I, Starčević B, Tomić M, Prskalo Z, Brizić I. Prevention of contrast-induced nephropathy with Na/K citrate. Eur Heart J. 2013 Aug;34(30):2362-7. doi: 10.1093/eurheartj/eht009. Epub 2013 Jan 24.

Department of Cardiology, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina.

AIMS: Contrast-induced nephropathy (CIN) is a frequent complication of many radiological procedures involving the application of contrast media. It represents a significant health problem that causes the increase in mortality, morbidity, and medical costs. For the prevention of CIN, a number of methods have been proposed to be effective. Among them, alkalinization of urine takes an important place. Although the Na/K citrate is a well-known agent for urine alkalinization, it has not been studied in the prevention of CIN. METHODS AND RESULTS: Two hundred and two patients who underwent coronary angiography were included in the study. They were randomized into groups receiving the drug Na/K citrate per os and to the control group. Serum creatinine and glomerular filtration rate were determined in all patients immediately before coronary angiography, and 48 h after the procedure. CIN criteria were a creatinine increase of >25%, reduction in the glomerular filtration rate by >25%, or an increase in serum creatinine of >44 μmol/L. The incidence of CIN in the group receiving Na/K citrate was significantly lower when compared with the control group (4% compared with 20%, P = 0.0001). Patients who had a urine pH <6 had a more than ten-fold higher incidence of contrast nephropathy compared with patients whose urine pH was >6. CONCLUSION: Alkalinization of urine using the Na/K citrate may reduce the incidence of CIN.

Markotić F, Černi Obrdalj E, Zalihić A, Pehar R, Hadžiosmanović Z, Pivić G, Durasović S, Grgić V, Banožić A, Sapunar D, Puljak L. Adherence to pharmacological treatment of chronic nonmalignant pain in individuals aged 65 and older. Pain Med. 2013 Feb;14(2):247-56. doi: 10.1111/pme.12035. Epub 2013 Jan 31.

Office for monitoring and quality improvement, Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina.

BACKGROUND: Medication nonadherence is a frequent problem in the treatment of chronic conditions. OBJECTIVE: To study the adherence to pharmacological treatment of chronic nonmalignant pain, as well as factors and patient attitudes related to nonadherence in patients aged ≥65 years. METHODS: The cross-sectional study was conducted with a self-administered questionnaire among 100 patients aged ≥65 years by five family physicians at the Health Care Centre Mostar, Bosnia and Herzegovina. RESULTS: According to their own statements, 57% of the patients were nonadherent, while 84% exhibited some form of nonadherence on the Morisky scale. The patients reported a mean pain intensity of 6.6 ± 2.2 on a visual analog scale. The most common deviation from the prescribed therapy was self-adjustment of the dose and medical regimen based on the severity of pain. Polymedication correlated positively with nonadherence. Nonsteroidal anti-inflammatory drugs were the most frequently prescribed medications. The majority of the participants (59%) believed that higher pain intensity indicates progression of the disease, and half of the participants believed that one can easily become addicted to pain medications. Nonadherence was associated with patient attitudes about addiction to analgesics and ability of analgesics to control pain. CONCLUSION.: High pain intensity and nonadherence found in this study suggest that physicians should monitor older patients with chronic nonmalignant pain more closely and pay more attention to patients' beliefs regarding analgesics to ensure better adherence to pharmacological therapy.

Martinović-Bevanda A, Radić N. Spectrophotometric sequential injection determination of D-penicillamine based on a

complexation reaction with nickel ion. Anal Sci. 2013;29(6):669-71.

Department of Chemistry, Faculty of Science and Education, University of Mostar, Bosnia and Herzegovina.

