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Isak Samokovlija (Goražde, September 3, 1889 - Sarajevo, January 15, 1955; a well-known doctor and writer from Bosnia and Herzegovina). With permission of Jelica Najdanović Bokonjić, granddaughter of Dr. Isak Samokovlija.

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Evaluation of SARS-CoV-2 Antibody Response Post Third Dose COVID-19 mRNA Vaccination at Universitas Indonesia Hospital

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Abstract

Objective. The longevity of vaccine effectiveness and antibody titer after the Moderna mRNA COVID-19 vaccination booster in healthcare workers in Indonesia is not known. **Materials and Methods.** We performed a prospective observational study of healthcare workers at the Universitas Indonesia Hospital after Moderna mRNA COVID-19 booster vaccination. An Immunology Analyzer with Chemiluminescence Immunoassay (CLIA) test was used to examine Anti SARS-CoV-2 S-RBD levels. Antibody levels were classified into two systems (3 categories, and 2 categories). **Results.** There were 31 male subjects (75.6%), 33 subjects (80.5%) aged 25-39 years, 17 subjects (41.5%) with overweight BMI, 35 subjects (85.4%) without comorbidities, and 29 subjects without previous history of COVID-19 infection (70.7%) who had antibody titer >1000 AU/ml. There were 27 subjects (65.9%) who had a booster shot ≥6 months after the second vaccination with antibody titer >1000 AU/ml. In this study, there was no significant correlation between antibody titer with factors such as gender, age, BMI, comorbidities, history of COVID-19 infection and time between the 2nd vaccination and booster vaccination. **Conclusion.** There is no significant correlation between antibody titer with factors such as gender, age, BMI, comorbidities, history of COVID-19 infection and time between the 2nd vaccination and booster vaccination

Key Words: COVID-19 ▪ SARS-CoV-2 ▪ Vaccine.

Introduction

On March 11, 2020, WHO declared COVID-19 infection a pandemic. COVID-19 is caused by SARS-CoV-2 and does not yet have a primary therapy that can directly kill the virus, making vaccine the main hope for stopping this pandemic (1). The SARS-CoV-2 vaccination has proven effective in inducing an immune response and progressively open the way to overcome the COVID-19 pandemic (2). The goal of SARS-CoV-2 vaccines is to produce anti-spike neutralizing antibodies (nAbs) that recognize the viral S protein. Theseanti-spike nAbs can prevent virus–human cell interaction and aid in the elimination of infection in its early stages (3). A previous study showed there are several factors that influence antibody levels, including age, infection history, and virus mutation (3). The effectiveness of vaccines in reducing the spread of COVID-19 has been proven. In Indonesia, along with the increase in the vaccination rate, the number of hospitalizations, deaths due to COVID-19 and confirmed positive cases decreased compared to 2020 when vaccination was not used in general (4).

Studies have already demonstrated that the third dose increases immunogenicity against SARS-CoV-2, reflected in a rapid and broad immune response to the third mRNA vaccine BNT162b2 dose (5). In July 2021, the third dose of vaccination was intended for all health workers. The third

dose or booster vaccination for health workers in Indonesia uses two types of vaccine, which are the CoronaVac vaccine and mRNA-1273 vaccine (Moderna vaccine). The first and second dose for health workers used the CoronaVac vaccine (4). Compared to the homologous boosting type, the heterologous boosting type of vaccine administration was expected to widen cellular and humoral immunogenicity against COVID-19 infection (6, 7). The heterologous boosting between Coronavac and mRNA vaccine gives better antibody response than other vaccine booster options, and also shows great protection against the delta and omicron variants (8-10). The Moderna vaccine is an mRNA-based vaccine that has several advantages, one of which is the fast and specific formation of immunogens. The Moderna vaccine showed efficacy of 94 after two doses in a phase 3 trial (11). Various side effects of mRNA vaccines can occur locally or systemically (3). Antibodies formed after the administration of the vaccine serve as biomarkers of immunity, so that the detection of specific antibodies can provide information about adaptive immunity against SARS-CoV-2. Quantitative assays for detecting anti-SARS-CoV-2 antibodies can help determine vaccine-specific antibody responses, individual antibody titer, and longitudinal monitoring of antibody responses. The test can also assess whether a person's antibody levels are the result of an adaptive immune response induced by infection, or a vaccine-induced response (12). A study by Ibarguengoitia et al. shows that the median antibody titer ranged from 379-2960 AU/ml in the group with negative COVID-19 history, and 590-3090 AU/ml in the group with positive COVID-19 history (13). The purpose of this study was to determine the SARS-CoV-2 antibodies response after the third dose of mRNA-based vaccination in health workers at Universitas Indonesia Hospital

Methods

Examination of IgG S-RBD SARS-CoV-2 Antibodies

The investigation was carried out at the Clinical Pathology Laboratory of Universitas Indonesia Hospital using the Mindray CL-900i Immunology Analyzer manufactured by Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China (14). The sensitivity value of the analyzer was 0.006ng/mL, with a measurement range of 0.006–50 ng/mL. The analyzer was calibrated at the beginning of the study. The quality control (QC) results of the analyzer were within QC limits. If subject's antibody was >1000 AU/ml, it was shown as it is, but if the antibody level was <1000 AU/ml, it showed as the exact number.

Study Design and Population

The research design was a prospective cohort method conducted in August 2021-January 2022 at Universitas Indonesia Hospital, one of the COVID-19 center hospitals in Indonesia. The target population in this study were all health workers at Universitas Indonesia Hospital receiving the third dose of Moderna vaccine, without any limitations in duration between the booster injection and laboratory testing. The research inclusion criteria were Universitas Indonesia Hospital health workers who had received the first and second doses with inactivated whole-virus CoronaVac vaccine, and the third dose with Moderna vaccine, and health workers who were registered as permanent employees and part timers at Universitas Indonesia Hospital. The exclusion criteria were Universitas Indonesia Hospital health workers who had a history of allergies to Moderna vaccine, and Universitas Indonesia Hospital health workers who received some other vaccine than Moderna as

the third dose. This study involved 49 Universitas Indonesia Hospital health workers who were tested for IgG S-RBD SARS-CoV-2 antibodies in their serum and plasma using the CLIA Anti-SARS-CoV-2 principle. A relatively small number of subjects was chosen as this study acts as a preliminary study. Our research divided antibody titer into two systems. In the first we divided antibody titers into categories: <500; 500-1000; >1000 AU/ml based on the tests.

Data Collection and Statistical Analysis

Respondents supplied their data using a form filled in directly by the research subject. The analytical study was conducted on 49 samples (Figure 1). The questionnaire data were entered into a Microsoft Excel sheet. Data were statistically analyzed using Microsoft Excel 2019 and SPSS 24. Nominal categorical data were displayed in percentage graphs, and numerical data presented along with mean data and standard deviations. Analysis of categorical data was carried out using the chi square, or alternatively Fisher's test if they did not meet the chi square rule. Meanwhile, analysis of numerical data was carried out using one way ANOVA or an unpaired T test for comparison with samples of less than two groups. If the data did not meet the normality test, the Kruskal Wallis and Mann Whitney tests were carried out.

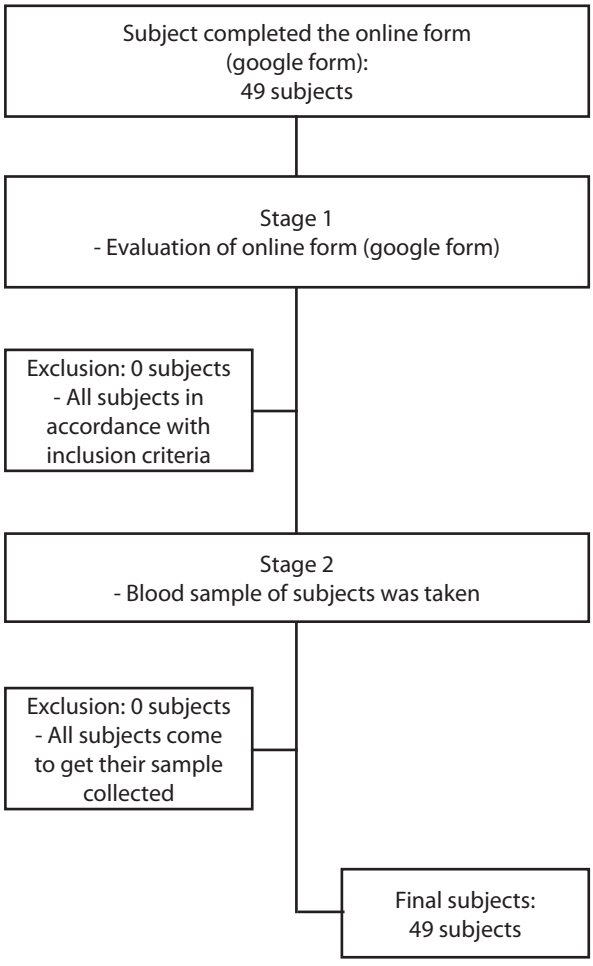

Figure 1. Subject recruitment process.

Ethics Statement

This research was approved by the Ethics Committee of Universitas Indonesia Hospital, approval number S-010/KETLIT/RSUI/II/2022, with protocol number 2021-09-099. This research also followed Declaration of Helsinki guidelines.

Results

In this study, a total of 49 subjects took part (Table 1). The majority of subjects were male, that is 36 subjects (73.5%). There are 40 subjects (81.6%) who were 25-39 years old. We divided their BMI into four groups: underweight, normal weight, overweight, and obese. The subjects were dominantly in the normal weight BMI group, that is 22 of them (44.9%), and 19 subjects (38.8%) were overweight. There are 7 subjects (14.3%) who had comorbidities, which were hypertension (3 subjects, 6.1%), diabetes mellitus (2 subjects, 4.1%), and coronary artery disease (CAD) and asthma with 1 subject each (2%). There are 13 subjects (26.5%) who had a previous history of COVID-19. Thirty-three subjects (67.3%) received the 3rd vaccine within 6 months after the 2nd vaccine.

Table 2 shows the evaluation of antibody titers after administration of the Moderna booster vaccine, using the first category, whereby we divided the antibody titer into three groups. There were 31 subjects (86.1) with antibody titer >1000 AU/

Table 1. Demographic Characteristics

Characteristic	N (%)	Mean (±SD)
Gender		
Female	13 (26.5)	-
Male	36 (73.5)	-
Age group (years old)		30.0 (±6.7)
<25	6 (12.2)	-
25-39	40 (81.6)	-
≥40	3 (6.1)	-
BMI (kg/m^2)		25.4 (±4.6)
Underweight <18.5	2 (4.1)	-
Normal weight 18.5-24.9	22 (44.9)	-
Overweight ≥ 25	19 (38.8)	-
Obese ≥30	6 (12.2)	-
Comorbidity		
Yes	7 (14.3)	-
No	42 (85.7)	-
Comorbidity		
Diabetes mellitus	2 (4.1)	-
Hypertension	3 (6.1)	-
CAD	1 (2)	-
Asthma	1 (2)	-
History of previous COVID-19 infection		
Yes	13 (26.5)	-
No	36 (73.5)	-
2nd to 3rd vaccine duration (months)		5.6 (±1.6)
< 6	16 (32.7)	-
≥6	33 (67.3)	-
Antibody titer (AU/mL)		
<500	4 (8.2)	-
500-1000	4 (8.2)	-
>1000	41 (83.7)	-

ml. Thirty-three subjects (82.5) aged 25-39 years had antibody titer >1000 AU/ml. Seventeen subjects (89.4) in the overweight BMI category had antibody titer >1000 AU/ml. Thirty-five subjects (83.4) with no comorbidities had antibody titer >1000 AU/ml. Twenty-nine subjects (80.6) with no previous history of COVID-19 infection had antibody titer >1000 AU/ml. In this study, 27 subjects (81.8) who had the 3rd booster vaccine ≥6 months from the second vaccine had antibody titer >1000 AU/ml. Four subjects (9.5) had antibody titer <500 AU/ml, while none of them had a comorbid disease. Of these 4 subjects, one had a history of previous COVID-19 infection. In this study, there was no significant correlation between antibody titer and gender, age, BMI, the presence of comorbid diseases, a previous history of infection with COVID-19, or the time between the 2nd and the booster vaccination.

Table 2. Antibody Titer for the First System

Characteristics	<500 AU/ml N (%)	500-1000 AU/ml N (%)	>1000 AU/ml N (%)	P*
Gender				
Female	2 (15.4)	1 (7.7)	10 (76.9)	0.54
Male	2 (5.6)	3 (8.3)	31 (86.1)	
Age (years)				
<25	1 (16.7)	0(0)	5 (83.3)	0.77
25-39	3 (7.5)	4(10)	33 (82.5)	
≥40	0 (0)	0 (0)	3 (7.3)	
BMI (kg/m^2)				
Underweight	0 (0)	0 (0)	2 (100)	0.68
Normal weight	3 (13.6)	3 (13.6)	16 (72.6)	
Overweight	1 (5.3)	1 (5.3)	17 (89.4)	
Obese	0 (0)	0 (0)	6 (100)	
Comorbidity				
Yes	0 (0)	1 (14.3)	6 (85.7)	0.59
No	4 (9.5)	3 (7.1)	35 (83.4)	
History of previous COVID-19 infection				
Yes	1 (7.7)	0 (0)	12 (92.3)	0.44
No	3 (8.3)	4 (11.1)	29 (80.6)	
2nd to 3rd vaccine duration (months)				
<6 months	2 (12.5)	0 (0)	14 (87.5)	0.28
≥6 months	2 (6.1)	4 (12.1)	27 (81.8)	

*Chi-square test.

Discussion

In this study, we found that age was not significantly related to antibody titer. This is in line with research conducted by Richards et al. (15) where there was no significant difference in antibody titer between subjects under 50 years old and those over 50 years old who received either Pfizer or Moderna vaccine. Sinto et al. (16) also found in their study that there was no significant correlation between antibody titer and age (16). A study conducted by Bates et al. (17) also showed no significant age-related trend among participants. Different results were found in the study by Cucunawangsih et al. (18) where age significantly correlated with antibody titer.

The study by Jalkanen et al. showed that after the first BNT162b2 mRNA vaccine dose, anti-S1 IgG antibody levels and neutralization titers were significantly lower in the older age group (55–65 years) compared to the younger age groups (20–34 and 35–44 years). However, after the second mRNA vaccine dose, the neutralization titers were similar in all the age groups (20–34, 35–44, 45–54, and 55–65 years) (19). Our subjects' mean age was 29 years old, younger than the subjects in the studies by Cucunawangsih et al. and Jalkanen et al.

Our study shows that antibody titer was not significantly correlated with gender. This result is also supported by the studies by Richards et al., (15), Sinto et al., (16) and Cucunawangsih et al. (18) who found in their studies that gender did not have a significant relationship with antibody titer. However, another study conducted by Ibarguengoitia et al. (13) showed a difference in terms of patient gender with significant correlations, since females had higher antibody titers. The study by Jalkanen et al. (19) showed that after the

second dose of BNT162b2 mRNA vaccine, female vaccinees had slightly higher neutralization titers than males, although the anti-S1 IgG antibody levels remained the same. Our study differs from the studies by Romero-Ibarguengoitia et al. and Jalkanen et al., because our study was dominated by male subjects and gender did not have any direct pathophysiology in the antibody-forming response (20).

In this study, we found no significant differences in antibody titers between the under-weight, normal weight, overweight and obese groups. In another study by Pellini et al. (21), it was also found that there was no significant difference in antibody levels after 7 days of giving the Pfizer mRNA vaccine booster in the underweight, normal weight, overweight, and obese groups. In another study by Yamamoto et al. (22), the antibody titer following the Pfizer mRNA vaccine was associated with BMI in the male gender group, where an increase in BMI in men was associated with a lower post-vaccination antibody titer, with P<0.001 for BMI <18.5 kg/m^2 and 27 kg/m^2. In women, an increase in BMI was not associated with post-vaccination antibody titer. Another study of the Pfizer mRNA vaccine by Nam et al. (23) showed that the antibody level was inversely correlated with weight, body mass index, body fat amount, and the body weight to height ratio in the Spearman correlation analysis. In multivariate analysis of categorized variables, a lower serum level of antibodies (<81.5%) was associated with weight ≥55 kg (OR: 9.01; 95 CI 1.44-56.40). The constant state of low-grade inflammation, present in overweight people, can weaken some immune responses, including those launched by T cells, which can directly kill infected cells (24). The increased adipose tissue causes leptin, TNF-α, and IL-6 to be overproduced, while adiponectin is decreased (25). An imbalanced adipocytokine profile can lead to chronic low-grade inflammation, which can induce B-cell immunosenescence, and impair antibody production post-vaccination (26).

