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THE INFLUENCE OF CO-FACTORS AND HPV INFECTION ON THE APPEARANCE OF CERVICAL LESION

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Abstract

The cervical cancer (CC) is in the second position in the world, by incidence and mortality, and in the first place in the developing countries. According to the information from literature different risk factors are more or less significant for the development of CC. One of the most important factor for the development of premalignant and malignant cervical lesion is infection with human papillomaviru (HPV). The strongest epidemic corelation was found in connection to the age at the time of first sexual intercourse, especially prior to the age of 18, and 21. The objective of this study is to determine the influence of etiological factors (smoking, age at the time of first sexual intercourse, number of intercourses, number of partners and status of circumcision) on the occurrence of pre-malignant and malignant cervical lesions among patients with persistent HPV infection.
Key words: *cervical lesion, HPV infection, cofactors*

The cervical cancer (CC), is in the second position in the world, by incidence and mortality (Anonymus-NIH Consensus Development Conference, 1997), and in the first place in the developing countries. After 1947, its incidence and the rate of mortality dropped by 50 to 70% in the developed countries, despite the sex revolution, numerous migrations and an increased prevalence of infection by the human papiloma virus (HPV). This fact is mainly explained by the results achieved in the implementation of cancer screaning (William, 1999). However, in some countries of the third world, CC is still the leading malignome and the cause of death among female population (William, 1999). This points to the necessity to apply screaning for cervical lesion already in the late teenage years, when this disease's rates are beginning to increase (Zarcone, 1998).

According to the information from literature (Smith et al., 1993; Haverkos et al., 2000) different risk factors are more or less significant for the development of CC. Ever since 1842 it has been recorded that

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the uterus, cervical cancer is much more frequent among married than among single women and that it is very rare among nuns. (Griffiths, 1991). Studies which were carried out at the beginning of the 20th century indicate that, in the epidemics card of this disease, an early marriage and low socio-economic status of women have an important role (Terris, 1980), with certain components related to a man. The strongest epidemic corelation was found in connection to the age at the time of first sexual intercourse, especially prior to the age of 18, and 21 (Koutsky, 1992). Visits to prostitutes, circumcision status, sexually transmitted diseases (STD) are considered to be important predicotrs of risk for cervical cancer to occur, linked primarily to the insufficient genital hygiene (Adam et al., 2000). Certain authors are also emphasising, within the combination of the said risk factors, the importance of over-consumption of alcohol, while smoking, according to the same authors, has a protective role towards persistent HPV infections (Ho et al.,1998).

The objective of this study is to determine the influence of etiological factors (smoking, age at the time of first sexual intercourse, number of intercourses, number of partners and status of circumcision) on the occurrence of pre-malignant and malignant cervical lesions among patients with persistent HPV infection.

Patients, material and methods

Patients

The survey included 101 patients of the Gineacological-obstetrician Clinic of the University Clinical Centre and the Institute for Health Care of Women within the Tuzla Health Care Centre. In the case of these women, detection and identification of HPV has been indicated because of a suspicious citological result. Each patient with an identified high risk lesion –HGSIL (CIN II, CIN III, CIS, SCC) has been recommended to get a histological confirmation of the change that is present. Surveyed women were at the ages between 20 to 52. The control group was made of 100 surveyed women with a good result of citological examination (examination results were within the normal limits or benignant celular change) and without a verified HPV infection.

Material and methods

The applied work method was that of a prospective study, carried out in the period since January 1999 until January 2000. Information about a patient's occupation, age, age at first sexual intercourse, number of partners, number of intercourses per week, and

circumcision of partners, has been collected by using a specially designed form at the time of first examination of a surveyed woman.

Samples of the cervical smear for the HPV analysis were being taken during routine gineacological examinations, by using sticks with cotton, taken from the Digene Specimen Collection Kit, from the whole surface of a portion, and by mild rotating moves from the outer cervical entrance.