A simple and sensitive spectrophotometric method, based on reaction between Ni(II) ion and D-penicillamine (PEN), was developed. The proposed SIA system enhanced the analytical applicability of the reaction of complexation, and allowed the determination of PEN in the concentration range of 3.0 × 10(-6) - 2.0 × 10(-4) mol L(-1) with a sampling rate of 200 h(-1). With the proposed SIA system, PEN could be accurately determinated up to 0.9 nmol quantity. The method was successfully applied to the determination of PEN in laboratory samples and pharmaceuticals.

Mirjanić-Azarić B, Rizzo M, Šormaz Lj, Stojanović D, Uletilović S, Sodin-Semrl S, Lakota K, Artenjak A, Marc J, Černe D. Atorvastatin in stable angina patients lowers CCL2 and ICAM1 expression: Pleiotropic evidence from plasma mRNA analyses. Clin Biochem. 2013 Jun 20. pii: S0009-9120(13)00283-X. doi: 10.1016/j.clinbiochem.2013.06.006. [Epub ahead of print]

Clinical Centre Banja Luka, Department of laboratory diagnostics Banja Luka, Republic of Srpska, Bosnia and Herzegovina.

OBJECTIVE: Statin pleiotropy is still an evolving concept, and the lack of clarity on this subject is due at least in part to the lack of a definitive biomarker for statin pleiotropy. Using plasma mRNA analysis as a novel research tool for the non-invasive in vivo assessment of gene expression in vascular beds, we hypothesised that atorvastatin lowers the plasma mRNA level from statin pleiotropy-target genes, and the reduction is independent of the reduction of low-density lipoprotein cholesterol (LDL-C). DESIGN AND METHODS: Forty-four patients with stable angina received atorvastatin therapy (20mg/day, 10weeks). Plasma chemokine (C-C motif) ligand 2 (CCL2) and intercellular adhesion molecule-1 (ICAM1) mRNA levels and their protein concentrations (MCP-1, sICAM-1) were analysed before and after the treatment. Plasma vascular adhesion molecule-1 (sVCAM-1) concentrations were also analysed. RESULTS: Atorvastatin lowered plasma mRNA levels (CCL2: -31.76%, p=0.037; ICAM1: -34.09%, p<0.001) and MCP-1 protein concentration (-18.88%, p=0.008) but did not lower sICAM-1 and sVCAM-1 protein concentrations, and the decreases appeared to be independent from the lowering of LDL-C. The plasma mRNA levels correlated with their protein concentrations following statin treatment only. CONCLUSION: Our results significantly strengthen the clinical evidence in support of statin pleiotropy. Furthermore, this unique simultaneous measurement of plasma mRNAs and their protein concentrations offers an advanced non-invasive in vivo assessment of the circulation pathology.

Nefić H, Handžic I. The effect of age, sex, and lifestyle factors on micronucleus frequency in peripheral blood lymphocytes of the Bosnian population. Mutat Res. 2013 Apr 30;753(1):1-11. doi: 10.1016/j.mrgentox.2013.03.001. Epub 2013 Mar 7.

Department of Biology, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, Sarajevo 71000, Bosnia and Herzegovina.

This study confirmed that the frequency of human lymphocyte biomarkers measured with the cytokinesis-block micronucleus cytome (CBMNcyt) assay, is associated with age, sex, and lifestyle factors. Cytogenetic analysis was carried out on samples from 100 healthy individuals living in Bosnia and Herzegovina. Cells were cytologically scored for viability status, defined by the proportion of necrotic and apoptotic cells; mitotic status, corresponding to the proportion and ratios of mono-, bi- and multinucleated cells; the nuclear division index and chromosomal damage, determined by the presence of micronuclei, nucleoplasmic bridges or nuclear buds of bi-nucleated cells. Ageing is positively associated with the frequency of cytogenetic biomarkers. The micronucleus frequency in females was significantly higher than the micronucleus frequency in males. The micronucleus frequency is positively associated with family history of cancer. Furthermore, it is positively correlated with smoking: the frequency is higher in male subjects with a smoking habit than in female smokers. However, alcohol is observed to decrease the frequency of apoptotic cells in human lymphocytes. The frequency of micronuclei was positively correlated with exposure to medication. Lastly, the frequency of nuclear buds and apoptotic and necrotic cells negatively correlated with exposure to radiation.