In our study, it was found that seven subjects had comorbid diseases. We found no significant correlation between antibody titer and the presence of comorbidities. This is in accordance with a study conducted by Choi et al. (27), where there was no significant difference in antibody titer nAb or S-IgG levels 6 months after of the second dose of SARS-CoV-2 mRNA vaccination in healthy individuals compared to individuals with comorbidities. Another study by Eliakim-Raz et al. (28) showed the same result. Their study reported the evaluation of anti-spike (anti-S) IgG antibody titer after administration of the third dose of mRNA (Pfizer) vaccine in a population over 60 years of age in Israel. They found no significant correlation between comorbidities and post-vaccination antibody titers (28). Pellini et al. (21) in their study found that antibody titer was not significantly correlated with hypertension. Different results were found in the research by Sinto et al. (16), where there was a significant correlation between antibody titer and cardio-vascular disease and diabetes, with p-values of 0.02 and 0.038 respectively. Our study showed there was no correlation between comorbidity and antibody titer. Even though comorbidities are a risk factor for progression of COVID-19 into a severe and critical stage, comorbidities are not significantly correlated to the booster vaccination.

Prior infection may enhance protection from vaccination, raising the question of hybrid immunity. In several studies, the results showed that vaccinations carried out in groups with a history of being infected with COVID-19 had a much higher antibody response than groups that had not been previously infected. Anichini et al. (29) reported that nAb levels following the second dose of vaccine in the group who were not infected with SARS-CoV-2 were lower than following the first dose in the group with a history of COVID-19 infection. Their study also found that there was a significant correlation in IgG levels between the 1-2 month group and the 2-3 month group. The IgG level in the 1-2 month group was higher than the 2-3 month group, while the nAb level in the >3 month group was the highest of all the other groups. This result indicates that the booster response is more effective when the vaccine is given more than 3 months after being infected with

COVID-19 (29). The study by Krammer et al. (30) also demonstrated that a faster immune response was found in the single-dose mRNA group with a history of infection than in the group without a history of infection who had received the full dose. Krammer also reported that SARS-CoV-2 antibodies formed more quickly in the group with a history of infection, where antibody titer had started to form within 0-4 days after vaccination, while in the group without a history of infection the average antibody titer began to form at 9-12 days after vaccination (30). A study conducted by Demonbreun et al. (31) on 33 people who had received a booster mRNA vaccine found that an antibody response formed within 6-10 days after receiving the booster, and the concentration of IgG in the group with a history of infection was higher than in the group without a history of infection.

In our study, we did not find a significant correlation between antibody levels and a history of COVID-19 infection. Although our data did not show any significant difference, from the percentage we can see there was a tendency for subjects with a history of COVID-19 infection to have a higher antibody titer than subjects without a history of COVID-19 , in line with other studies (5, 11). The difference may not be seen as statistically significant due to the small number of samples.

Zhao et al. (32) found in their research that the levels of antibody formation against the omicron variant of COVID-19 were 62, 56, and 100, respectively, for recipients of the inactivated virus booster vaccine, recipients of the protein subunit ZF2001 at a one-month interval, and recipients of the protein subunit ZF2001 at a four-month interval. In addition, antibody levels to the omicron variant compared to the SARS-CoV-2 prototype were 5.1 times lower in the inactivated virus vaccine group, 10.26 times in the ZF2001 protein subunit vaccine group at a one month interval, and 3.1 times in the ZF2001 protein subunit vaccine group at a four month interval. This shows that a longer interval to booster administration is directly proportional to the increase in antibody titer (32). This result is expected because antibody maturation time is better in the group with a longer interval (33).

Our study did not show any significant difference in the antibody titer between intervals less than six months and more than six months. Our study used an interval duration of 6 months because in Indonesia's national program the administration of boosters is at an interval of 8 months after the first dose of vaccination in health workers. However, we compared the percentages that showed that subjects with boosters <6 months were better than subjects with a ≥6 months booster, with an average interval of almost 6 months. This contrasts with Zhao's study and a meta-analysis by Cromer et al. that showed that the administration of a booster at a six-month interval gave a 4.9-fold increase in titer compared to administration at a one-month interval, where it only increased by 1.3-2.1-fold (32, 34). Our study could have shown different results if we had more subjects and checked their antibody titers in the first, third, and sixth months.

There are concerns that the efficacy of the previous two doses vaccines might decrease due to weakening antibody levels and the appearance of new variants of SARS-CoV-2, with amino acid changes in the spike protein and elsewhere in the viral genome (5). A study in UK conducted by Andrews et al. showed evidence of a substantial increase in protection against symptomatic COVID-19 disease after a booster dose of BNT162b2 or mRNA-1273 vaccine during the period when the Delta variant was the dominant strain in that country. Very high levels of protection were seen against hospitalization or death with a BNT162b2 booster (35). According to the Ministry of Health's weekly report on COVID-19 of October 2021, in Indonesia, the Delta Variant sequence still dominated the reported variants. Sequencing results showed the Variant of Concern (VoC) Delta in as many as 98.9 cases (274/277) (36). However, we do not know the COVID-19 variant for sure because we did not perform genome sequencing at the time in the patients infected with COVID-19. We consider that 3 months should pass before giving the booster vaccination if the delta variant is the most common variant in Indonesia.

Limitations of the Study

The main limitation of this study is the relatively small number of subjects and that it is limited to one center. Data about the time duration between booster injection time and the antibody laboratory testing would improve the results of this study.

Conclusion

In this study, it was found that the antibody titer after receiving the Moderna mRNA vaccine booster was sufficient within 3 months after vaccination. There were 41 subjects (83.7) with antibody titer >1000 AU/ml. S-IgG antibody levels were maintained for 3 months after the booster vaccination. In this study, antibody titers did not have a significant correlation with the variables of gender, age, BMI, comorbidities, history of being infected with COVID-19 and time after vaccination. Further studies are required to better understand the factors that can affect antibody titers when using the Moderna vaccine. In addition, a similar study with more subjects and multi-centered research would be beneficial and may provide more significant correlations.

What Is Already Known on This Topic:
Vaccine is beneficial against some diseases. There are some options for COVID-19 vaccine with some differences in efficacy. Study of COVID-19 booster vaccination is limited in Indonesia. Moderna shows relatively low efficacy in comparison with other vaccines in terms of main dose administration (2).

What This Study Adds:
The efficacy of Moderna vaccine as a booster dose is shown. This study can be a guide for booster administration, mainly in Indonesia.

Authors' Contributions: Conception and design: RH, APM, FA, ZD, DDW, RAP, HML, VD, GCF, AW, MHA, Y, SA, MR, NDI and AG; Acquisition, analysis and interpretation of data: RH, APM, FA, ZD, DDW, RAP, HML, VD, GCF, MR, NDI and AG; Drafting the article: RH, APM, FA, ZD, DDW, RAP, HML, VD and GCF; Revising it critically for important intellectual content: RH, AW, MHA and Y; Approved final version of the manuscript: RH, AW, MHA and Y.

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

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The Potential of Cinnamon Extract (*Cinnamomum burmanii*) as Anti-insomnia Medication through Hypothalamus Pituitary Adrenal Axis Improvement in Rats

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Abstract

Objective. This study aimed to explore the efficacy of cinnamon extract as an anti-insomnia medication in experimental animals by evaluating the levels of hormones and neurotransmitters related to insomnia. **Materials and Methods.** A total of 30 male Wistar rats were divided into six groups. Induction of insomnia in animal models was done by administration of p-chloro-phenylalanine (PCPA) compounds. Estazolam was administrated to the positive control group. Cinnamon extract administration was divided into 3 doses, namely: 25 mg/kg BW, 50 mg/kg BW and 100 mg/kg BW. Evaluation of the organ coefficient was conducted to evaluate drug toxicity to the organs. The enzyme-linked-immunoassay method assessed hormones and neurotransmitters in the serum and hypothalamus related to insomnia. **Results.** There was a decrease in the adrenal coefficient in the cinnamon extract group compared to the PCPA group (0.011+0.001, P<0.05). In addition, there was a decrease in the corticotropin-releasing hormone, adrenocorticotropin hormone, and corticosterone levels in the serum of animals who received cinnamon extract. Our study found a dose of cinnamon extract of 50 mg/kg BW was the best dose to balance neurotransmitter levels in insomniac rats. **Conclusion.** The cinnamon extract increased serotonin and melatonin levels and decreased norepinephrine levels in the insomnia-induced group. Cinnamon extract has potential as an anti-insomnia medication through hypothalamus-pituitary-adrenal axis improvement and brain neurotransmitter regulation in an animal model of insomnia.

Key Words: *Cinnamomum burmanii* ▪ Corticosterone ▪ Corticotropin-Releasing Hormone ▪ Oxidative Stress ▪ Serotonin.

Introduction

Insomnia is a sleep disorder that can affect a person's quality of life, both physically and mentally (1). The increased frequency of insomnia in the last decade is influenced by various life stressors that are high in the millennial era (2). These life stressors are caused by job, school, or social problems. Insomnia is a clinical condition characterized by difficulty falling asleep and maintaining sleep, and decreased sleep quality (2). The diagnosis of insomnia also requires the presence of daytime impairment or consequences associated with the night time sleep complaints. Insomnia is reported to be experienced by nearly 30% or nearly a third of the world's population (3).

Disruption of neurotransmitters or endogenous sleep-regulating molecules is associated with insomnia. Neurotransmitters related to sleep regulation and circadian rhythms include gamma amino butyric acid (GABA), serotonin, melatonin, histamine, prostaglandins, and hypocretin or orexin (4). In addition, dysfunction of the hypothalamus-pituitary-adrenal gland (HPA) axis is thought to increase the disruption of the sleep-wake cycle, leading to insomnia (5). The HPA axis involves endogenous molecules such as corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and corticosterone (CORT) (5).

Management of insomnia is carried out using two approaches, namely pharmacological, and non-pharmacological approaches according to the cognitive behavioral therapy (2). Drugs used in the pharmacological approach act on gamma-aminobutyric acid (GABA) receptors, melatonin, histamine or hypocretin (2). Drugs that act on these four receptors are currently a modality of insomnia therapy, but the available drugs have unwanted side effects and none of these drugs creates a sleep cycle that is similar to natural sleep (6). Of course, this is a new problem that will trigger new problems related to insomnia. Herbal medicine is one of the traditional treatments for insomnia. However, the efficacy of herbal remedies for treating this disorder are currently unknown.

Cinnamon (*Cinnamomum burmanii*) is a plant that is well known by the Indonesian people as a cooking spice and has been used for generations in overcoming various health problems, including to help overcome insomnia (7, 8). Cinnamaldehyde is the main compound believed to play a role in improving sleep quality in insomnia (9). Cinnamon is also believed to be able to improve neurotransmitter activity in cases of insomnia.

This study is the first initial study to explore the efficacy of cinnamon extract as an anti-insomnia medication by evaluating the effect of cinnamon extract administration on experimental animals with induced insomnia. Exploration of serum levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), corticosterone (CORT), serotonin, norepinephrine and melatonin were carried out to test the efficacy of the cinnamon extract in sleep regulation in the animal model.

Methods

Cinnamon Extract Preparation

Cinnamon bark was obtained from the Tawangmangu Medicinal Plant Development Center, Karanganyar, Central Java, in September 2021, and was identified and classified at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indonesia. The cinnamon bark was washed, cleaned and dried at 60° C for 36 hours. Further, the cinnamon was mashed so that it became dry simplicia. A total of 500 grams of dry simplicia were extracted by the maceration method using 70% ethanol (1:10) solvent for 36 hours. The macerate was then evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) to obtain a thick extract of 124 grams (24.8% yield).

Animals and Treatment

This study was conducted at the Eureka Research Laboratory, Palembang, Indonesia. The experimental animals used were 30 male Wistar rats of 150-200 grams weight and 3 months of age. This study was approved by the Animal Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya (Ref. No. 234/FKUNSRI/IX/2021). The animals were kept in a standardized rearing room (temperature 25 ± 2°C, humidity 50±5%, 12 hour light and dark cycle) with standard feed and water ad libitum. Induction of insomnia in the animal model was performed by administration of p-chloro-phenyl alanine (PCPA) compounds (Sigma, St. Louis, MO, USA; No: C6506) (10). PCPA was dissolved in weak alkaline saline (pH 7-8) and injected intraperitoneally (400 mg/kg BW) once daily for two days. After acclimatization, the experimental animals were grouped into six groups (N=5 per group): a control group (untreated, received only saline), the PCPA group (400 mg/kg BW), the PCPA + estazolam group (0.5 mg/kg BW), the PCPA + cinnamon extract group (50 mg/kg BW), the PCP + cinnamon extract group (100 mg/kg BW), and the PCPA + cinnamon extract group (200 mg/kg BW). The experimental animal treatment was conducted for 7 days. After injection with PCPA within 28-30 hours, there was a change in the circadian rhythm and sleep latency of the rats, and it was significantly different from the control group that was not injected with PCPA, which indicated the success of the induction method.

Organ Coefficient

The experimental animals were weighed for seven days of the treatment. Experimental animals were anaesthetized with 10% intraperitoneal chloral hydrate. Furthermore, the evacuation of the adrenal organs was carried out after taking blood from the abdominal aorta. The brain organs are then evacuated and put on ice, and the brain coefficient was determined. The organ coefficient is the ratio of organ weight to the bodyweight of the experimental animals. Changes in the value of organ coefficients indicate drug toxicity to the organs.

Assessment of CRH, ACTH and CORT Serum Levels

Before treatment, the rats were anaesthetized by injection of 10% intraperitoneal chloral hydrate. Blood was drawn through the abdominal aorta, then centrifuged at 3500 rpm at 4°C for 15 minutes. CRH), ACTH and CORT levels in the serum were assessed using the enzyme-linked immunoassay (ELISA) method according to the guidelines and kit instructions (CloudClone, Wuhan, China).

Assessment of Serotonin, Norepinephrine and Melatonin Levels in the Hypothalamus

The animals were anaesthetized by intraperitoneal injection of 10% chloral hydrate. Then the brain was evacuated and put on ice, and the hypothalamus evacuated with precision forceps. The supernatant from the hypothalamus was homogenized and centrifuged at 5000 g for 10 minutes, and then the levels of serotonin, norepinephrine and melatonin were measured by the ELISA method, according to the guidelines and kit instructions (CloudClone, Hanzhou, China).

Statistical Analysis

The results of the study are presented as mean ± standard deviation (SD). One way ANOVA was used to compare the results between multiple groups. $P<0.05$ showed a significant difference, and all data processing was carried out using the SPSS 25.0 program (IBM, Armonk, USA).

Results

The results showed a difference in the addition of the adrenal coefficient between the control and PCPA groups (Table 1). The increase in body weight and brain coefficient was significantly different from the treatment group that received cinnamon extract. The decrease in adrenal coefficient in the group that received cinnamon extract compared to the PCPA group was also quite significant.

Table 1. Effect of Cinnamon Extract on Brain Coefficient and Adrenal Coefficient in PCPA-induced Insomnia

Group	Brain coefficient (Mean±SD)	Adrenal coefficient (Mean±SD)
Control	0.850±0.030	0.01±0.003
PCPA*	0.830±0.040	0.016±0.002
PCPA + estazolam	0.900±0.040†	0.014±0.002
PCPA + cinnamon extract (25 mg/kg BW‡)	0.870±0.030	0.014±0.002
PCPA + cinnamon extract (50 mg/kg BW‡)	0.890±0.030†	0.011±0.001†
PCPA + cinnamon extract (100 mg/kg BW‡)	0.890±0.020	0.011±0.001†

*P-chloro-phenyl alanine; †P<0.05 compared with PCPA group. ‡Body weight.

The PCPA group showed a significant increase in CRH, ACTH, and CORT serum levels compared to the control group (Table 2). Giving cinnamon extract for seven days could reduce levels of CRH, ACTH and CORT. As a comparison, this study used estazolam, a benzodiazepine group that works by binding to the benzodiazepine receptor and strengthening the effect of GABA.