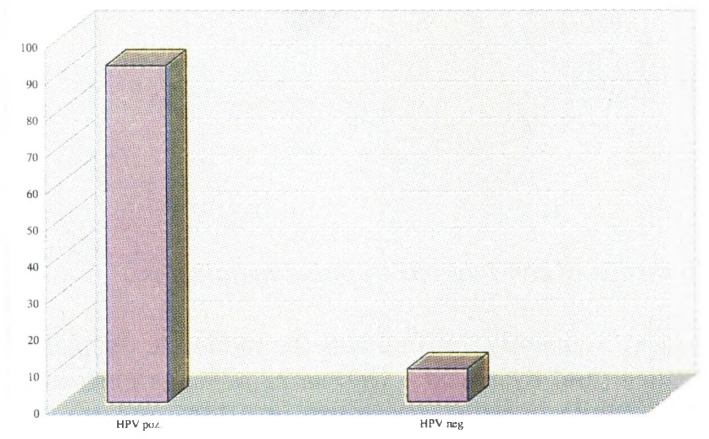
Detection of the presence of HPV in the cervical smear was done by the Digene HPV Test-Hybride Capture II (Hagenesee, 1999). The morphological changes found by using light-microscope method were classified according to the WHO classification of cervical lesion (XX).

For all the citological examination results, suspicious for either the presence of koilocytes in the specimen as a cito-morphologic characteristic of HPV infection, or for the weight of the citologically found lesia, a histologic verification of changes was carried out. All the morphological changes found by using light and microscope have been classified according to the WHO classification of the cervical lesion. Bioptical material phornaline fixed, paraphine embeded, has been cut into standard 5μ cuts and coloured by applying the standard procedure for HE (hematoxsiline eozin) colouring, and then mounted by using Canada balsam.

Statistical processing

The obtained indicators were processed at the level of counting the percentages of occurrence and numerical values, which then represented their co-relation.

Results

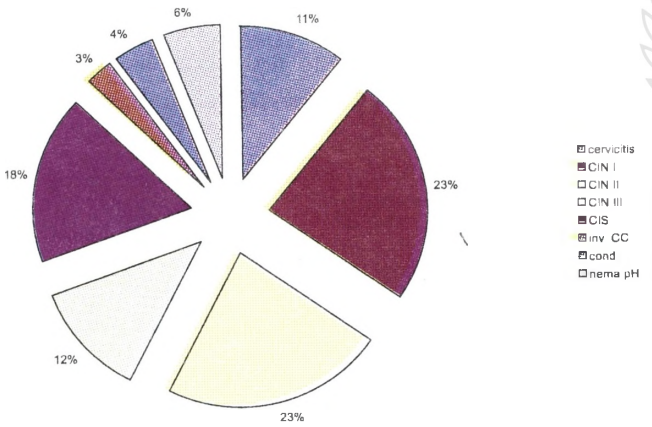


Picture 1. *The ratio of HPV positive and negative patients*

Picture 1 presents the ratio between HPV positive and negative patients. Out of 101 patients 92 (91,08%) were HPV positive, while 9 (8,91%) were HPV negative.

Histological verification of changes has been indicated for all cytological examination results, suspicious either because of the presence of koilocytes in the specimen as cito-morphologic characteristic of HPV infection, or because of the weight of a citologically found lesion. Histological verification was not done for 6 out of 92 HPV positive patients, or it was done after the study period for the examined group. Cytological examination of these 6 patients in 4 cases pointed to High Grade Squamous Intraepithelial Lesion (HGSIL), and in 2 cases to Low Grade Squamous Intraepithelial Lesion (LGSIL).

Distribution of pato-histologically verified lesias of cervix, irrespective of the age and presence of HPV infection, is presented in Graph 2. In the samples which were pato-histologically analysed, 10,89% chronic inflammations of cervix were verified, 3,96% condyloma, 23,76% mild displasias – CIN I, 22,77% moderate dysplasia-CIN II, 17, 82% Carcinoma in Situ – CIS, 11,88% severe dysplasias – CIN III, and 2,97% invasive cancers of the surface epithels of the cervix.