Obrdalj EČ, Sesar K, Šantić Ž, Klarić M, Sesar I, Rumboldt M. Trauma symptoms in pupils involved in school bullying--a cross sectional study conducted in Mostar, Bosnia and Herzegovina. Coll Antropol. 2013 Mar;37(1):11-6.

University of Mostar, School of Medicine, Department of Family Medicine, Mostar, Bosnia and Herzegovina.

To determine the association between involvement in school bullying and trauma symptoms and to find whether children with presence of trauma symptoms participate in school bullying more as victims, as bullies or as bully/victims. The study included 1055, 6th to 8th grade (12-14 years of age) elementary school pupils from the western part of Mostar, The pupils were self-interviewed using a Questionnaire on School Violence developed in 2003 and validated in Croatia, and Trauma Symptoms Check List for Children (TSCC). The pupils involved in the school violence, either as victims, bullies, bully/victims had significantly more trauma symptoms than the not involved. Involvement in school bullying as a bully/ victim was a strong indicator of trauma symptoms, particularly anxiety, anger, posttraumatic stress, dissociation, obvious dissociation, and dissociation fantasy symptoms, while the victims of school violence had the highest odds ratio for the development of depressive symptoms. There is strong association between bullying and trauma symptoms in young adolescents. From our results, emphasis should be placed at the regularly screening on bullying in praxis of family physicians and regularly conduction of preventive measures and early intervention in every primary school.

Pašić F, Salkić NN. Predictive score for anastomotic leakage after elective colorectal cancer surgery: a decision making tool for choice of protective measures. Surg Endosc. 2013 May 25. [Epub ahead of print]

Department of Surgery, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

BACKGROUND: Anastomotic leakage is the most severe complication after colorectal surgery and a major cause of postoperative morbidity and mortality. We aimed to identify a predictive score for postoperative leakage after colorectal cancer surgery and to evaluate its usefulness in assessing various protective measures. METHODS: A total of 159 patients were divided into test (79 patients) and validation (40 patients) groups in order to identify the risk factors and construct the predictive score. The remaining 40 patients (intervention group) were prospectively evaluated with the application of protective measures guided by risk stratification according to the predictive score. RESULTS: A total of 23 of 159 (14.5 %) patients had anastomotic leakage, with 7 of 23 (30.4 %) of them needing reoperation. 11 of 159 (6.9 %) patients died, with 10 (6.3 %) deaths directly associated with anastomotic leakage. The rate of leakage in the test and validation groups (nonintervention group) was 22 of 119 (18.5 %), while the rate of leakage in the intervention group was 3 of 40 (7.5 %). The odds ratio for anastomotic leakage in the intervention group was 0.23 compared to the nonintervention group, with a relative risk reduction of 73 % for unfavorable event. The number needed to treat was 8 patients. There were also 10 of 119 (8.4 %) deaths in the nonintervention group compared to 1 of 40 (2.5 %) in the intervention group (Fisher's test; p = 0.18). CONCLUSIONS: Our simple predictive score may be a valuable decision making tool that can help surgeons reliably identify patients at high risk for postoperative anastomotic leakage and apply guided intraoperative protective measures.

Pojskić N, Silajdžić E, Kalamujić B, Kapur-Pojskić L, Lasić L, Tulić U, Hadžiselimović R. Polymorphic Alu insertions in human populations of Bosnia and Herzegovina. Ann Hum Biol. 2013 Mar;40(2):181-5. doi: 10.3109/03014460.2012.756063. Epub 2013 Jan 30.

Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8 Kampus, 71000 Sarajevo, Bosnia and Herzegovina.