The PCPA-induced group showed decreased serotonin and melatonin levels in the hypothalamus compared to the control group (Table 3). Norepinephrine levels in the group receiving PCPA showed a significant increase compared to the control group. The cinnamon extract increased serotonin and melatonin levels and decreased

Table 2. Effect of Cinnamon Extract on Serum Levels of CRH, ACTH and CORT in PCPA-induced Insomnia

Group	CRH† (ηg/mL) (Mean±SD)	ACTH‡ (ρg/mL) (Mean±SD)	CORT§ (ηg/mL) (Mean±SD)
Control	8.150±0.400	423.100±15.140	30.100±2.140
PCPA*	12.120±0.900	587.500±23.210	46.100±3.650
PCPA + estazolam	8.130±0.400‖	442.600±18.670‖	38.100±2.140‖
PCPA + cinnamon extract (25 mg/kg BW¶)	9.870±0.300‖	436.700±16.740‖	41.600±1.540‖
PCPA + cinnamon extract (50 mg/kg BW¶)	7.900±0.300‖	433.700±19.720‖	33.400±2.120‖
PCPA + cinnamon extract (100 mg/kg BW¶)	7.540±0.200‖	429.500±17.180‖	32.500±1.640‖

*P-chloro-phenyl alanine; †Corticotropine releasing hormone; ‡Adrenocorticotropine hormone; §Cortisone; ‖P<0.05 compared with PCPA group; ¶Body weight.

Table 3. Effects of Cinnamon Extract on Serotonin, Norepinephrine and Melatonin Levels in PCPA-induced Insomnia

Group	Serotonin (ηg/mL) (Mean±SD)	NE* (ηg/mL) (Mean±SD)	MT† (ρg/mL) (Mean±SD)
Control	6.150 ±0.400	0.320±0.010	6.100±0.400
PCPA‡	3.120±0.200	0.650±0.040	3.400±0.200
PCPA + Estazolam	6.130±0.400§	0.350±0.010§	5.100±0.400§
PCPA + cinnamon extract (25 mg/kgBW‖)	7.170±0.300§	0.470±0.020§	4.600±0.400§
PCPA + cinnamon extract (50 mg/kgBW‖)	7.850±0.300§	0.400±0.020§	5.400±0.100§
PCPA + cinnamon extract (100 mg/kgBW‖)	7.930±0.200§	0.380±0.010§	6.200±0.300§

*Norepinephrine; †Melatonin; ‡P-chloro-phenyl alanine; §P<0.05 compared with PCPA group; ‖Body weight.

norepinephrine levels in the insomnia-induced group. Our study found a dose of cinnamon extract of 50 mg/kg BW was the best dose to balance neurotransmitter levels in insomnia rats.

Discussion

Our study showed that in the treatment group that received cinnamon extract, there was an increase in the adrenal coefficient, indicating the cellular repair of the brain and adrenals. In accordance with this finding, a previous study found improvement and reduction of inflammation in the rats' brains after treatment with cinnamon extract (11). Another study revealed cinnamaldehyde in cinnamon extract has beneficial effects against oxidative stress and nitric oxide metabolites in rats' adrenal glands and brains (12).

Injection of p-chloro-phenyl alanine (PCPA) is the most commonly used model to establish an insomnia model (13). In our study, induction of insomnia by administration of PCPA caused a significant increase in hypothalamus-pituitary-adrenal gland axis (HPA axis) hormone levels compared to the control group. Furthermore, cinnamon extract treatment in our study caused a decrease in HPA axis hormone levels (cortisol, ACTH and CRH), which showed the efficacy of the cinnamon extract in improving regulation of the HPA axis. Another study found a decrease in the hormone levels of the HPA axis after administration of cinnamon extract (14).

Our results further demonstrated an increase in serotonin levels and decreased norepinephrine levels in the insomnia-induced group after cinnamon extract treatment. Serotonin and melatonin are essential neurotransmitters that play a role in the initiation of sleep, while norepinephrine is a neurotransmitter that keeps a person alert. A PCPA injection in animal models causes a decrease in serotonin and melatonin activity which causes sleep disturbances, and a PCPA injection causes an increase in norepinephrine activity, which stimulates the rats to remain awake. A previous study showed increased melatonin activity can improve sleep quality in human (15). Disruption of neurotransmitters is one of the factors that play a role in the pathogenesis of insomnia. Several

neurotransmitters, including serotonin, norepinephrine, dopamine and GABA, play an essential role in regulating sleep and wakefulness. In our study cinnamon extract treatment was shown to increase serotonin and melatonin activity, and reduce norepinephrine activity. Our study also revealed the efficacy of cinnamon extract as anti-insomnia medication in an animal model.

Conclusion

On the basis of our results, it was concluded that cinnamon extract has potential as an anti-insomnia medication through HPA axis improvement and regulation of brain neurotransmitters in an animal model of insomnia. To understand further and evaluate the active compound of *Cinnamomum burmanii* extract for insomnia, isolation of the active compound, and toxicity and safety tests are needed.

What Is Already Known on This Topic:

Cinnamon (Cinnamomum burmanii) is a plant that is well known as a cooking spice and has been used for generations in overcoming various health problems, including to help overcome insomnia. Cinnamaldehyde is the main compound believed to play a role in improving sleep quality in insomnia. Cinnamon is believed to be able to improve neurotransmitter activity in cases of insomnia.

What This Study Adds:

This study is the first, initial study to explore the efficacy of cinnamon extract as an anti-insomnia medication by evaluating the effect of cinnamon extract administration on experimental animals with induced insomnia. In this study, cinnamon extract treatment for seven days reduced levels of corticotropin hormone, adrenocorticotropin hormone and cortisone. The cinnamon extract increased serotonin and melatonin levels, and decreased norepinephrine levels in insomnia-induced animals.

The study was conducted at the Eureka Research Laboratory, Palembang and Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

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

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Partial Superficial Parotidectomy Versus Extracapsular Anatomical Dissection for the Treatment of Benign Parotid Tumors

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Abstract

Objectives. Superficial benign parotid tumors are a common neoplasm of the salivary glands. Different surgical procedures have been applied for partial superficial parotidectomy (PSP) and extracapsular dissection (ECD), which are the two predominant surgical techniques. Our study aimed to evaluate PSP versus ECD for benign parotid tumors, in relation to post-operative complications and recurrence rates. **Materials and Methods.** 266 patients who underwent parotidectomies of benign superficial parotid tumors were evaluated retrospectively. The first group (PSP group) was composed of 143 patients who underwent PSP, and the second group (ECD group) was composed of 123 patients who underwent ECD. **Results.** In the ECD group the rate of patients presenting with total postoperative permanent facial nerve paralysis, House-Brackmann grade III, was 0.8%, whereas in the PSP group it was 1.4%. Frey's syndrome was only reported in the PSP group. Salivary fistula occurred in both groups at similar rates. Sensation dysfunction due to greater auricular nerve division occurred in 72% patients in the PSP group and 10.6% in the ECD group. No statistical difference regarding recurrence rates was found between the two groups. **Conclusions.** Both ECD and PSP procedures are safe surgical options for superficial parotidectomy in the treatment of benign tumors, with similar recurrence rates and post-surgical complications, apart from sensation abnormalities due to more extensive auricular nerve division.

Key Words: Extracapsular ▪ Parotidectomy ▪ Superficial ▪ Complication ▪ Facial Nerve.

Introduction

The development of salivary glands starts between 6 and 8 weeks of intrauterine life, with a common embryogenesis as their development origins stem from the growth of oral epithelium into the underlying mesenchyme. The parotid gland is encapsulated after the submandibular and sublingual glands, although is the first gland to develop (1).

This salivary gland is divided into superficial and deep lobes, with a border consisting of the facial nerve, which is not visible on preoperative imaging examination. Consequently, the retromandibular vein comprises a landmark for ultrasound, separating the deep and superficial lobes of this salivary gland, since it is usually situated superiorly to the trunk of the facial nerve. Although the extracranial part of the facial nerve may in some cases be visualized on high-resolution Magnetic Resonance Imaging (MRI), the retromandibular vein is commonly the anatomic landmark between the two lobes on preoperative Computerized Tomography (CT) scans and MRI examinations of parotid neoplasms (2).

Parotidectomy is basically an anatomical dissection, especially when the facial nerve needs to be identified. The landmarks most often used for facial nerve identification are the tympanomastoid

suture, the mastoid process, the tragal pointer, the stylomastoid foramen, the posterior belly of the digastric, and in some cases the peripheral branches of the facial nerve. Both procedures, partial superficial parotidectomy and extracapsular parotidectomy, are performed when a benign tumor is situated externally to the facial nerve.

Parotid tumors constitute 3% of head and neck tumors (3, 4). The majority of them are benign, and the most common histological type is a pleomorphic adenoma, which accounts for approximately 70% of all benign parotid tumors (3, 5). Pleomorphic adenomas tend to recur more often, therefore total resection is of paramount importance (3, 4, 6). Different surgical techniques have been used to treat benign superficial parotid tumors over the past century (7). In the early 20th century, intracapsular removal was reported as the most popular surgical procedure, due to the low rates of facial nerve damage despite the subtotal removal of the tumor capsule (7). However, with this technique, recurrence rates were as high as 45%. Therefore, more radical techniques were needed (7). About 50 years later, superficial parotidectomy replaced enucleation, consisting of the removal of the entire tumor, along with the surrounding superficial lobe of the parotid gland (5). Some years later, superficial parotidectomy became the most popular procedure due to the reduction in tumor recurrence rates (2%), despite the increased rates of Frey's syndrome, loss of facial sensation and, of course, facial nerve paralysis (7). Extracapsular removal has been performed for the last 25 years as a surgical procedure for which identification of the facial nerve is not needed and only the tumor is removed, including its capsule, without any normal glandular tissue (7).

Moreover, surgical techniques were implemented to dissect the facial nerve trunk and branches anatomically from the gland, to ensure the nerve's preservation, as well as to perform complete superficial gland removal. Recurrence rates were remarkably low, about 0-5% (5). Complete superficial parotidectomy was associated with a higher risk of facial nerve palsy, Frey's syndrome, neuroma, seroma, hematoma and loss of facial sensation (5, 7-10). Consequently, partial superficial parotidectomy (PSP) has replaced total superficial parotidectomy, since the entire tumor is removed, along with about 1-2cm of normal parotid tissue, and the severity of complications is minimized as less parotid tissue is resected (5). In recent years, surgical techniques for benign parotid tumors have been developed in the anatomical direction of less invasive procedures (11). Experienced salivary gland surgeons have taken this approach one step further by performing extracapsular dissection (ECD). An important aspect of ECD is that no dissection of the main trunk of the facial nerve is attempted. ECD is a surgical technique with reduced incidence of facial nerve paralysis, Frey's syndrome, recurrence rates and shorter operation time (12).

The main objective of this review is to evaluate both PSP and ECD procedures for superficial benign parotid tumors regarding post-surgical complications, as well as to address the most appropriate technique by evaluating these outcomes, as mentioned in the current literature.

Patients and Methods

In our study we included patients with benign tumors of the superficial parotid gland, treated with partial superficial parotidectomy (PSP) or extracapsular dissection (ECD) between 2000 and 2020 at the ENT clinic of "Metaxa" Memorial Anticancer Hospital in Piraeus, Greece. Preoperative assessment included U/S (ultrasound), and in many cases Computer/Tomography or Magnetic Resonance Imaging scans. In all patients FNA (fine needle aspiration) had been performed in order to ensure the benignity of the tumors. Since the facial nerve travels into the parotid gland all patients included in the study had normal facial nerve function preoperatively on the House-Brackmann Scale (the House-Brackmann scale is a nerve grading system for clinical evaluation of nerve function from I: normal to V: No facial motion, introduced by Los Angeles ENTs Dr. John W. House and Dr. Derald E. Brackmann in 1985). All tumors extracted in this study were additionally confirmed as benign by histological

reports following the surgical procedures. This study includes patients who were naïve regarding parotidectomy, with only one lesion detected, and without spillage or rupture of the removed tumor during the operation. Both surgical procedures were performed by experienced surgeons.

Surgical Techniques

Partial Superficial Parotidectomy

A lazy-S incision is performed and a superficial cervicofacial flap is raised to the anterior border of the parotid mass or the parotid gland. Identification is undertaken of the great auricular nerve and skeletonisation of the anterior border of the sternocleidomastoid muscle, as well as of the posterior part of the digastric muscle and the cartilage of the external auditory canal, up to the pointer and mastoid tip. Identification is made of the common branch of the facial nerve and dissection undertaken of the tumor, controlling nerve function using nerve stimulation. After partial superficial parotidectomy, hemostasis, installation of high-vacuum drainage and non-resorbable sutures for the skin take place.

Extracapsular Dissection

The identical incision and superficial cervicofacial flap are made. Skeletonisation of the anterior border of the sternocleidomastoid muscle follows. Finally, the tumor is dissected, with preservation of the tumor capsule, after identification of the great auricular nerve. Facial nerve identification is not required during this surgical technique. Hemostasis is ensured while high-vacuum drainage and non-resorbable sutures for the skin are inserted.

Nerve stimulation is used in both surgical procedures for all patients. The results of the PSP and ECD procedures were compared in terms of recurrence rates and postoperative complications. A total of 143 patients (PSP group) underwent partial superficial parotidectomy (PSP) of a benign parotid tumor as the primary intervention. Sixty-three (44%) of them were female, while 80 (56%) were male (Table 1). The youngest patient was 19 years old and the oldest 86 years old (mean age: 52.5 years old) (Table 1). A total of 123 patients (ECD group) underwent extracapsular dissection (ECD) of a benign parotid tumor as the primary intervention. The 123 patients consisted of 98 (79.7%) females and 25 (20.3%) males aged between 22 and 91 years (mean age: 54.65 years old) (Table 1).

The first follow up visit was within a week after surgery. The second follow up was scheduled six months after the operation. Further follow up examinations were also scheduled one and two years after surgery. All the post-surgical complications mentioned were reported within the follow up period.

Table 1. Baseline Characteristics

Characteristics	ECD* N (%)	PSP† N (%)	Total N (%)
Age			
<20	-	2 (1.4)	2 (0.75)
21-40	30 (24.4)	40 (28)	70 (26.3)
41-60	43 (35)	50 (35)	93 (35)
61-80	38 (30.9)	36 (25.1)	74 (27.8)
>80	12 (9.7)	15 (10.5)	27 (0.15)
All ages (N; Mean±SD)	123 (54.65±18.05)	143 (52.5±18.71)	266 (53.5±18.41)
Sex			
Men	25 (20.3)	80 (56)	105 (39.5)
Women	98 (79.7)	63 (44)	161 (60.5)

*Extracapsular dissection; †Partial superficial parotidectomy.

Statistical Analysis

Statistical analysis was performed using the SPSS 25.0 statistical software package (SPSS Inc., Chicago, Illinois, USA). Categorical variables were presented as the number and percentages of the corresponding population. Pearson's chi-square test was used to compare categorical variables between groups. A P-value <0.05 was considered a statistically significant difference.

Results

In the PSP group, the most common histological type was pleomorphic adenoma (81/143; 56.6%), followed by Warthin's tumor (46/143; 32.2%) and 16 other benign lesions (16/143; 11.2%), including cysts (8/143; 5.6%), intraparotid lymph nodes (7/143; 4.9%) and oncocytoma (1/143; 0.7%) (Table 2).

Postoperatively, in (7/143; 4.9%) cases of permanent facial nerve weakness occurred (not completely resolved over a period of 6 months to 1 year). Five of these patients (5/143; 3.5%) exhibited weakness of only a marginal branch of the facial nerve (House-Brackmann grade II), whereas two patients (2/143; 1.4%) suffered from total paresis (House-Brackmann grade III) (Table 3).

Facial nerve weakness was diagnosed using clinical signs (lip and eyelid movement), and electromyographic examination. Facial nerve weakness immediately after the operation was not measured. Two patients (2/143, 1.4%) developed Frey's syndrome, four patients (4/143; 2.8%) reported salivary fistula, six patients (6/143; 4.2%) seroma, and eight patients (8/143, 5.6%) hematoma. 103/143 patients (72%) reported referred hypoesthesia due to division of the great auricular nerve (Table 3). Local relapse was diagnosed in three patients (3/143; 2%). All three patients (3/143; 2%) with local relapse visited the clinic (one patient during the follow up period and two patients after several years) (Table 3).

In the ECD group the distribution of histological types was pleomorphic adenomas (55/123; 44.8%), Warthin's tumors (42/123; 34.1%) and 26 other benign lesions (26/123; 21.1%) including cysts (13/123; 10.7%), intraparotid lymph nodes (6/123; 4.9%), oncocytoma (3/123; 2.4%), hemangioma (3/123; 2.4%) and kimura disease (1/123; 0.8%) (Table 2).