Picture 2. *Presence of cervical intra-epithel neoplasias*

The most frequently verified was the moderate dysplasia – CIN II with 25%, then mild dysplasia – CIN sa 22,82%, carcinoma in situ – CIS sa 16,30%, while invasive carcinoma at 2,17% and condyloma at 4,34% are less frequent. 4 out of 6 patients without pato-histologically verified lesion have had a HGSIL citological examination result, 2 of

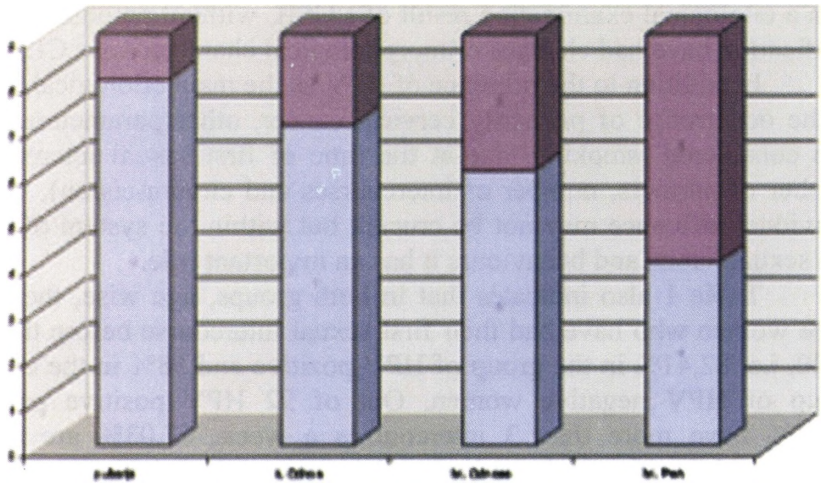
which towards CIN III and 2 towards CIS or heavier lesion. 2 patients with a citological examination result of LGSIL without patohistological verification have had changes of morphological characteristics CIN I.

In addition to the presence of HPV as the main etiological factor in the occurrence of primarily cervical cancer, other parameters were also considered (smoking, age at the time of first sexual intercourse, number of partners, number of intercourses and circum-cision), whose individual influence may not be crucial, but within the system of daily and sexual habits and behaviours it has an important role.

Table 1 also indicates that in both groups, age wise, there are more women who have had their first sexual intercourse before the age of 20, i.e. 82,41% in the group of HPV pozitive and 58% in the control group of HPV negative women. Out of 92 HPV positive patients 91,20% have more than 3 intercourses a week, 67,03% are active smokers while 46,15% have had or have more than 1 partner. In the control group the situation is almost completely the opposite; patients are non-smokers in u 66% cases, 74% have one relationship and sex partner, 71% of them have 1 to 2 intercourses per week. In both the examined and the control group male partners are circumcised (68,13% in group), and 67% (in the control group).

Table 1. *Other risk factors and their presence in the case of HPV positive women*

HPV positive		Factor	HPV negative	
N	%		N	%
61	(67,03)	yes ---smoking --- yes	34	(34,00)
31	(34,65)	No no	66	(66,00)
75	(83,41)	≤ 20 --- 1st intercourse --- ≤ 20	58	(58,00)
17	(18,68)	≥ 20 ≥ 20	42	(42,00)
50	(54,94)	1 --- No of partners ---- 1	74	(74,00)
42	(46,15)	≥ 2 ≥ 2	26	(26,00)
9	(9,89)	≤ 2 --- No of intercourses --- ≤ 2	71	(71,00)
83	(91,20)	> 3 > 3	29	(29,00)
62	(68,13)	yes --- Circumcision --- yes	67	(67,00)
29	(31,86)	No no	33	(33,00)
92	(100,00)	Total	100	(100,00)