BACKGROUND: From a demographic and genetic perspective, Bosnia and Herzegovina is interwoven with a number of differentially isolated local populations of indigenous people with different population and religious backgrounds. AIM: In order to estimate their genetic structure, this study investigated the frequencies of 10 Alu polymorphic loci in 10 regional populations distributed across Bosnia and Herzegovina. Genetic differentiation among the three major population groups in Bosnia and Herzegovina was estimated. SUBJECTS AND METHODS: DNA from 506 unrelated individuals was extracted from buccal swabs using the salting-out extraction method. Each DNA sample was PCR-amplified using locus-specific primers. RESULTS: Gene diversity values showed similarity in all analysed populations and ranged from 0.305-0.328. FST values for all loci showed that most variability is found within populations. Overall FST for all loci and AMOVA indicated that most variability was detected within populations. CONCLUSION: Results of this study are in agreement with the previous studies, indicating that the three populations inBosnia and Herzegovina have the same genetic background. There is no significant differentiation among regional populations, pointing to absence of geographic influence. The Bosnian population is clearly located within the European gene pool.

Salkić NN, Alibegović E, Jovanović P. Endoscopic appearance of duodenal mucosa in Whipple's disease. Gastrointest Endosc. 2013 May;77(5):822-3; discussion 823. doi: 10.1016/j.gie.2013.01.016. Epub 2013 Mar 8.

Department of Gastroenterology and Hepatology, University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina.

No abstract available.

Skenderi F, Krakonja F, Vranić S. Infarcted fibroadenoma of the breast: report of two new cases with review of the literature. Diagn Pathol. 2013 Feb 27;8:38. doi: 10.1186/1746-1596-8-38.

Department of Pathology, Clinical Center of the University of Sarajevo, Bolnička 25, Sarajevo, Bosnia and Herzegovina.

INTRODUCTION: Fibroadenomas are the most common benign breast tumors in young women. Infarction is rarely observed in fibroadenomas and when present, it is usually associated with pregnancy or lactation. Infarction can exceptionally occur as a complication of previous fine-needle aspiration biopsy or during lactation and pregnancy. MATERIALS AND METHODS: Retrospective review of 650 cases of fibroadenomas diagnosed at our institution during the 8-years period identified two cases of fibroadenomas with infarction (rate ~0.3%). RESULTS: Two partially infarcted fibroadenomas were diagnosed on core biopsy and frozen section in an adolescent girl (13 years old) and in a young woman (25 years old), respectively. No preceding fine-needle aspiration biopsy was performed in these cases, nor were the patients pregnant or lactating at the time of the diagnosis. CONCLUSION: Spontaneous infarction within fibroadenoma is a rare phenomenon in younger patients. The presence of necrosis on core biopsy or frozen section should be cautiously interpreted and is not a sign of malignancy.

Smajlović D, Salihović D, Ibrahimagić OC, Sinanović O. Characteristics of stroke in young adults in Tuzla Canton, Bosnia and Herzegovina. Coll Antropol. 2013 Jun;37(2):515-9.

University of Tuzla, Tuzla University Clinical Centre, Medical Faculty, Department of Neurology, Tuzla, Bosnia and Herzegovina.