Nine patients (9/123, 7.3%) exhibited facial nerve weakness immediately after the operation. Most cases of the cases of paresis (8/9; 88.9%) were House-Brackmann grade II and only one (1/9; 11.1%) House-Brackmann grade III. 7/9 of those patients (7/123; 5.7%) had total restoration of facial nerve functionality over a period of 14 days to 6 months (House-Brackmann grade I). 2/9 patients with post-operative facial nerve paresis (2/123; 1.6%) had permanent facial nerve paresis that persisted for a period of 6 months, and was therefore considered as permanent impairment of facial nerve function (Table 3). One of these patients presented weakness of only the marginal branch of the facial nerve (House-Brackmann

Table 2. Type of Benign Tumors

Benign tumors	ECD* N (%)	PSP† N (%)	Total N (%)
Warthin's	42 (34.1)	46 (32.2)	88 (33.1)
Pleomorphic adenoma	55 (44.8	81 (56.6)	136 (51.1)
Cysts	13 (10.7)	8 (5.6)	21 (7.9)
Lymph nodes	6 (4.9)	7 (4.9)	13 (4.9)
Oncocytoma	3 (2.4)	1 (0.7)	4 (1.5)
Hemangioma	3 (2.4)	0 (0)	3 (1.1)
Kimura disease	1 (0.8)	0 (0)	1 (0.4)

*Extracapsular dissection; †Partial superficial parotidectomy.

Table 3. Post-Surgical Complications

Complications	PSP* N (%)	ECD† N (%)	P‡
Facial nerve paralysis	7 (4.9)	2 (1.6)	0.141
Frey's syndrome	2 (1.4)	0 (0)	0.188
Fistula	4 (2.8)	3 (2.4)	0.856
Seroma	6 (4.2)	8 (6.5)	0.401
Hematoma	8 (5.6)	4 (3.3)	0.391
Great Auricular nerve division	103 (72)	13 (10.6)	<0.0001
Local Relapse	3 (2)	1 (0.8)	0.391

*Partial superficial parotidectomy; †Extracapsular dissection

grade II paresis), and one patient presented with House-Brackmann grade III paralysis. Patients with post-operative facial nerve dysfunction underwent further investigation in the form of clinical and electromyographic examination at regular intervals (2 weeks, 3 months, 6 months and 1 year after surgery).

With the ECD method of parotidectomy, thirteen patients (13/123; 10.6%) reported disturbance of sensation, eight (8/123; 6.5%) developed seroma, four (4/123; 3.3%) hematoma, fistula in three cases (3/123; 2.4%) and local relapse in one (1/123; 0.8%), with a follow up interval of two years (Table 3).

No statistical significance was reported between the two surgical procedures relating to post-operative complications in terms of relapse (P=0.391), hematoma (P=0.391), seroma (P=0.401), salivary fistula (P=0.856) and Frey's syndrome (P=0.188), or facial nerve permanent weakness (P=0.141) (Table 3). A statistically significant difference was observed between the two surgical procedures in hypoesthesia due to great auricular nerve division (P<0.0001) (Table 3).

Discussion

In our study, in the PSP group there was a higher rate of reported sensation abnormalities since the greater auricular nerve was divided, while in the ECD group there was a significantly lower percentage of this postoperative complication. Additionally, the rate of complications in terms of permanent facial nerve weakness in the main trunk or peripheral branch (not completely resolved over a period of 6 months to 1 year) was higher in the PSP group than the ECD group. In the PSP group all facial nerve paralysis was permanent, while in the ECD group 22.2% was permanent. However, in our study no statistically significant difference was found in terms of permanent facial nerve disorders between the two different surgical approaches. In PSP facial nerve function is at higher risk since, apart from the peripheral branches, its main trunk must be dissected (13).

Frey's syndrome is a complication of parotidectomy as a result of the regeneration of the postganglionic parasympathetic nerve fibers in relation to the severed postganglionic sympathetic fibers. In our study, this syndrome seems to occur at low rates with the PSP method and it was not observed in ECD procedures. Salivary fistulas, as well as hematoma, also occur at higher rates, but without statistical significance in PSP, as a greater amount of parotid parenchyma is removed.

According to the current literature, ECD is a safe surgical approach, offering early post-operative recovery and better preservation of salivary function (14). As already reported earlier, the long-term outcomes of the ECD technique could be related to the less radical nature of this surgical technique (12). As reported in the literature, ECD has fewer postoperative complications than PSP (15, 16). A meta-analysis by Martin et al. (2020) reported that ECD shows a lower rate of facial nerve paralysis, Frey's syndrome and recurrence (12). In our study, within 2 years of follow up, the percentage of patients with local recurrence requiring re-operation in the PSP group was similar to the ECD group.

Limitations of the Study

The limitations of our study are the short follow up interval since it has been reported that at least 10 years is necessary to assess the recurrence rate reliably (17). As far as local recurrence concerns, it depends on the integrity of the tumor capsule (6). However, as reported in our study, surgical procedures were excluded in which the intraoperative rupture of the pleomorphic adenoma capsule occurred, with potential tumor spillage into the surgical field (ruptures occurred in approximately 5% of cases). Recurrence, especially of pleomorphic adenoma, is related to surgery and directly linked to tumor spillage or/and capsular exposure, as well as to tumor factors, such as histological subtype, incomplete capsule and pseudopodia (18). It is important to mention that the ECD procedure is not considered as an appropriate treatment for malignant tumors (3, 8, 13). A small malignant tumor (<3 cm) could masquerade as a benign one. In such cases, a CT scan or MRI and FNAC should be performed pre-operatively. It is reported that in

some cases FNAC might not be diagnostic regarding an underling malignancy since it may fail to target a small diameter tumor (8, 13). This study is a retrospective non-randomized study. It is important to underline that tumor size and the location of tumors were not considered as a selection factor for the ECD or PSP surgical techniques. Another limitation of this retrospective study is considered to be the lack of data regarding retrograde facial nerve dissection. According to the current literature, antegrade and retrograde facial nerve dissection did not demonstrate any significant advantage regarding surgical outcomes (19). Therefore, there is still insufficient evidence regarding which dissection approach produces the best results in the treatment of parotid tumors (19).

Conclusion

The recommendation of extracapsular anatomical dissection or partial superficial parotidectomy as the gold standard for treatment of superficial benign tumors of the parotid gland cannot be entirely supported by the literature. Our study aimed to evaluate both procedures in terms of post-operative complications, and concluded that both procedures are safe options, with a significant difference regarding a higher rate of sensation abnormalities due to the division of the great auricular nerve during partial superficial parotidectomy. This statistically significant difference had as a result the change of the surgical technique used in our Institution and therefore extracapsular anatomical dissection is the preferred choice for Warthin's tumors due to the lower rate of great auricular nerve division, in combination with the extremely low recurrent rate of this benign tumor.

What Is Already Known on This Topic:

Benign parotid tumours of the parotid gland present as an asymptomatic mass in the pre-auricular region. The surgical techniques used most often are extracapsular parotidectomy and partial superficial parotidectomy. Many studies have been published describing both surgical procedures without directly comparing them. There is currently no gold standard for benign parotid tumors situated externally to the facial nerve, and it remains unclear which surgical technique is the most appropriate choice.

What This Study Adds:

Both surgical excisions are valuable and safe options for the treatment of benign parotid tumors. The results of this publication tend to suggest that extracapsular parotidectomy is the more appropriate method, with a statistically significantly lower rate of sensation abnormalities caused by the division of the great auricular nerve. Therefore, surgical practice in our Institution has been changed and ECD is the preferred technique for Warthin's tumors. However, according to the literature, ECD cannot be nominated as the gold standard due to various limitations.

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

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Morphometric Analysis of the Supraorbital Foramen and Notch in the Population of Bosnia and Herzegovina

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Abstract

Objective. The aim of this study was to learn about the morphological characteristics of the supraorbital foramen and to determine its precise position in relation to the surrounding anatomical landmarks in the adult population of Bosnia and Herzegovina. **Material and Methods.** For this purpose, 60 skulls from the Bosnia and Herzegovina population of known sex (32 males and 28 females), taken from the osteological collection of the Department of Human Anatomy of the Medical Faculty in Sarajevo, were subjected to morphological and morphometric analysis. Morphometric measurements were performed using a digital vernier caliper (Mitutoyo Corporation, Japan). **Results.** The study showed that most supraorbital nerves exit the orbit through the supraorbital notch (73.8%) and the rest through the foramen (26.2%). Of this number, bilateral supraorbital notches were recorded in 58.33% of cases, a bilateral supraorbital foramen in 18.34% of cases, while in 23.33% of cases a notch was recorded on one side and a foramen on the contralateral side. Morphometric measurements performed to determine the exact position of the supraorbital foramen relative to the surrounding landmarks showed different values in males and females. An accessory foramen was also observed on the examined skulls in 16.67% of cases. **Conclusion.** Detailed knowledge of anatomical variations of the supraorbital foramen is required for safe and successful administration of regional anesthesia, in order to avoid iatrogenic nerve injuries during orbitofacial region surgery.

Key Words: Supraorbital Foramen ▪ Supraorbital Notch ▪ Supraorbital Nerve ▪ Craniofacial Surgery.

Introduction

The supraorbital margin is formed entirely by the squamous part of the frontal bone, which is interrupted at the junction of its sharp lateral 2/3rd and rounded medial 1/3rd by the supraorbital foramen/notch (1). In 25% individuals, the notch is converted into a foramen by ossification of the periosteal ligament crossing it (2), and has been referred to as a supra orbital ligament in the literature (3). The supraorbital notch or supraorbital foramen (SON/SOF) is the passage for the supraorbital artery, veins and nerves in the frontal bone. The supraorbital artery, a branch of the ophthalmic artery, leaves the orbit through the SON/SOF, and divides into superficial and deep branches to supply the skin and muscles of the upper eyelid, forehead and scalp.

The supraorbital nerve is one of the main cutaneous nerves supplying the forehead and scalp region, and may be injured during various invasive procedures. This nerve is the larger terminal branch of the frontal nerve, and after exiting through the SON/SOF it divides into medial and lateral branches to supply the upper eyelid, conjunctiva and skin of the scalp, up to the lambdoid suture. Supraorbital nerve blocks are commonly performed in the region of the supraorbital foramen during procedures such as closure of facial wounds, biopsies and scar revisions, as an absolute but temporary treatment for supraorbital

neuralgia, and other cosmetic cutaneous procedures. Effective and precise analgesia can only be achieved if one is aware of the most frequent location of the exit of the nerve in this region. Knowledge of the location of the supraorbital nerve is also essential during various endoscopic procedures, which are increasingly being used for cosmetic facial surgery (4, 5). However, cosmetic surgeons are generally reluctant to perform brow lifts and other open, as well as endoscopic surgical procedures in this region, for fear of injuring the supraorbital nerve and subsequent sensory loss (6, 7). Excessive dissection and retraction close to such neurovascular bundles can cause scarring, which may lead to entrapment neuropathies and painful neuralgias (8, 9). According to the standard descriptions in anatomy textbooks, the supraorbital notch/foramen is situated at the junction of the lateral two-thirds and medial third of the supraorbital margin (10, 11).

However, most published studies report that the position and morphometric characteristics of the SON/SOF are not constant. In some skulls, cases have been reported of incomplete foramina, double foramina, a double notch or the absence of all of them (12-18). In the absence of supraorbital foramina or notches, the supraorbital vessels and nerves are more prone to injury due to the sharp supraorbital margin.

Traumatic or iatrogenic injury to the neurovascular bundle at the point of emergence through the foramina may result in bleeding and hypoesthesia, paraesthesia or even anesthesia in the region of supply, depending on the degree of injury (19, 20). Despite its significance, little is known about the morphological details and location of the supraorbital notch/foramen in the adult population of Bosnia and Herzegovina.

Hence, this study was carried out to elucidate the number, dimensions, orientation and position of the supraorbital notch/foramen in relation to the surgically encountered anatomical landmarks in an adult population in Bosnia and Herzegovina.

Material and Methods

Sixty adult human skulls (28 male and 32 female) were used as material in this study. The skulls were taken from the osteological collection of the Department of Human Anatomy, Faculty of Medicine, University of Sarajevo. Skulls of known sex and age (53±21), without visible gross pathology, deformities or traumatic lesions were included in the study.

Both sides of the skull were analyzed visually, and the presence of supraorbital foramina (SOF) or notches (SON) was noted, as well as the presence of accessory foramina or notches (SONA/SOFA), and their number. The position of the SON/SOF relative to the infraorbital foramen (IOF) was noted as lying in the same vertical plane as the IOF or lying laterally or medially from this plane.

Morphometric measurements were performed using digital vernier calipers 0-1000mm, 0.05mm, Metric 530-502 (Mitutoyo Corporation, Japan), with a margin of error of 0.01mm. All the measurements were repeated three times, and the mean was taken for further analysis. Furthermore, the measurements were recorded by the same person, to minimize errors in the methodology.

The following is a description of all the measurements taken (Figure 1):

1. Maximum transversal diameter of the supraorbital foramen (SOF-TD),
2. Maximum vertical diameter of the supraorbital foramen (SOF-VD),
3. Vertical distance between the lower edge of the supraorbital foramen and the upper orbital edge (SOF-SOM),
4. Distance from the medial edge of the supraorbital notch and foramen (SON/SOF) to the midline of the face (SON/SOF -FM),
5. Distance from the lateral edge of the SON/SOF to the temporal crest of the frontal bone (SON/SOF -TCFB),
6. Distance from the SON/SOF side edge to the frontozygomatic suture (SON/SOF -FZS).

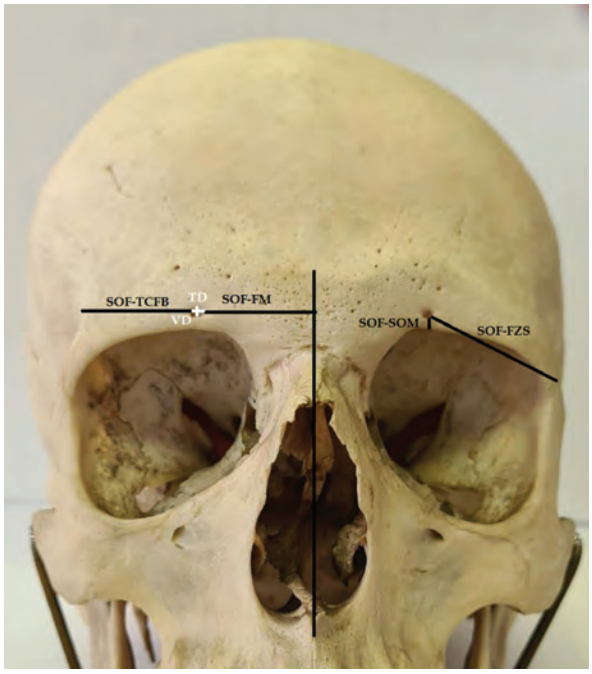

Figure 1. A skull showing the measurements taken to determine the position and dimensions of the supraorbital foramen. SOF=Supraorbital foramen; SOM=Supraorbital margin; FM=Facial midline; TCFB=Temporal crest of the frontal bone; FZS=Frontozygomatic suture; VD=Vertical diameter; TD=Transversal diameter.

Statistical Analysis

All parameters were analyzed using SPSS version 19 (SPSS Inc., Chicago, IL, USA), and data were compiled in Microsoft Excel 2020 (Microsoft Corp., Redmond, WA, USA) and shown in tables. Descriptive analysis was used to estimate the mean and standard deviation. A comparison of the mean values between sides and genders was performed using the paired and unpaired samples t-test. A P-value less than 0.05 was accepted as the level of statistical significance for this study.

Results

In the present study, supraorbital openings were found in the form of notches (73.8%) or foramina (26.2%) on all 120 sides of the skulls examined. Bilateral supraorbital notches were found in 58.33% of cases, and bilateral supraorbital foramina in 18.34%, while in 23.33% of cases a notch was recorded on one side and a foramen on the contralateral side (Table 1).

An accessory supraorbital foramen was observed in 10 (16.67%) skulls (Table 1), and of these, in three cases accessory foramina were present bilaterally. The most common position of accessory supraorbital foramina in relation to the main SON/SOF was lateral in 61.2% of sides, followed by medial in 28.3%, and superior in 10.5%.

The dimensions of the supraorbital foramen and linear measurements from the SON / SOF to different anatomical landmarks in relation to sex and sides are summarized in Tables 2 and 3, respectively.

In our study, it was noted that the SON/SOF distance from the midline of the face was 24.45 ± 2.75 and 23.79±3.45 mm on the right and left sides, and 24.87±3.63 and 22.46±3.07 mm in males and females, respectively.