Picture 3. *Influence of co-factors on the CIN among HPV negative patients*

Table 2. *Presence of other risk factors among HPV positive women*

Factor		Lesion		
		ASCUS*	LGSIL**	HGSIL***
Smoking	N	11	26	53
	Yes	8	14	33
	No	5	9	17
	Earlier	-	3	-
1st intercourse	N	13	48	95
	≤ 20 yrs.	11	26	53
	≥ 20 yrs.	2	22	42
No of partners	N	11	26	53
	> 1	6	14	30
	≥ 4	6	12	21
No of intercourses	N	13	26	53
	≤ 2	2	1.	6
	≥ 3	24	15	18
	> 5	5	9	26
	≥ 10	2	1	3
Circumcision	N	13	26	53
	Yes	7	17	38
	No	6	9	15

* Atypical squamous cell undetermined significant
** Low- grade squamous intraepithelial lesion
*** High- grade squamous intraepithelial lesion

Out of 9 HPV negative patients in the examined group with a present lesion of cervix 5 (83,33%) were smokers, with more than 5 intercourses a week or 66,66% and with < 20 years of age at the time of first intercourse or 77,7%. This can be noted in Picture 3.

The influence of individual risk factors in accordance with the present lesion has also been monitored among HPV positive patients, irrespective of the type of virus. Numerical presence and co-relations between different risk factors in relation to lesion can be seen in table 2.

Overall, patients are monogamous with 53,84% in the LGSIL and 56,60% in the HGSIL, but with a higher frequency of intercourses per week, or over 5 for ASCUS in 38,46% cases, and 49,05% for HGSIL, while patients with LGSIL have had a slightly lesser number of intercourses, between 3 and 5, with 57,69% and mainly with circumcised men. Irrespective of the present lesion, the largest number of patients are active smokers at 61,53% in ASCUS lesions, 53,84% in LGSIL and 62,26% in HGSIL. As in the previous analysis, these are mostly the cases where sexual life started early before the age of 20, which in the ASCUS and LGSIL groups stands at 84,61%, and in the HGSIL group at 79,24%.

Discussion

The fact that infection by humane virus of papilloma leads to different lesions of cervix, not to the same speed and degree of progression, but even to a spontaneous regression of certain changes under specific preconditions, points to the importance of other, additional factors; environment or endogenic factors, quite possibly able to modulate the evolution of HPV in the cervix tissue (Bosch at al., 1997.). A woman's risk from HPC infection is determined by her age, behaviour and both her own and her partner's sexual habits. The change in age limits is evident though, and so an increased CIN incidence was observed among younger women, especially that of high degree (Blohmer and sar,1999). In relation to the age this is the first consideration, age at the time of first sexual intercourse. According to our data, it is evident that irrespective of the presence of HPV, beginning of sexual intercourses at an early age considerably affects the creation and development of different lesions of cervix. At the same time, when comparing HPV positive and HPV negative patients with respect to the age factor, there is a more obvious discrepancy between HPV positive patients who started with sexual activity prior to the age of 20 and those who started it after the age of 20 (Iljazović-Latifagić, 2000). Biological basis for influence of age on the HPV prevalence is not known as yet. One explanation could be the reduced exposure to the virus, as a consequence of change in sexual behaviour at an older age (Ho and assts.., 1998;

Hildesheim and assts.,1993). Further, certain gained immunity mechanisms can develop after a period of exposure which can lead to a reduction in HPV prevalence at an older age (Schiffman, 1994). Persistent infection can increase the risk of development and lasting of squamous intraepithelial lesion. According to the study of multi-factorial influences on the lesions of cervix, Susane Kruger-Kjaer, among HPV positive and HPV negative women, it is exactly the early sexarha that is the crucial factor for creation of either ASCUS, LGSIL or HGSIL, and especially HGSIL at an early age. (Kruger-Kjaer,1998). The fact that female population of certain peoples or countries has a high incidence of cervical cancer, despite certain traditional norms of sexual behaviour, focuses the interest on the role of "the male factor" as an important element in the rate of risk for development of cervical lesion among women (Kruger-Kjaer and assts., 1991).