The aim of the study was to analyze stroke in young adults in Tuzla Canton, Bosnia and Herzegovina. From January 2001 to December 2005, 3864 patients with first-ever stroke were admitted at the Department of Neurology Tuzla. A retrospective analysis of risk factors, stroke types, severity and one month outcome in all young adults (18-45 years of age) with first-ever stroke was carried out. Out of total, there were 154 (4%) young adults with stroke. Mean age was 38.8 +/- 5.7 years and 47% were women. The leading risk factors were smoking (56%) and hypertension (45%). Subarachnoid hemorrhage (SAH) was more frequent in young adults compared with older patients (> 45 years of age) (22% vs. 3.5%, p < 0.0001), intracerebral hemorrhage (ICH) was similar in both groups (16.9% vs. 15.8%, p = 0.7), but ischemic stroke (IS) was predominant stroke type in the older group (61% vs. 74%, p = 0.0004). Young adults had more frequent lacunar stroke (26.6% vs. 16.1%, p = 0.01) and stroke due to other etiology (8.5% vs. 1.8%, p = 0.0004) than stroke patients over 45 years of age. Stroke severity at admission was lower in young adults than in older patients (p < 0.0001), as well as mortality at one month (11% vs. 30%, p < 0.0001). Favorable outcome (modified Rankin Scale < or = 2) had 71% of young adults compared with only 53% of patients in the older group (p = 0.0003). Stroke in young adults in Tuzla Canton is rare. Risk factors profile, stroke types, severity and outcome at one month in young adults are different from those in older patients.

Subasi A. Classification of EMG signals using PSO optimized SVM for diagnosis of neuromuscular disorders. Comput Biol Med. 2013 Jun 1;43(5):576-86. doi: 10.1016/j.compbiomed.2013.01.020. Epub 2013 Feb 27.

International Burch University, Faculty of Engineering and Information Technologies, Francuske Revolucije bb. Ilidza, Sarajevo, 71000, Bosnia and Herzegovina.

Support vector machine (SVM) is an extensively used machine learning method with many biomedical signal classification applications. In this study, a novel PSO-SVM model has been proposed that hybridized the particle swarm optimization (PSO) and SVM to improve the EMG signal classification accuracy. This optimization mechanism involves kernel parameter setting in the SVM training procedure, which significantly influences the classification accuracy. The experiments were conducted on the basis of EMG signal to classify into normal, neurogenic or myopathic. In the proposed method the EMG signals were decomposed into the frequency sub-bands using discrete wavelet transform (DWT) and a set of statistical features were extracted from these sub-bands to represent the distribution of wavelet coefficients. The obtained results obviously validate the superiority of the SVM method compared to conventional machine learning methods, and suggest that further significant enhancements in terms of classification accuracy can be achieved by the proposed PSO-SVM classification system. The PSO-SVM yielded an overall accuracy of 97.41% on 1200 EMG signals selected from 27 subject records against 96.75%, 95.17% and 94.08% for the SVM, the k-NN and the RBF classifiers, respectively. PSO-SVM is developed as an efficient tool so that various SVMs can be used conveniently as the core of PSO-SVM for diagnosis of neuromuscular disorders.

Šerifović-Trbalić A, Demirović D, Cattin PC. Intensity-based hierarchical elastic registration using approximating splines. Int J Comput Assist Radiol Surg. 2013 Jun 23. [Epub ahead of print]

Faculty of Electrical Engineering, University of Tuzla, Tuzla, Bosnia and Herzegovina.

PURPOSE: We introduce a new hierarchical approach for elastic medical image registration using approximating splines. In order to obtain the dense deformation field, we employ Gaussian elastic body splines (GEBS) that incorporate anisotropic landmark errors and rotation information. Since the GEBS approach is based on a physical model in form of analytical solutions of the Navier equation, it can very well cope with the local as well as global deformations present in the images by varying the standard deviation of the Gaussian forces. METHODS: The proposed GEBS approximating model is integrated into the elastic hierarchical image registration framework, which decomposes a nonrigid registration problem into numerous local rigid transformations. The approximating GEBS registration scheme incorporates anisotropic landmark errors as well as rotation information. The anisotropic landmark localization uncertainties can be estimated directly from the image data, and in this case, they represent the minimal stochastic localization error, i.e., the Cramér-Rao bound. The rotation information of each landmark obtained from the hierarchical procedure is transposed in an additional angular landmark, doubling the number of landmarks in the GEBS model. RESULTS: The modified hierarchical registration using the approximating GEBS model is applied to register 161 image pairs from a digital mammogram database. The obtained results are very encouraging, and the proposed approach significantly improved all registrations comparing the mean-square error in relation to approximating TPS with the rotation information. On artificially deformed breast images, the newly proposed method performed better than the state-of-the-art registration algorithm introduced by Rueckert et al. (IEEE Trans Med Imaging 18:712-721, 1999). The average error per breast tissue pixel was less than 2.23 pixels compared to 2.46 pixels for Rueckert's method. CONCLUSION: The proposed hierarchical elastic image registration approach incorporates the GEBS approximation scheme extended with anisotropic landmark localization uncertainties as well as rotation information. Our experimental results show that the proposed scheme improved the registration result significantly.