The mean SON/SOF distance from the temporal crest of the frontal bone in the Bosnia and Herzegovina population was 30.04±3.48 mm on

Table 1. The Morphological Features of the Supraorbital Nerve Exits

Morphological features	Male: N=28	Female: N=32	Total: N=60
	N (%)	N (%)	N (%)
Bilateral supraorbital notches	20 (71.43)	15 (46.87)	35 (58.33)
Bilateral supraorbital foramen	2 (7.14)	9 (28.13)	11 (18.34)
Unilateral notch and foramen	6 (21.43)	8 (25.00)	14 (23.33)
Bilateral accessory foramen	2 (7.14)	1 (3.13)	3 (5.00)
Unilateral accessory foramen	5 (17.86)	2 (6.25)	7 (11.67)

Table 2. Morphometric Measurements of the Supraorbital Notches/Foramina in Relation to Gender

Measurements	Males (N=28)			Females (N=32)			P†
	Mean±SD*	Min.	Max.	Mean±SD*	Min.	Max.	
Maximum transverse diameter of SON/F (TD)	3.75±1.36	2.39	3.69	3.58±1.04	2.29	2.69	0.940
Maximum vertical diameter of SON/F (VD)	1.98±0.76	1.08	2.73	1.83±0.73	1.02	1.82	0.383
Distance from SON/F to SOM	2.98±1.70	0.77	8.07	2.78±1.31	1.94	4.14	0.071
Distance from SON/F to FM	24.87±3.63	17.89	30.49	22.46±3.07	17.31	31.11	0.002
Distance from SON/F to TCFB	29.71±3.58	20.4	35.6	27.89±3.26	20.63	39.33	0.007
Distance from SON/F to FZS	28.30±2.56	22.88	35.88	27.16±2.74	22.14	32.44	0.015

*SD=Standard deviation; †Unpaired Samples *t*-test; SON/F= Supraorbital foramina or notches; TD= Transversal diameter; VD=Vertical diameter; SOM=Supraorbital margin; FM= Facial midline; TCFB=Temporal crest of the frontal bone; FZS=Frontozygomatic suture.

Table 3. Morphometric Measurement of the Supraorbital Notch/foramina in Relation to Side

Measurements	Total (n=60)			Right (n=60)			Left (n=60)			P†
	(Mean±SD)* mm	Min	Max	(Mean±SD)* Mm	Min	Max	(Mean±SD)* mm	Min	Max	
Maximum transverse diameter of SON/ F (TD)	3,65±1,35	2,32	3,25	3.66±1.44	2,15	3,65	3 .63±1.25	2,48	2,84	0.486
Maximum vertical diameter of SON/F (VD)	1,99±0,69	1,14	2,39	2.09±0.74	1,03	2,73	1.89±0.64	1,24	2,04	0.081
Distance from SON/F to SOM	2,84±1,43	2,18	6,78	2.88±1.31	3,37	5,27	2.79±1.54	0,98	8,28	0.648
Distance from SON/F to FM	24,12±3,1	15,59	28,79	24.45±2.75	15,43	29,23	23.79±3.45	15,74	28,34	0.383
Distance from SON/F to TCFB	29,54±3,5	18,31	32,96	30.04±3.48	16,81	36,31	29.04±3.52	19,81	29,61	0.361
Distance from SON/F to FZS	27,82±2,64	21,99	33,09	27.99±2.83	22,16	35,66	27.64±2.45	21,81	30,51	0.229

*SD=Standard deviation; †Paired Samples *t*-test; SON/F= Supraorbital foramina or notches; TD= Transversal diameter; VD=Vertical diameter; SOM=Supraorbital margin; FM= Facial midline; TCFB=Temporal crest of the frontal bone; FZS=Frontozygomatic suture.

the right and 29.04±3.52 mm on the left side, and 29.71±3.58 mm in males and 27.89±3.26 mm in females.

The mean recorded value of the SON/SOF distance from the frontozygomatic suture was 27.64±2.45 mm on the right and 27.99±2.83 mm on the left side, and 29.71±3.58 mm in males and 27.16±2.74 mm in females.

In males, higher values of SOF dimensions and linear distances from the SON/SOF to anatomical landmarks were observed compared to females. However, a statistically significant difference was only observed in the distance from the SON/SOF to the temporal crest of the frontal bone, and the distance from the medial edge of the SON/SOF to the midline of the face (Table 2).

Higher values of these dimensions were recorded on the right side compared to the left side, but without statistical significance (Table 3).

The position of the SOF relative to the position of the IOF is shown in Table 4. According to the results of this study, most supraorbital foramina (78.4%) are located medially of the infraorbital foramen, where only 15.8% of subjects had both foramina in the same sagittal plane, and in 5.8% of subjects the supraorbital foramen was located laterally from the infraorbital foramen.

Table 4. Frequency of the Location of the SON/SOF in Relation to the Position of the IOF

Position of SON/SOF in relation to the position of IOF	N (%)
Medial to the IOF	94 (78.4)
Lateral to the IOF	7 (5.8)
In the same vertical plane as IOF	19 (15.8)

SON/SOF=Supraorbital notch/foramen; IOF=Infraorbital foramen.

Discussion

This study provides valuable data on the morphometry and relative location of the supraorbital notch or foramen in the adult population of Bosnia and Herzegovina. Precise identification of the supraorbital notch or foramen is important in therapeutic, diagnostic, anesthetic and surgical procedures of the maxillofacial region (21-24).The appearance of a supraorbital notch (73.8%) was more frequent than the appearance of a foramen (26.2%), which is a result that supports the observations made in some studies (14, 20, 25), but contradicts the observations of Lima et al. (26).

The mean distances from the supraorbital notch or foramen to the anatomical landmarks mentioned above were significantly greater in Bosnia and Herzegovina males than in females. Our results support the gender differences in the position of the supraorbital notch or foramen reported in previous studies (12, 13, 27). Gender differences in the relative position of the supraorbital notch or foramen emphasize the importance of applying data on anatomical variations to an individual subject in a specific population (13).

The mean distances from the supraorbital notch or foramen to the midline of the face, the temporal crest of the frontal bone, and the frontozygomatic suture observed in this study were consistent with those reported in studies by Agthong et al., Gupta et al., and Nanayakkar et al. (13, 14, 28), while differing from those reported in the studies by Webster et al., Chrcanović et al., Chung et al. and Smith et al. (15, 18, 29, 30). It is speculated that these differences could be caused by ethnic or climatic factors, which confirms the results of previous research (12, 13, 27, 31, 32, 33, 34).

We cannot say with certainty which of the above-mentioned factors influenced the various measurements in males and females of the Bosnian-Herzegovinian population, because for such conclusions it is necessary to conduct much more extensive research that would include a larger number of samples and the collection of samples from the entire territory of Bosnia and Herzegovina. The insufficient number of samples in the present study is a limiting factor that prevents the results from being applied to the entire population of Bosnia and Herzegovina. For these reasons, the goal of our future research will be to conduct a more extensive analysis on the given topic.

Population-specific linear measurements have clinical implications as they may aid in precise localization, thereby avoiding injury to the neurovascular bundle exiting through the supraorbital foramen or notch. Surgically, it can be difficult to locate the midline of the face accurately, and in such cases the distance from the temporal crest of the frontal bone is considered a better anatomical landmark (12).

The standard anatomy texts describe the location of supraorbital and infraorbital foramina on the same sagittal plane (1, 10, 11). Although this is in accord with some European populations, it ignores a large body of evidence with reference to other populations (27, 29). Such diversity in the location of the supraorbital foramen may be attributed to ethnic factors (12, 29). According to the results of this study, the majority of supraorbital foramina (78.4%) were located medially to the infraorbital foramen, only 15.8% of the study subjects displayed both foramina in the same sagittal plane, and in 5.8% of subjects the supraorbital foramen was located laterally from the infraorbital foramen. Our findings are consistent with the corresponding figures of Thais (27) and Koreans (29), highlighting the racial differences in the modal position of the supraorbital foramen in relation to the infraorbital foramina observed in different populations.

The incidence of accessory supraorbital foramina in this population from Bosnia and Herzegovina was found to be 16.67%. The occurrence of multiple supraorbital foramina was shown to vary widely between different populations (14, 17, 25, 33, 35). Multiple facial foramina have been associated with the branching of nerves during development, and may explain cases of failure of infiltrative anesthesia for maxillofacial procedures (36). Furthermore, the existence of multiple foramina in a minority of patients also has clinical

implications, as injury to any branch of the supraorbital nerve that exits through these foramina may result in sensory deficit (34, 37).

Determining the position of neurovascular bundles that pass through the appropriate openings on the face, on the basis of the surrounding landmarks, increases the success of surgical interventions, and reduces the risk of iatrogenic damage to these bundles. For this reason, it is necessary to make a detailed analysis of SON/SOF variations for each population individually.

Conclusion

As far as we know, this is the first study to address the anatomical variations of supraorbital notches or foramina in the Bosnia and Herzegovina population. It is important to emphasize that supraorbital notches or foramina show numerous morphological and morphometric variations, so special attention should be paid during surgical procedures to determine their exact location and thus avoid iatrogenic injuries of the neurovascular structures passing through them.

What Is Already Known on This Topic:

A supraorbital foramen (SOF) or notch (SON) is present at the junction of the lateral two-third and medial one third of the supraorbital margin. According to previous studies, in 25% of cases the supraorbital notch is converted into a foramen by ossification of the periosteal ligament bridging it. The supraorbital foramen/notch transmits neurovascular structures, namely the supraorbital artery, veins and nerve, and supplies the area around the eye, and the skin over the forehead. These neurovascular structures are prone to injury during various procedures performed in their areas of supply, and this will lead to damage of the structures being supplied by them.

What This Study Adds:

Despite its significance, little is known about the morphometric details of supraorbital foramina in the population of Bosnia and Herzegovina. Hence this study was carried out to elucidate the number, dimensions, position and orientation of supraorbital foramina in relation to the anatomical landmarks in dry skulls from Bosnia and Herzegovina.

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

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Comparison of Effectiveness between Wycope Video Laryngoscope, C-MAC Video Laryngoscope, and Direct Laryngoscope in Intubation of Elective Surgery Patients

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Abstract

Objective. Airway management has undergone a dramatic transformation since the arrival of video laryngoscope (VL). VL has higher intubation success rate on first try and lower complications in comparison to direct laryngoscope (DL). The use of VL is recommended in intubating COVID-19 patients to speed up intubation time and reduce failure rate. A team from Airlangga University developed Wycope Video Laryngoscope (Wycope VL), a VL with Wi-Fi connection to smartphones for an easier VL with low cost. This study aimed to compare the effectiveness of Wycope VL, C-MAC Video Laryngoscope (C-MAC VL), and DL. **Materials and Methods.** This study was an analytic observational study with a cross sectional design, involving 63 patients who were divided into 3 groups based on the type of laryngoscope, namely Wycope VL, C-MAC VL, and DL. Intubation is carried out by 4th year anaesthesiology resident. Research subjects were patients who will undergo elective surgery at Dr. Soetomo General Hospital under general anaesthesia using orotracheal tube. Inclusion age of 19-64 years, PS ASA 1-2, no anatomical abnormalities of the airway, did not have difficult airway, and was willing to participate in the study. **Results.** All patients were successfully intubated without complications. C-MAC VL (5.33±1.42 seconds) and Wycope VL (5.95±0.74 seconds) was significantly faster in seeing vocal folds and glottis compared to DL (7.14±0.72 seconds) with P=0.000. DL was significantly faster in average time of intubation (15.52±5.90 seconds) compared to C-MAC VL (16.95±1.11 seconds) and Wycope VL (20.29±2.81 seconds) with P=0.000. **Conclusion**. DL was faster compared to VL in speed of intubation while C-MAC VL and Wycope VL was faster in viewing the vocal folds and glottis compared to DL.

Key Words: Laryngoscope ▪ Airway Management ▪ Intubation.

Introduction

The video laryngoscope (VL) is rapidly gaining popularity as an intubation device in a variety of scenarios and clinical settings among airway management specialists. In Sakles' study, the success of intubation using a C-MAC video laryngoscope (C-MAC VL) was higher than direct laryngoscope (DL) both in the first and second intubation attempt (1). Lewis' study showed that video laryngoscopy improved the ease of laryngeal visualization in patients without a predicted difficult airway (OR 6.77, 95% CI 4.17-10.98) and with a predicted difficult airway (OR 7.13, 95% CI 3.12-16.31) compared to DL (2). Difficult intubation is associated with serious complications such as brain damage from hypoxia and hypercarbia. Other side effects include dental injury caused by repeated attempts at intubation, laryngeal spasm, and bronchial spasm (3). Based on data from the American Society of Anesthesiologists, the incidence of difficult airway and failed airway in the operating room is 1.2%-3.8% and 0.13-0.30% and can increase by 20% in other rooms such as the Intensive Care Unit (ICU) (4). Griesdale in his study found that the incidence of difficult intubation was 6.6% in the ICU of the Vancouver General Hospital (VGH) and 39% of patients experienced complications (5).

The act of intubation itself poses a risk of transmission of infectious diseases including COVID-19 from the patient's mouth to a specialist in Anesthesiology and Intensive Therapy. The use of a video laryngoscope is recommended as a strategy for intubating COVID-19 patients with the aim of speeding up intubation time and reducing intubation failure thus decreasing the possibility of complications and aerosol disease transmission (6).

Visualization using a video laryngoscope improves visualization of the glottis, including suspected or encountered difficult intubation, thereby reducing the rate of failed intubation, accelerating intubation time and complications of airway trauma, thus increasing the effectiveness of intubation (2). However, successful visualization does not always results in successful endotracheal intubation, there were cases in which intubation attempt failed despite good visualization of the glottis.

The innovations made on the video laryngoscope itself are not new at Airlangga University. We hope to create better, cheaper, and superior video laryngoscopes as part of technological advances. Wycope video laryngoscope is one of the innovations developed by a team from Airlangga University. The use of Wycope video laryngoscope is expected to facilitate intubation with low production costs, so that this tool can be said to have good effectiveness.

Figure 1. Wycope Video Laryngoscope.

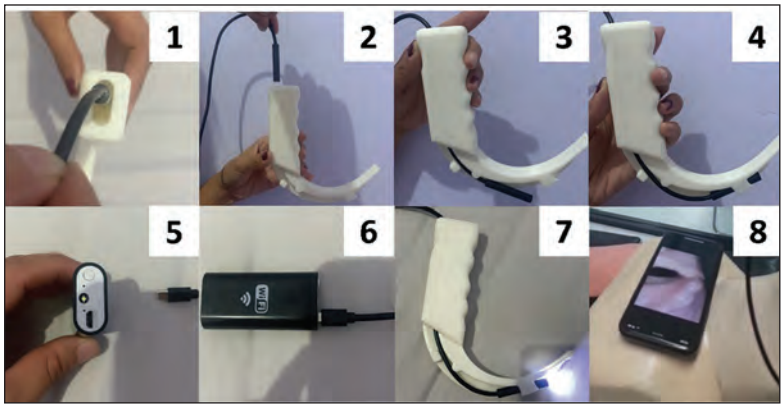

Figure 2. Setting up the Wycope Video Laryngoscope. The camera is connected to a cellphone via Wi-Fi connection. The cellphone can be placed on top of patient's chest or held by an assistant or placed anywhere else as convenient.

The Wycope video laryngoscope consists of 2 main components, namely the frame of the laryngoscope and the laryngoscope camera cable. The Wycope laryngoscope frame is a combination of a handle and a blade. This blade from Wycope has a 60 degree angle similar to the Glidescope but with a different camera placement, design and material. The laryngoscope frame is made of PLA (Polylatctic Acid) which is printed with a 3D printer. PLA itself has been widely used in the manufacture of medical equipment in handling COVID-19 patients due to its physical properties (7). The camera cable used here is a Wi-Fi endoscope camera which is commonly used for endoscopy. The choice of this camera is because the camera used is very good and clear and has sufficient lighting. The Wi-Fi connection used is easy to connect with cellphones from Android or IOS systems. The application used as a liaison is inskam. In using the application there is no delay from the

connection. The use of this Wi-Fi uses a battery attached to the cable and can be recharged. The price of this Wi-Fi endoscope is relatively affordable and can be replaced if it is damaged. The cost of a full set is around 75 USD. The frame is disposable, it cost around 25 USD. Sharing one frame for more than one patient is not recommended.