Primarily, and in relation to the mentioned age factor, the number of sexual partners is also important. According to our study of HPV negative patients, they are mainly monogamous (74%) with a lesser frequency of intercourses, which is probably an important preventive measure for STD. HPV positive patients are sexually more active (91,20% with more than 3 intercourses/week) and are almost in the same position in the examined groups whether with one or with more partners, which emphasises the role of a man. Certain characteristics of male sexual behaviour, such as genital hygiene and circumcision, STD and smoking, greatly affect the time of appearance and the development stage of a created cervical lesion.

Brinton and co-workers reported that men much more frequently have more sexual partners until they establish a permanent relationship, an earlier sexarha. And more frequently get STD, which, with joint sexual habits with a permanent sexual partner increases the risk of pre-malignant and malignant lesions (Brinton et al.,1989; Bosch et al.,1996). This position could, to a certain degree, be an explanation for high prevalence of HPV and lesions in our study. According to our results, an early sexual activity, more partners and higher frequency of intercourses are mutually unrelated factors in the creation of cervical cancer (de Sanjose and assts., 1997). The said factors and the high HPV prevalence is expected among the population of lower social-economic status (Ferrera and asst.., 1999; de Sanjose and assts, 1997) which may be a consequence of less developed preventive health care.

According to some studies, insufficient penilane hygiene, presence of STDs and bad education, i.e. "a highly risky man", increases the risk of his partner to get a disease of cervix. (Svare,1998 and Kruger-Kjaer, 1991). Although it has been proven that circumcision protects a men from HIV infection, from penilene cancer, infection of urinary system and ulcerous STDs (Moseset et al.,1998), our results as

well as the results in Panama, Costa Rica, Bogota,Columbia and Mexico City (Brinton,1991) with difficulty define the role and influence of circumcision on the creation and progressing of lesion, where there are more sexual partners and a highly risky sexual behaviour (not using condoms, more partners). In other words, it appeared that circumcision does not have a protective role in cases of highly risky sexual behaviour.

An almost identical percentage of circumcised partners (68% and 67%) in both groups of our study (HPV positive and negative patients) with specific difference in the presence of other risk factors, points to the importance of sexual habits/behaviour, as a factor in the creation of cervical intraepithel neoplasias and invasive cancer.

Certain earlier studies indicated that smoking was a protective factor for persistent HPV infection, without knowing if the protective mechanism was biological or some other unknown effect (Ho and assists., 1998). The percentage of 67,03% active smokers in the HPV group of positive patients in relation to 34% active smokers in the HPV group of negative patients in our study, points to an absolutely opposite effect and points to tobacco as a very potent carcinogene, able to lead to neoplastic progression and its significant joining with the cervical cancer (Ngelangel etal., 1998).

The presented results, as well as the results of numerous studies conducted world-wide point to the central role of the HPV in the pathogenesis of cervical lesions with an etiologically important co-action with other mutually independent factors, primarily factors related to sexual habits/behaviour. The factors from this group, in the frame of environment of highly risky sexual behaviour and without the presence of HPV, considerably affect the creation of cervical lesions.

Apstrakt

Cervikalni karcinom(CC) je, i po incidenci I mortalitetu drugi u svijetu, a prvi u zemljama u razvoju. Prema podacima iz literature, različiti faktori su manje ili više značajni u njegovom nastanku. Jedan od najznačajnijih faktora u nastanku ne samo malignih nego I premalignih lezija grlića jeste infekcija humanim virusom papiloma (HPV). Najjača epidemiološka korelacija nađena je za dob u vrijeme prvog odnosa, naručito prije 18, odnosno 21 godine života. Cilj ove studije je da determiniše uticaj različitih etioloških faktora (Pušenje, dob u vrijeme prvog odnosa, broj odnosa, broj partnera I status cirkumcizije) na nastanak premalignih I malignih lezija grlića kod pacijenata sa infekcijom HPV.

Ključne riječi: *cervikalne lezije, infekcija HPV, kofaktori nastanka*

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