Štrbac B, Jokić VS. Evaluation of set-up errors in head and neck radiotherapy using electronic portal imaging. Phys Med. 2013 Jan 2. pii: S1120-1797(12)00208-6. doi: 10.1016/j.ejmp.2012.12.001. [Epub ahead of print]

International Medical Centres, Centre for Radiotherapy, Dvanaest beba bb, 78000 Banja Luka, Bosnia and Herzegovina.

INTRODUCTION: The aim of this study was to evaluate three-dimensional (3D) set-up errors and propose optimum margins for planning target volume (PTV) coverage in head and neck radiotherapy. METHODS: Thirty-five patients were included in the study. The total number of portal images studied was 632. Population systematic (Σ) and random (σ) errors for the patients with head and neck cancer were evaluated based on the portal images in the caudocranial longitudinal (CC) and left-right lateral (LR) direction measured in the anterior-posterior (AP) field, as well as from the images in the caudocranial longitudinal (CC) and dorsoventral lateral (DV) direction measured in the lateral (LAT) field. The values for the clinical-to-planning target volume (CTV-PTV) margins were calculated using ICRU Report 62 recommendations, along with Stroom's and van Herk's formulae. RESULTS: The standard deviations of systematic set-up errors (Σ) ranged from 1.51 to 1.93 mm while the standard deviations of random set-up (σ) errors fell in between 1.77 and 1.86 mm. The mean 3D vector length of displacement was 2.66 mm. PTV margins calculated according to ICRU, Stroom's and van Herk's models were comprised between 1.95 and 6.16 mm in the three acquisition directions. DISCUSSION AND CONCLUSIONS: Based on our results we can conclude that a 6-mm extension of CTV to PTV margin, as the lower limit, is enough to ensure that 90% of the patients treated for head and neck cancer will receive a minimum cumulative CTV dose greater than or equal to 95% of the prescribed dose.

Tomić V, Petrović O, Crnčević Orlić Ž, Mandić V. Gestational diabetes and pregnancy outcome - do we have right diagnostic criteria? J Matern Fetal Neonatal Med. 2013 Jun;26(9):854-9. doi: 10.3109/14767058.2013.776530. Epub 2013 Apr 12.

Department of Gynecology and Obstetrics, Mostar Clinical Hospital, Kralja Tvrtka b.b., Mostar, Bosnia and Herzegovina.

OBJECTIVE: To determine thresholds of maternal glycemia at which specific adverse pregnancy outcomes occur in high-risk population. METHODS: A total of 1002 pregnant women with risk factors for gestational diabetes mellitus (GDM) underwent an originally modified glucose tolerance test (OGTT) with 75 g of glucose. Information on OGTT results and pregnancy outcomes were collected from database and medical records. RESULTS: Large for gestational age (LGA) newborn, infant's stay in the neonatal intensive care unit (NICU) >24 h, neonatal hyperbilirubinemia and cesarean section due to cephalopelvic disproportion were identified as specific GDM adverse outcomes. In the study group of participants with one or more specific GDM adverse outcomes, mean glycemic values

during the modified OGTT (4.2 ± 1.0 mmol/L at 0 min, 6.8 ± 1.7 mmol/L at 30 min, 7.9 ± 2.1 mmol/L at 60 min, 7.7 ± 2.3 mmol/L at 90 min and 7.5 ± 2.3 mmol/L at 120 min) according to Student's t-test for independent samples were significantly higher than mean glycemic values in the control group of participants without specific adverse outcomes ($p < 0.001$, $p = 0.02$, $p < 0.001$, $p < 0.001$, $p < 0.001$). CONCLUSION: This study provides additional data that support the acceptance of the newly recommended outcome-based GDM diagnostic criteria.