This study was conducted to find out whether there are significant differences in time required to see the vocal folds and glottis, intubation time, and use of BURP (back, upward, right lateral, pressure) maneuver between Wycope VL, C-MAC VL, and DL. Aim of this study was to compare the effectiveness of using all three laryngoscopes for intubation

Materials and Methods

This research is an analytic observational study. This study involved 63 patients who were divided into 3 groups based on the type of laryngoscope, namely Wycope VL, C-MAC Video laryngoscope (C-MAC VL), and DL. The research subjects were

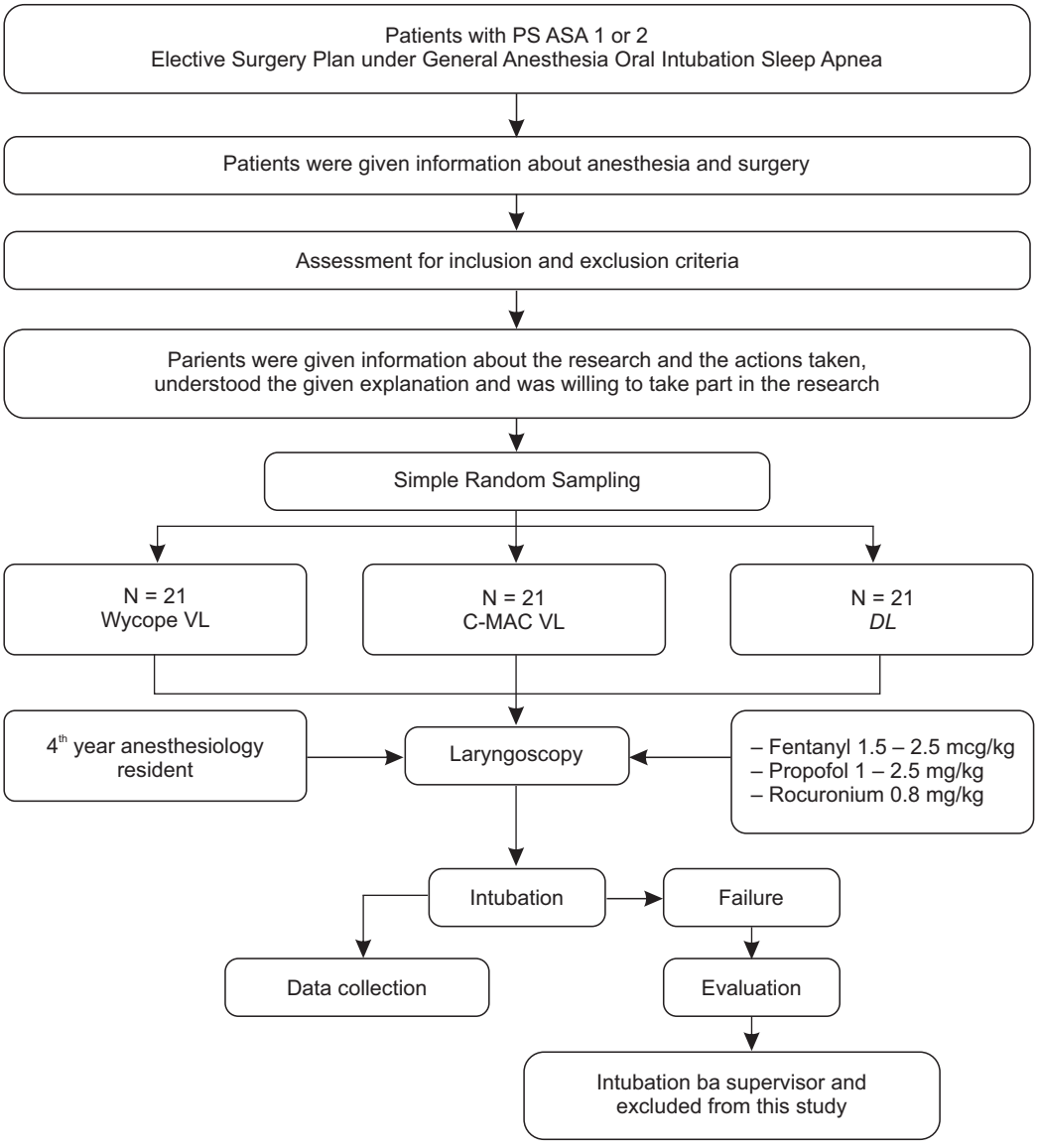

Figure 3. Study overview.

patients who would undergo elective surgery at Dr. Soetomo General Hospital under general anesthesia with oral intubation within April-June 2021. All patients met the inclusion criteria and none of them met the exclusion criteria. Intubation was carried out by three 4th year anesthesiology residents who had undergone residency in the same period of time and had attended the same trainings for each laryngoscopes.

Operator who would perform the intubation did the pre-surgery assessment the day before the surgery. Inclusion criteria were age of 19-64 years, PS ASA 1-2, normal airway anatomy, and was willing to participate in the study. Exclusion criteria for the study included difficult airway and failure of intubation by the resident and was replaced by the supervisor. The patients had predicted difficult airway if they had one or more of the followings: obesity, Mallampati score of 4, incisor distance less than 3 cm, mentohyoid distance less than 3 cm, hyothyroid distance less than 2 cm, limited neck range of motion, or history of difficult intubation.

Patients and their families were explained about the information on surgical and anesthetic procedures and the research carried out. Written informed consent was obtained from participants 1 day before the surgery. The process and protocol of this study were approved by the Ethics Committee of Dr. Soetomo General Hospital.

All patients who met the inclusion criteria and did not have difficult airway were numbered sequentially from number one. Among all of them 63 numbers/patients were chosen randomly. These chosen numbers represented chosen patients. Chosen numbers were arranged from the smallest to the biggest and then assigned to Wycope VL group, C-MAC VL group, and DL group consecutively from the smallest number to the biggest one.

Patients were positioned supine and preoxygenation was given. Drugs administered were fentanyl 1.25-2.5 mcg/kg body weight, propofol 1-2.5 mg/kg bodyweight, and rocuronium 0.8 mg/kg bodyweight. Stylet and non-kink endotracheal tube was used in all intubation. Blades used were type C Macintosh blades for DL and C-MAC VL. For DL, blades used were blades with 60 degrees arch similar to type D blade. Monitoring included blood pressure, peripheral oxygen saturation, electrocardiogram, and end-tidal carbon dioxide (ETCO2). Assistant only assisted by doing BURP (back, upward, right lateral, pressure) maneuver when needed. All assistants had a minimum of 4 years of experience working in operating theatre or critical care unit.

Characteristics data of research subjects include gender, PS ASA, age, body mass index (BMI), height, weight, and Cormack-Lehane degree. Main outcomes of this study were: (1) time required to see the vocal folds and glottis counted from insertion of the laryngoscope tip through the patient's lips until operator could assess the Cormack-Lehane grade and said "seen", (2) intubation time counted from insertion of the laryngoscope tip through the patient's lips, insertion of the endotracheal tube, until the laryngoscope were completely taken out and its tip already passed through the patient's lips, and (3) number of patients whom BURP (back, upward, right lateral, pressure) maneuver was done during the intubation process.

Statistical Analysis

Data collected was processed using different tests. Normality of ratio scale data was tested with Shapiro-Wilk test. Then it was analyzed with Mann-Whitney test because the data was not normally distributed. For nominal scale data, it was processed using the Fisher's Exact test because of the small sample size. Data analysis was performed using SPSS ver.26.0. A value of $P<0.05$ was considered significant.

Results

This study involved 63 subjects divided into 3 groups according to the type of laryngoscope. Subjects were homogeneous in terms of gender, PS ASA, age, body mass index (BMI), height, weight, and Cormack-Lehane degree as seen in Table 1.

Table 1. Characteristics of Research Subjects

Characteristics	Wycope VL	C-MAC VL	DL	P value
Gender				
Male (N; %)	8 (38.1)	8 (38.1)	10 (47.6)	0.77
Female (N; %)	13 (61.9)	13 (61.9)	11 (52.4)	
PS ASA				
1 (N; %)	5 (23.8)	6 (28.6)	8 (38.1)	0.589
2(N; %)	16 (76.2)	15 (71.4)	13 (61.9)	
Age (year)	42.29±11.42	40.81±11.90	33.90±13.07	0.054
BMI	23.33±2.67	22.80±2.81	22.73±3.26	0.843
Grade CL				
1 (N; %)	15 (71.4)	16 (76.2)	16 (76.2)	0.283
2 (N; %)	6 (28.6)	5 (23.8)	3 (14.3)	
3 (N; %)	0 (0.0)	0 (0.0)	2 (9.5)	
Weight (kg)	58.67±9.32	59.76±9.284	60.00±10.84	0.896
Height (cm)	158.38±7.304	161.29±9.93	161.81±8.19	0.381

Wycope VL=Wycope Video Laryngoscope; C-MAC VL=C-MAC Video Laryngoscope; DL=Direct Laryngoscope; PS ASA=Physical Status American Society of Anesthesiologists; BMI=Body Mass Index; CL=Cormack-Lehane Degree.

Table 2. Time Required to See the Vocal Folds and Glottis Using Wycope DL, C-MAC VL, and DL

Statistics	Wycope VL	C-MAC VL	DL
Mean+SD (seconds)	5.95±0.74	5.33±1.42	7.14±0.72
Median (seconds)	6	5	7
Min-max (seconds)	5–7	3–9	6–8

Wycope VL=Time required to see the vocal folds and glottis using the Wycope Video Laryngoscope; C-MAC VL=Time required to see the vocal folds and glottis using the C-MAC Video Laryngoscope; DL=Time required to see the vocal folds and glottis using the Direct Laryngoscope.

Observation of the difference in time required to see the vocal folds and glottis between DL and C-MAC VL, DL and Wycope VL, C-MAC and Wycope VL showed significant result with P value 0.000, 0.000, and 0.19 respectively. The average time in the DL group was 7.14±0.72 seconds with a range of 6-8 seconds and a median of 7 seconds. In the C-MAC VL group, the average time was 5.33±1.42 seconds with a range of 3-9 seconds and a median of 5 seconds. While in the Wycope VL group, the average time was 5.95±0.74 seconds with a range of 5-7 seconds and a median of 6 seconds (Table 2 and 5).

Data of intubation time was not normally distributed due to lack of variation. The median time in the DL group was 15 seconds, in the C-MAC VL group was 17 seconds, and in the Wycope VL group was 19 seconds. In the Wycope VL group, the range of intubation time was 17-28 seconds with an average time of 20.29±2.81 seconds. In the C-MAC VL group, the range was 15-19 seconds with an average time of 16.95±1.11 seconds. Meanwhile, in the DL group, the range was 11-36 seconds with an average time of 15.52±4.90 seconds. Comparison analysis between DL and C-MAC VL, DL and Wycope VL, C-MAC and Wycope VL showed there was significant difference in the time required for intubation with P=0.000 (Table 3 and 5).

BURP maneuver was used in 1 patient (4.8%) in the Wycope VL group, 3 patients (14.3%) in the C-MAC VL group, and 9 patients (42.9%) in the

Table 3. Intubation Time Using Wycope VL, C-MAC VL, and DL

Statistics	Wycope VL	C-MAC VL	DL
Mean+SD (seconds)	20.29±2.81	16.95±1.11	15.5±4.90
Median (seconds)	19	17	15
Min-max (seconds)	17–28	15–19	11–36

Wycope VL=Intubation time using the Wycope Video Laryngoscope; C-MAC VL=Intubation time using the C-MAC Video Laryngoscope; DL=Intubation time using the Direct Laryngoscope.

Table 4. BURP Maneuver

BURP Maneuver	Wycope VL	C-MAC VL	DL
Used	1 (4.8%)	3 (14.3%)	9 (42.9%)
Not used	20 (95.2%)	18 (85.7%)	12 (57.1%)

BURP Maneuver=Back, upward, right lateral, pressure maneuver; Wycope VL=Wycope Video Laryngoscope; C-MAC VL=C-MAC Video Laryngoscope; DL=Direct Laryngoscope.

Table 5. Statistical analysis using Mann-Whitney test and Fisher's Exact test

Outcomes	P value	DL and C-MAC VL	DL and Wycope VL	C-MAC and Wycope VL
Time required to see the vocal folds and glottis	Test*	0.000	0.000	0.190
Intubation time	Test†	0.000	0.000	0.000
Use of BURP maneuver	Test‡	0.085	0.009	0.606

Wycope VL=Intubation using Wycope Video Laryngoscope; C-MAC VL=Intubation using C-MAC Video Laryngoscope; DL=Intubation using Direct Laryngoscope; BURP Maneuver=Back, upward, right lateral, pressure maneuver; *Analyzed using Mann-Whitney test; †Analyzed using Mann-Whitney test; ‡Analyzed using Fisher's Exact test.

DL group. Statistical analysis showed significance between Wycope VL and DL with P=0.009, and no significance between C-MAC VL and DL also Wycope VL and C-MAC VL with P=0.085 and 0.606 respectively (Table 4 and 5).

Discussion

This study aimed to compare the effectiveness of using all three laryngoscopes for intubation, therefore the factors which might affect the intubation process should be made as similar as possible. Those factors included anatomy of the airway, head position, preoperative anesthesia, drugs, operator and assistant, as well as the preparation of other intubation equipment (8).

Anatomy of the airway was made homogenous by excluding the patients who were predicted to have a difficult airway on physical examination. All

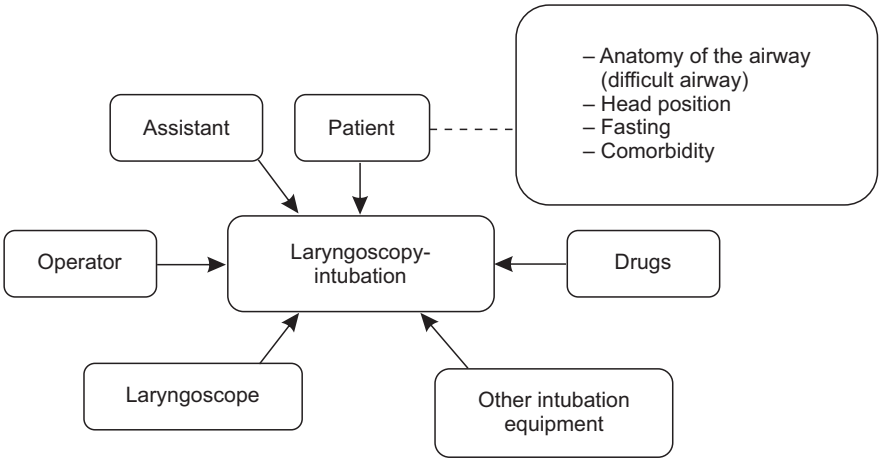

Figure 4. Factors affecting laryngoscopy and intubation.

patients in this study were given the same relaxant drug, dosage was adjusted according to body weight. The operators' skill was equalized before to maintain the homogeneity of this study (8).

All subjects were homogeneous in terms of gender, PS ASA, age, body mass index (BMI), height, weight, and Cormack-Lehane degree. Cormack-Lehane degree was used for assessment of possible complications during intubation (8).

Comparison of average laryngoscopy time using Wycope VL, C-MAC VL, and DL was statistically significant even though the difference was less than a second to two seconds. Both C-MAC VL and Wycope VL were significantly faster than DL. C-MAC VL was significantly faster than Wycope VL. These result was in accordance to the theory that VL facilitates laryngoscopy compared to DL thus time efficiency could be achieved without having to do a sniffing position and BURP maneuver. Difference of time needed using Wycope VL and C-MAC VL matched the theory regarding the operator's experience or skill as use of C-MAC VL was more often than Wycope VL (2, 8-11).

In this study, we found that DL was significantly faster in intubation time compared to C-MAC VL and Wycope VL. C-MAC VL was significantly faster than Wycope VL. Data of intubation time was not normally distributed due to the lack of variation. The median time in the DL group was 15 seconds, in the C-MAC VL group it was 17 seconds. Meanwhile, in the Wycope VL group, the median time was 19 seconds. We concluded that

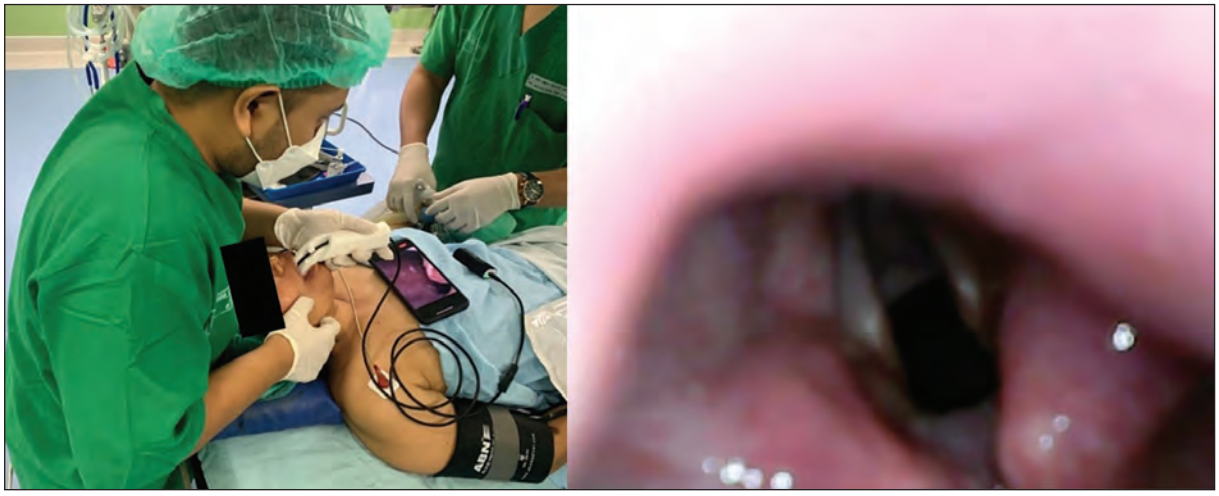

Figure 5. Intubation using Wycope Video Laryngoscope.

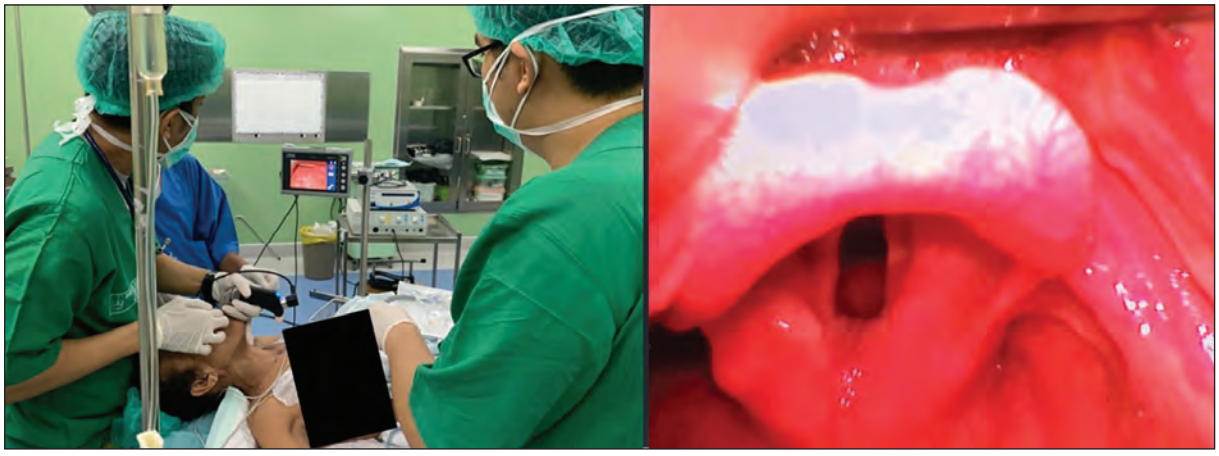

Figure 6. Intubation using C-MAC Video Laryngoscope.

in this study the use of DL for intubation was faster than VL. As to why intubation using Wycope VL took the longest time was because the operator had not used Wycope VL for a long time in comparison to DL and C-MAC VL. DL was most frequently used by 4th year residents, so the time required was shortest. The image quality of the camera also affected the intubation time. The use of VL requires experience/expertise from the operator especially in eye and hand coordination when inserting the endotracheal tube through the vocal fold opening. There was a difference in the field of view provided by the C-MAC VL and the Wycope VL due to different distance of the camera to the tip of the blade and blade curvature between the Wycope VL and C-MAC VL. Emergency conditions or elective surgeries also affected intubation procedure in terms of patient preparation and medication hence this study which was in elective surgery setting might not have the same results as studies carried out in emergency settings. Intubation time with DL was faster in this study because all of the patients did not have difficult airway whereas VL presented better laryngeal visualization and could aid intubation especially in patients with difficult airway. Wycope VL could aid intubation process by offering easier laryngeal visualization than DL with lower cost compared to C-MAC VL. The convenience offered by the VL might be significant if the operators had equal experiences on using both DL and VL (2, 8-11).

Observations on 63 subjects divided into three treatment groups showed that all subjects were successfully intubated and no complications of airway trauma were found in all three groups. This was due to several things, including: the homogeneity of the research subjects' characteristics, there was no patient with difficult airway, setting of the study which was elective surgery, and all operators were 4th year anesthesiology residents therefore they had sufficient skill and experience on all three laryngoscopes at the same level (2, 8, 11-13). The use of BURP maneuver between Wycope VL and DL showed significant difference. BURP maneuver was used less frequently in VL as VL provides better visualization. BURP maneuver was performed to aid visualization of epiglottis and vocal chords (8, 10, 11).

Limitations of the Study

Limitation of this study was the small sample size as it might lead to a biased result. Other limitations were this study only conducted in elective surgery setting but not in emergency conditions and all patients who had difficult airway were excluded thus we could not analyze the success rate of each device. Assessment for difficult airway was done, however data of Mallampati score, incisor distance, mentohyoid distance, hyothyroid distance, neck range of motion, and history of difficult intubation were not collected despite being checked before enrolling patients to this study. Although all operators had sufficient skill and experience in using DL, C-MAC VL and Wycope VL, the use of DL was still more frequent so they were more experienced in using DL. They also had more experience and was more accustomed to using C-MAC VL as it was used more often compared to Wycope VL. Future study with larger sample size, in emergency setting, with operators who has equal experiences on using DL, C-MAC VL, and Wycope VL might show different result as bias will be minimalized and subjects will be more heterogeneous.

Conclusion

This study showed that there were significant differences in the speed of laryngoscopy and intubation using the three laryngoscopes. The DL was faster than VL in speed of intubation while the C-MAC VL and Wycope VL was faster in viewing the vocal folds and glottis compared to DL.

What Is Already Known on This Topic:
Video laryngoscope is a rising device in airway management especially in intubation. Difficult intubation is related to serious complications one of which is brain damage from hypoxia and hypercarbia. Studies showed VL had better visualization and time required in laryngoscopy in comparison to DL.

What This Study Adds:
In this study we compare the effectiveness of Wycope VL, C-MAC VL, and DL in adult patients who did not have difficult airway and in

elective surgery setting. Wycope VL is a VL developed by a team from Airlangga University to aid laryngeal visualization in intubation with lower cost. C-MAC VL and Wycope VL was faster in viewing the vocal folds and glottis compared to DL although DL was faster in speed of intubation as all of the operators used DL more frequently compared to C-MAC VL and Wycope VL.

Authors' Contributions: All authors contributed equally to the article.

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

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Narrative Review
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Researching the Phenomenon of Poor Ovarian Responders and Management Strategies in IVF: A Narrative Review

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Abstract
This narrative review aims to summarize all the latest studies published between 2015-2021 concerning the management protocols adopted for poor ovarian response (POR) cases. Patients defined as "poor responders" show minimal response to controlled ovarian hyperstimulation, although there is no standard definition for POR. Although infertility specialists are endeavoring to improve cycle outcomes in poor responders by adopting multiple management strategies, still the estimated risk of cycle cancellation is about 20%. All the studies performed during this study period were evaluated and their results were recorded. The latest published protocols to improve oocyte retrieval in poor responders include: anti-Müllerian hormone, clomiphene citrate, co-enzyme $Q_{10,}$ corifollitropin, dehydroepiandrosterone, double stimulation, Follicle Stimulation Hormone, Growth Hormone, Gonadotropin-releasing hormone agonists, letrozole, human chorionic gonadotropin, Luteinizing Hormone, progesterone and testosterone. **Conclusion.** Although many strategies have been suggested to manage POR, none has been proven superior to the others. Further large-scale randomized studies are needed to validate experimental techniques leading towards successful individualized treatment regimens.

Key Words: Poor Ovarian Response ▪ Assisted Reproduction Technology ▪ Controlled Ovarian Hyperstimulation ▪ Bologna Criteria ▪ POSEIDON (Patient-Oriented Strategies Encompassing Indivindualized Oocyte Number) Classification.

Introduction

Assisted reproduction technology (ART) has given hope to millions of couples suffering from infertility since the first In Vitro Fertilization (IVF) baby was born in 1978. Infertility experts strive to maximize reproduction success rates (1). However, IVF has lower success rates in women who respond poorly to Controlled Ovarian Hyperstimulation (COH), and they are described as "poor responders" (2). The proposed mechanism behind this condition seems to be a diminished ovarian reserve due to advanced maternal age, as well as a lower number of follicles sensitive to Follicle Stimulation Hormone (FSH) (2). Other causes have also been documented, from follicles insensitive to exogenous gonadotrophins, or suboptimal exposure attributed to obesity (2). Poor ovarian response has a high occurrence rate. According to the existing literature, poor responders have an incidence rate ranging from 9% to 24% (3). This considerably significant percentage could be attributed to the heterogeneity of the population of poor responders, since every IVF center adopts different criteria to categorize them (3). Data pooled from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology (ASRM/SART) Registry recorded a 14.1% cancellation rate of IVF

cycles, with 50% representing women classified as poor responders (4).

Regarding ovarian aging, irreversible damage accumulates on the molecular level due to oxidative stress in the normal ovarian metabolism (5). Researchers consider the subject of therapy for poor ovarian response to be most pressing, as the rate of pregnancy decreases proportionally with the decreasing number of female gametes that can be isolated (3). Notably, advanced reproductive age, along with potential ovarian functional decline, may be associated with Reactive Oxygen Species (ROS) accumulated due to oxidative stress, one of the most important factors of cellular injury (5). In this context, the reduced potential of gametes developed in advanced female age has also been investigated (3).

The classification of a poor responder was first attempted by Garcia et al. in 1983 (6), who defined it as a person who, on a standard stimulation regimen (150 IU human menopausal gonadotrophin), had a peak estradiol concentration of <300 pg/mL and who had poor follicle production, leading to a smaller number of eggs retrieved and transferred. Currently, there is no consensus on the definition of the poor responding patient, or any effective treatment protocol for their management (7). Poor ovarian response is responsible for 20% of cancellations in assisted conception treatment cycles (3). However, existing evidence suggests that GnRH agonist protocols may reduce this rate significantly (8, 9).

This narrative review aims to provide an overview of the currently available management strategies for POR for IVF.

Definition and Aetiology of Infertility

Infertility is the failure of conception after 12 months of intercourse without contraceptive precautions (10). It affects 15% of couples worldwide (10). Primary infertility refers to the infertility of a couple who have never been able to conceive, whereas secondary infertility is the failure to conceive following a previous pregnancy (11). According to the WHO, more than 187 million married women suffered from primary or secondary infertility in the developing world in 2002 (11).

The most common causes of female infertility can be classified into categories based on the anatomy of the female genital tract, and are therefore categorized as: uterine, tubal, cervical or due to ovulation abnormalities. Uterine abnormalities include: leiomyomas, adenomyosis, intrauterine adhesions, endometrial polyps and Mullerian anomalies. Tubal abnormalities include: tubal occlusion, endometriosis and PID (Pelvic Inflammatory Disease). Cervical abnormalities include infertility due to cervical factors, such as cervical stenosis. Ovulatory abnormalities include: androgenic disorders, polycystic ovarian syndrome, premature ovarian failure and ovarian dysgenesis (12).

Definition of POR; the Bologna Criteria

In 1983, the definition of patients as "poor responders" was first described (6), as mentioned above. After this, various researchers adopted different criteria in order to provide a definition of POR. POR has been defined as the presence of at least two of the following criteria (13):

a) age at least 40 years old or other risk factors for POR.
b) previous POR episodes (3 or less oocytes collected with a standard stimulation protocol).
c) Antral Follicle Count (AFC) less than 5-7 follicles or anti-Müllerian hormone (AMH) less than 0.5-1.1 ng/mL or an Ovarian Reserve Test with abnormal results.

The above criteria were introduced by the European Society of Human Reproduction and Embryology (ESHRE) as the "Bologna Criteria" in 2011 during an attempt to standardize the diagnosis of POR. However, since then the validity of these criteria has been questioned. The diagnosis of poor ovarian response encompasses a wide range of sub-populations due to different associated mechanisms. In a systematic review by Polyzos et al. in 2011, at least 41 different definitions of POR were reported in 47 randomized trials (11). AFC and AMH levels were included, with flexible cut-off values, due to the lack of consistency between them regarding the population of poor

responders. Notably, the Bologna criteria have failed to address the issue of oocyte quality versus quantity (14).

The Shift from Bologna to Poseidon Criteria

In an attempt to overcome these issues, the novel POSEIDON (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) criteria were proposed for classification of "low prognosis" patients (15). The POSEIDON criteria include female age, ovarian reserve markers (AMH, AFC, or both), as well as the number of oocytes retrieved in previous cycles of conventional ovarian stimulation, in order to classify poor prognosis patients into four groups (Table 1). The documentation of at least two POR episodes is sufficient to define a patient as a poor responder (7). Ferraretti et al. in 2011 suggested that at least two episodes of POR after maximal stimulation are sufficient to define a patient as a poor responder, in the absence of advanced maternal age or abnormal ORT (7). The lack of a universally established definition highlights the complexity of proposing the ideal ovarian stimulation protocol, in relation to younger or advanced age poor responders (16). The majority of the current strategies proposed in the international literature for ovarian stimulation are discussed below and summarized in Table 2.

Table 1. POSEIDON Classification

Group 1	Patients younger than 35 years old, presenting with adequate values of AFC ≥5, AMH≥1.2 ng/ml, and poor ovarian response
1a	With less than 4 oocytes retrieved, after standard ovarian stimulation
1b	With 4-9 oocytes aspirated/retrieved, after standard ovarian stimulation
Group 2	Patients older than 35 years old, presenting with adequate values of AFC ≥5, AMH≥1.2 ng/ml, and poor ovarian response
2a	With less than 4 oocytes retrieved, after standard ovarian stimulation
2b	With 4-9 oocytes aspirated/retrieved, after standard ovarian stimulation
Group 3	Patients younger than 35 years old, presenting with poor values of AFC<5, AMH<1.2 ng/ml, and poor ovarian response
Group 4	Patients older than 35 years old, presenting with poor values of AFC<5, AMH<1.2 ng/ml, and poor ovarian response

Table 2. Selected Studies (2015-2020) and Pharmacological Treatments for Ovarian Stimulation in PORs

Authors	Year	Study design	Sample size	Study population	Treatment	Outcome
Magnusson *et al.* (17)	2017	RCT	308	PORs	Adding AMH to a conventional protocol with GnRH agonist and recombinant FSH	The addition of AMH did not alter the rate of targeted ovarian response, 5-12 oocytes, or decreased the rate of ovarian hyperstimulation syndrome (OHSS) or cancelled cycles due to POR
Xu Y. *et al.* (22)	2018	RCT	186	POSEIDON Group 3 PORs	Pretreatment with coenzyme Q10	Better ovarian response to stimulation and embryological parameters
Kolibianakis EM *et al.* (25)	2015	RCT	79	Previous POR to stimulation (≤4 COCs) after maximal stimulation	Substitution of 150 μg corifollitropin alfa with 450 IU follitropin beta during the first 7 days of ovarian stimulation	The number of COCs (cumulus oocyte complexes) retrieved was not statistically different between the corifollitropin alfa and the follitropin beta groups
Elprince *et al.* (31)	2020	RCT	50	PORs	DHEA supplementation (25 mg/8 h for two consecutive cycles before induction of ovulation)	DHEA treatment showed a statistically significant improvement compared to the control group

Authors	Year	Study design	Sample size	Study population	Treatment	Outcome
von Horn *et al.* (38)	2017	RCT	80	IVF patients	Follicular flushing with the modified flushing system.	No increase in the number of oocytes was reported, only an increase in the duration of the procedure.
Madani *et al.* (40)	2018	Prospective Clinical Trial	121	PORs	Double stimulation by Letrozole, Clomid, hMG and GnRH-agonist	No significant difference between the number of oocytes retrieved after the first stimulation (combination of clomiphene and LZ) and the second stimulation (LZ alone)
Lefebvre *et al.* (43)	2015	Prospective randomized controlled nonblinded study	356	PORs	450 vs 600 IU/d gonadotropin	Gonadotropin of 600 IU/d does not improve outcome of IVF cycles compared with 450 IU/d.
Lattes *et al.* (46)	2015	Prospective, self-controlled study	106	PORs	GH supplementation (0.5 IU/day)	Increase in pregnancy rates
Bassiouny *et al.* (47)	2016	RCT	145	PORs	GH supplementation to the microflare stimulation protocol	The numbers of oocytes collected, metaphase II oocytes, and fertilized oocytes increased
Dakhly *et al.* (48)	2016	Prospective RCT	287	PORs	GH as an adjuvant therapy added to either long or short agonist protocol, miniflare or antagonist protocols	Long/GH showed significantly higher levels in the number of fertilized oocytes, than the short/GH and antagonist/GH protocols
Maged *et al.* (51)	2015	RCT	160	PORs	Delayed start protocol against the conventional gonadotropin (Gn)-releasing hormone antagonist protocol	Improved clinical pregnancy rate and IVF cycle parameters
Merviel *et al.* (53)	2015	RCT	440	PORs	Contraceptive pill + flare-up GnRH-a protocol compared to the multidose GnRH antagonist protocol.	Pregnancy rates per embryo transferred were not significantly different with the contraceptive pill + flare-up GnRH-a protocol compared to the multidose GnRH antagonist protocol.
Schimberni *et al.* (54)	2016	Trial	250	PORs	Clomiphene citrate plus a high dose of gonadotropins and GnRH antagonist, flexible GnRH antagonist protocol and a short GnRH agonist protocol.	Significantly higher pregnancy rate than the clomiphene and the GnRH antagonist protocol, a higher number of mature oocytes collected, estradiol levels and endometrial thickness.
Toftager *et al.* (57)	2016	Trial	1099	PORs	Risk assessment of severe OHSS in the long GnRH agonist group compared with the short GnRH antagonist protocol.	Patients at risk of OHSS particularly benefit from the short GnRH antagonist treatment
Siristratidis *et al.* (57)	2017	RCT	58	PORs	Mild versus conventional GnRH-agonist and antagonist protocols	Mild ovarian stimulation is inferior to conventional protocols in terms of the numbers of COCs retrieved.