Vidic A, Ilić Z, Benedik L. Recent measurements of 234U/238U isotope ratio in spring waters from the Hadzici area. J Environ Radioact. 2013 Jun;120:6-13. doi: 10.1016/j.jenvrad.2013.01.005. Epub 2013 Feb 11.

Institute for Public Health FBIH, M. Tita 9, 71000 Sarajevo, Bosnia and Herzegovina.

The Hadzici area has become interesting for investigation since depleted uranium ammunition had been employed in 1995 during the NATO air strike campaign in Bosnia and Herzegovina. The purpose of this study is to determine uranium concentration and (234)U/(238)U activity ratio in the spring waters of this area and to investigate their relationship, as well as spatial variations. The spring water samples were taken at 18 sites in total. For the determination of uranium radioisotopes, radiochemical separation procedure followed by alpha-particle spectrometry was applied. Uranium concentration in analyzed waters range from 0.15 to 1.12 μg/L. Spring waters from carbonate based sediments have a lower uranium concentration of between 0.15 and 0.43 μg/L, in comparison to waters sampled within sandstone-based sediments ranging from 0.53 to 1.12 μg/L. Dissolved uranium shows significant spatial variability and correlation with bedrock type confirmed by Principal Component Analysis and Hierarchical Cluster Analysis. The majority of the analyzed waters have a (234)U/(238)U activity ratio ranging from 1.02 to 1.90, of which half of the results range between 1.02 and 1.16. No apparent depleted uranium (DU) contamination was observed, as (234)U/(238)U activity ratio is dependent on geochemical conditions in the environment. Even though the tested spring waters demonstrate significant variability in uranium concentration, (234)U/(238)U activity ratio and (234)U excess, waters with similar uranium isotopic signatures are observable within the region. The guidelines on the spatial redistribution of dissolved uranium (corresponding to (238)U mass concentration), along with (234)U/(238)U activity ratios were provided by the Inverse Distance Weighting (IDW) method. Waters having similar isotopic signature have been delineated.

Vranić S, Hes O, Grossmann P, Gatalica Z. Low frequency of HIF-1α overexpression in germ cell tumors of the testis. Appl Immunohistochem Mol Morphol. 2013 Mar;21(2):165-9. doi: 10.1097/PAI.0b013e31825e00b7.

Department of Pathology, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Cellular hypoxia is a hallmark of cancer. Hypoxia-inducible factor-1α (HIF-1α) and von Hippel-Lindau protein (pVHL) are the key mediators of cellular response to hypoxia. Little is known about their role in germ cell tumors of the testis. We therefore examined their status in a cohort of germ cell tumors of the testis. Thirty-six primary germ cell tumors of the testis (11 seminomas, 24 mixed germ cell tumors, and 1 case of pure intratubular germ cell neoplasia) were included in the study. HIF-1α and pVHL expression were studied using immunohistochemical (IHC) methods in the tumor and adjacent benign tissue. Selected cases with a low pVHL expression were further tested for genetic alterations using polymerase chain reaction. HIF-1α protein expression was not detectable in adjacent atrophic seminiferous tubules. In contrast, HIF-1α was expressed in one third of the malignancies, but in a low percentage of cells (mean, 3%; range, 0% to 20%). No difference in HIF-1α expression was observed between seminomas and non-seminomas ($P=0.71$). pVHL was expressed in atrophic tubular epithelium and in the Leydig cells, whereas a substantial loss of pVHL expression was observed in germ cell tumors regardless of the histologic type (mean, 45.6%; range, 0% to 100%). No genetic alterations of the VHL gene were observed in the cases with low pVHL expression. No significant correlation between HIF-1α and pVHL expression was observed ($P=0.16$). Germ cell tumors of the testis, regardless of the histologic type, are characterized by consistently low HIF-1α protein overexpression and a partial loss of pVHL without underlying VHL gene alterations. Further studies are necessary to clarify the functional importance of such alterations in testicular germ cell tumors.

Vranić S, Schmitt F, Sapino A, Costa JL, Reddy S, Castro M, Gatalica Z. Apocrine carcinoma of the breast: A comprehensive review. Histol Histopathol. 2013 Jun 17. [Epub ahead of print]

Department of Pathology, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina; and Department of Medical Sciences, University of Turin, Turin, Italy.

Apocrine carcinoma of the breast is a rare, special type of breast carcinoma showing distinct morphologic, immunohistochemical and molecular genetic features. Apocrine epithelium has a characteristic steroid receptor profile that is estrogen receptor and progesterone receptor negative and androgen receptor positive. This combination of morphologic and immunohistochemical characteristics is essential for the proper recognition of the apocrine carcinomas. Strictly defined, apocrine carcinomas express either Her-2/neu or EGFR, which along with androgen receptor positivity make patients with the apocrine carcinoma eligible for targeted therapies.

Zerem E, Omerović S. Comments on the article about the treatment of infected pancreatic necrosis. Surg Endosc. 2013 Jun 19. [Epub ahead of print]

Department of Gastroenterology, University Clinical Center Tuzla, Trnovac bb, 75000, Tuzla, Bosnia and Herzegovina.

No abstract available.

Zerem E, Omerović S, Imširović B. Is surgical cholecystectomy better than percutaneous in treatment of acute cholecystitis in patients unfit for surgery? J Gastrointest Surg. 2013 Aug;17(8):1542-3. doi: 10.1007/s11605-013-2205-6. Epub 2013 Apr 20.

Department of Gastroenterology, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

No abstract available.

by Nerma Tanović

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Acknowledge. Anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. List the source(s) of funding for the study and for the manuscript preparation in the acknowledgements section.

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Sample references

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Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

More than six authors:

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

Organization as author:

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension. 2002;40(5):679-86.

No author given:

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

Volume with supplement:
Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42(Suppl 2):S93-9.

Issue with supplement:
Glauser TA. Integrating clinical trial data into clinical practice. Neurology. 2002;58(12 Suppl 7):S6-12.

Issue with no volume:
Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop. 2002;(401):230-8.

Letters or abstracts:
Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. Eur Respir J. 2002;20(1):242.;
Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. Drug Alcohol Depend. 2002;66 Suppl 1:S105.

Article republished with corrections:
Mansharamani M, Chilton BS. The reproductive importance of P-type ATPases. Mol Cell Endocrinol. 2002;188(1-2):22-5. Corrected and republished from: Mol Cell Endocrinol. 2001;183(1-2):123-6.

Article with published erratum:
Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: a critical review. Clin Ther. 2000;22(10):1151-68; discussion 1149-50. Erratum in: Clin Ther. 2001;23(2):309.

Article published electronically ahead of the print version:
Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sacderived precursor cells. Blood. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Books and other monographs

Personal author(s):
Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:
Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Organization(s) as author:
Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. Compendium of nursing research and practice development, 1999-2000. Adelaide (Australia): Adelaide University; 2001.

Chapter in a book:
Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Conference paper:
Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Dissertation:
Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Other published material

Newspaper article:
Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:
Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

Electronic material

CD-ROM:

Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Audiovisual material:

Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

Journal article on the Internet:

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm.

Monograph on the Internet:

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap. edu/books/0309074029/html/.

Homepage/Web site:

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of a homepage/Web site:

American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html.

Database on the Internet:

Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000 – [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.as.

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