Authors	Year	Study design	Sample size	Study population	Treatment	Outcome
Ashrafi *et al.* (52)	2018	RCT	250	PORs	Delayed start versus standard GnRH-antagonist protocol	Higher fertilization rate and better quality of embryos; lower cycle cancellation but no significant effect on clinical pregnancy rate.
Haas *et al.* (59)	2019	RCT	33	PORs	Double Trigger (GnRH agonist + HCG)	Significant increase in the number of top quality embryos, with a reasonable clinical pregnancy rate, compared to the conventional HCG trigger or the GnRH-ag trigger.
Mak *et al.* (60)	2016	RCT	49	PORs	Recombinant LH (rLH) supplementation vs urinary human chorionic gonadotrophin (uHCG) supplementation when using a fixed GnRH antagonist protocol	No statistically significant difference in cycle cancellation rates, numbers of oocytes retrieved per cycle initiated, fertilization rates, the numbers of embryos obtained per cycle initiated, implantation, clinical pregnancy or live birth rates.
Bastu *et al.* (63)	2016	RCT	95	PORs	Adding letrozole to protocol	Mild stimulation with addition of letrozole was as effective as stimulation with higher doses of gonadotropins alone.
Gizzo *et al.* (65)	2015	RCT	40	PORs	Optimal timing of recombinant luteinizing hormone (rLH) supplementation in GnRH-antagonist treatment	Improved ovarian response, embryo quality and pregnancy rate were achieved by administering rLH independently from the total dose administered.
Humaidan *et al.* (67)	2017	Randomized Clinical Trial	949	PORs	Fixed-ratio combination of recombinant human FSH plus recombinant human LH (follitropin alfa plus lutropin alfa; r-hFSH/r-hLH) vs r-hFSH monotherapy	r-hFSH/r-hLH was associated with a higher live birth rate, whereas r-hFSH was associated with a higher live birth rate for those with mild POR.
Llácer J *et al.* (68)	2020	RCT	60	PORs	Luteal phase ovarian stimulation (LPOS) versus follicular phase ovarian stimulation (FPOS)	LPOS was as effective as FPOS
Caprio *et al.* (71)	2015	Prospective controlled observational trial,	72	PORs	Myoinositol therapy	MI improves ovarian response to gonadotropins
Chen *et al.* (76)	2017	Controlled clinical trial	204	PORs	Minimal stimulation with progestin	Better ovulation control of the dominant follicle but no effect on the quality of oocytes
Bosdou *et al.* (34)	2016	RCT	48	PORs	Pre-treatment with transdermal testosterone	Ovarian response: no more than 1.5 oocytes

RCT=Randomized Clinical Trial; PORs=Poor Ovarian Responders; POR=Poor Ovarian Response; AMH=Anti-Mullerian Hormone; GnRH=Gonadotropin-Releasing Hormone; FSH=Follicle-Stimulating Hormone; OHSS=Ovarian Hyperstimulation Syndrome; COCs=Cumulus Oocyte Complexes; DHEA=Dehydroepiandrosterone; IVF=In Vitro Fertilization; hMG=Human Menopausal Gonadotropin; LZ=Letrozole; GH=Growth Horomone; HCG=Human Chorionic Gonadotropin: LH=Luteinizing Hormone; rLH=Recombinant LH; uHCG=Urinary HCG; r-Hfsh=Recombinant Human FSH; r-Hlh=Recombinant Human LH; LPOS=Luteal Phase Ovarian Stimulation; FPOS=Follicular Phase Ovarian Stimulation; MI=Myoinositol.

POR Management Protocols/Adjuvant Therapies

Anti-Müllerian Hormone

Serum AMH is regarded as a highly sensitive biomarker for ovarian response, with the interpretation of its levels being a useful clinical tool to guide infertility counselling. Magnusson et al. in 2017 performed a randomized controlled trial (RCT) to specify the effect of anti-Müllerian hormone in ovarian response (17). Patients regulated with GnRH agonists and stimulated with recombinant FSH demonstrated neither better ovarian response rate than without administering AMH, nor any decrease in ovarian hyperstimulation syndrome (OHSS), or even the number of cycle cancellations. However, there are no international standards for defining the cut-off values of AMH accordingly to well-established reference intervals, so extrapolation of clinical data to POR population should be made with caution. (17). In this regard, instead of assessing AMH levels alone before ovarian controlled stimulation, it is important to assess both AMH and AFC to predict POR (18).

Clomiphene Citrate

Chemically, clomiphene is a nonsteroidal triphenylethylene derivative that exhibits both estrogen agonist and antagonist properties (19). Song et al. in 2016 conducted a meta-analysis to establish the efficiency of clomiphene citrate in a mildly controlled hyperstimulation protocol (20). The selected four RCTs showed that both live birth and clinical pregnancy occurrence rates were similar, either with clomiphene or without (20).

On the other hand, Kamath et al. in 2017 examined the effectiveness of oral induction medication, such as clomiphene citrate versus gonadotropin-only regimens (21). According to their systematic review, although the use of clomiphene led to a reduction in the amount of gonadotropins required, no conclusive evidence suggested that it could be associated with a significant increase in the incidence of cycle cancellations (21).

Coenzyme Q_{10}

Investigating the effect of anti-oxidant pre-treatment with coenzyme Q_{10}, Xu et al. in 2018 performed an RCT in order to address its beneficial effects in ovarian response and embryo quality (22). Moreover, the combination of dehydroepiandrosterone (DHEA) and coenzyme Q_{10} compared with DHEA alone, during vitro fertilization cycles, improved ovarian response, but no associated improved clinical outcome was demonstrated (23).

Corifollitropin Alpha

A single injection of CFα, a synthetic recombinant glycoprotein, can replace daily FSH injections for the first seven days of controlled ovarian stimulation (COS) as required for IVF (24). Kolibianakis et al. in 2015 carried out a RCT comparing the substitution of follitropin beta by corifollitropin alpha, thus demonstrating that the number of cumulus oocyte complexes (COCs) retrieved was similar in all the groups examined (25). It was then suggested that Corifollitropin alfa simplifies IVF treatment when administered in a GnRH antagonist protocol, since it replaces seven daily FSH injections with a single dose of long acting FSH (25). Comparing the effectiveness of corifollitropin alfa versus daily gonadotropins in PORs undergoing controlled ovarian stimulation according to AFC, Adrisani et al. in 2019 suggested that corifollitropin alfa may be as effective as gonadotropins when AFC >5, while it might be less effective than gonadotropins when AFC ≤5 (26).

Androgens: Dehydroepiandrosterone (DHEA)-Testosterone

Dehydroepiandrosterone (DHEA), is an important precursor of androgen, and has been extensively studied for improving the outcome measures of ovarian stimulation in POR (27). Numerous studies have been published on DHEA supplementation in POR patients. Li et al. in 2015 conducted a meta-analysis evaluating the effects of DHEA on women with diminished ovarian reserve (DOR) who underwent in vitro fertilization with intra

cytoplasmic sperm injection (ICSI) (28). The use of DHEA increased the clinical pregnancy rate, while the impact of DHEA on oocyte retrieval, implantation, and abortion were not significant (28). Nagels et al. in 2015 in their Cochrane review concluded that pre-treatment with DHEA, or its derivative testosterone, may be associated with improved live birth rates in assisted reproductive technology (29). Androgen replacement in advanced-age women with diminished ovarian reserves might improve outcomes. Although DHEA doses range from 50 to 90 mg/day, with a treatment duration ranging from 1 to 12 months, the optimal dose and duration of DHEA remains to be defined (30). Elprince et al. in 2020 studied the effect of DHEA supplementation on improving ovulation among poor responders, and showed a statistically significant effect in the treatment group (31). This may be attributed to the increasing expression of androgen receptor and FSH receptor in granulosa cells after DHEA supplementation (32).

Transdermal testosterone prior to ovarian stimulation significantly increases percentages of live birth and reduces the doses of FSH required (33). In a systematic review and meta-analysis conducted by González-Comadran et al. in 2012, 113 women who were pretreated with transdermal testosterone achieved significantly higher live birth rates and clinical pregnancy rates, and required significantly lower doses of exogenous FSH compared with 112 in the control group (33). Specifically, the RCT of Bosdou et al. in 2016 suggested that pretreatment with 10 mg of transdermal testosterone for 21 days does not improve ovarian response by more than 1.5 oocytes, however, higher doses of testosterone may be more effective (34). Overall, interpreting the results of the meta-analysis by Noventa et al. in 2019 demonstrated that adjuvant testosterone treatment is associated with increased live birth rates and clinical pregnancy rates, as well as the total number of oocytes retrieved (35).

Double Lumen Needle Follicular Flushing System

Despite limited evidence supporting the use of follicular flushing (36), it continues to be common practice in many infertility clinics (37). Von Horn et al. in 2017 examined a double-lumen needle follicular flushing system, comparing it with a single-lumen aspiration needle in IVF patients with poor ovarian response (38). Unfortunately, follicular flushing did not produce a higher oocyte number, while it doubled the duration of the procedure (38).

Double Ovarian Stimulation

Double ovarian stimulation in the same ovarian cycle (DuoStim) starting in the luteal phase could provide more opportunities for retrieving oocytes in a short period of time (39). Madani et al. in 2019 attempted to compare the effectiveness of double stimulation during the follicular and luteal phases in poor responding women (40). According to them, the number of oocytes retrieved after the first and second stimulations did not differ significantly, but the oocytes retrieved after the first stimulation were of better quality (40). Conversely, Vaiarelli et al. in 2019, after reviewing the evidence of DuoStim, suggested that it could be adopted as an effective strategy to maximize the number of oocytes retrieved and subsequently the number of competent embryos in a short timeframe, which is crucial for these patients (41). Although no serious concerns have been raised regarding the safety of DuoStim, Labarta et al. in 2020 underlined the need for further randomized studies to analyze whether similar results could be obtained from two consecutive cycles of ovarian stimulation (42).

Follicle-Stimulating Hormone (FSH)

Lefebvre et al. in 2015 carried out a prospective randomized controlled study to identify the optimal FSH dose for controlled ovarian stimulation in poor responders (43). No major differences were found between the two groups tested (supplementation of 450 IU versus 600 IU gonadotropin per day), regarding the number of oocytes retrieved, pregnancies, implantation or fertilization rate (43). Van Tilborg et al. in 2016 summarized the existing evidence on FSH dosage for PORs treated by a GnRH agonist protocol (44). An individualized

gonadotropin dose ranging from 100-600IU/day depending on basal FSH or AFC does not improve the rates of cycle cancellation, pregnancy, or live births (44). An attempt was made to define an individualized standard FSH dose based on the measurement of various biomarkers, including basal FSH (bFSH), AFC, and AMH (45). However, this study was inconclusive about whether the standard dose of 150 IU could be effective, or a higher dose is needed for ovarian stimulation (45).

Growth Hormone (GH)

GH is described as an adjuvant therapy in in vitro fertilization for poor ovarian responders, but evidence on IVF outcomes has been conflicting. Lattes et al. in 2015 studied the effects of a small dose of GH administered during an IVF cycle in poor responding patients (46). They conducted a prospective self-controlled study in which 64 PORs were administered a small dose of GH, using the same protocol and gonadotropin dose (46). Finally, high pregnancy rates were achieved with no side-effects and at low cost (46). Addition of GH to a conventional IVF protocol with a GnRH antagonist, should be approached with caution (47). In this RCT, one group of patients was given GH in addition to the antagonist protocol, which not only lowered the treatment duration of hMG and GnRH, but also increased the number of oocytes collected and fertilized (47). On the other hand, the small difference in the rate of clinical pregnancy and the low statistical power of the study implied that GH should be supplemented with caution (47). Dakhly et al. in 2016 adopted a different approach (48). The aim of their randomized prospective trial was to define the most suitable protocol including GH for treating PORs (48). The patients involved were allocated into four groups. The group that demonstrated the best outcomes regarding the number of oocytes retrieved and fertilised was the one that received the long agonist protocol with GH (48). Li et al. in 2017 attempted to examine the effectiveness of a GH protocol in relation to the outcome of treating poor responding women (49). Supplementation of GH to the IVF protocol did not provide any significant improvement in the clinical pregnancy and live birth rates, not to mention that the timing of GH administration, as well as the collocation of medications, may have also affected the outcome (49).

Gonadotropin Releasing Hormone (GnRH)

GnRH antagonist administration in the early follicular phase can decrease gonadotrophin levels, which may improve synchronization of follicles, improving ovulation stimulation (50). Maged et al. in 2015 compared a delayed start protocol with a standard protocol that used a gonadotropin releasing hormone antagonist (GnRH antagonist) in poor responding patients (51). The two groups of patients in this RCT either started receiving the GnRH on the first day, or it was delayed until day 8. The results showed an improvement in the IVF cycle parameters and the rate of clinical pregnancy in the delayed group as opposed to the group that began the GnRH dose immediately (51). In this direction, Ashrafi et al. in 2018 attempted to present the differences between a delayed start GnRH protocol and a standard one (52). The trial showed a statistically significant difference in fertilisation rates, in favour of the delayed start protocol. Given the small study sample, further evaluation of their evidence should be performed (52). Merviel et al. in 2015 adopted a more direct approach: among PORs for whom a standard long agonist GnRH protocol had failed, they applied and compared the results of two different protocols: a contraceptive pill with a flare-up agonist GnRH, and a GnRH antagonist (53). Even though the embryo transfers were greater with the first protocol than the second, their pregnancy and implantation rates were similar in relation to the woman's age and lifestyle. Since the prognostic factors for this protocol were maternal age <36, no tobacco consumption, a total FSH/hMG dose <5,000 IU, and endometrial thickness >10 mm, customizing the policy of ovarian stimulation according to the woman's age and lifestyle could certainly improve outcomes (53). Likewise, Schimberni et al. in 2016 examined three different protocols on poor responders: a short

GnRH agonist, a GnRH antagonist with high doses of gonadotropins and clomiphene citrate, and a flexible GnRH antagonist, respectively (54). Of the three groups, the one that had the short GnRH agonist had the highest rate of pregnancy and lowest cost of therapy, in contrast to clomiphene citrate which should be avoided due to its very low success rate and high costs (54). The challenge of selecting the gonadotropin starting dose was met with success by Li et al. in 2021 (9). Although several nomograms have been developed to estimate the appropriate gonadotropin starting dose in GnRH agonist protocols adopted in IVF, no nomogram was suitable for GnRH antagonist protocols (9). Another comparison between two different protocols was performed by Siristratidis et al. in 2017 (55). In their study, they evaluated the effect of a mild GnRH agonist/antagonist protocol in comparison to a conventional protocol. The outcome, however, was that the number of COCs was lower than with conventional stimulation, thereby showing its inferiority to the conventional protocol (55). Similarly, Lambalk et al. in 2017 investigated which would be the better protocol of a long agonist GnRH and a GnRH antagonist (56). To answer this question, they carried out a systematic review and meta-analysis. For poor responders, it was revealed that the antagonist protocol was associated with a smaller incidence of OHSS and similar rates of pregnancy (56). Notably, OHSS is a possible side-effect of a GnRH protocol. For that reason, Toftager et al. in 2016 performed a study to assess the risk of OHSS when using the short antagonist and the long agonist GnRH protocols (57). Women less than forty years old and infertile were randomly allocated to GnRH antagonist or agonist protocols. OHSS, rated as severe, moderate or mild, appeared less often in the antagonist group than the agonist, so patients at risk of OHSS particularly benefited from the short GnRH antagonist treatment (57).

Human Chorionic Gonadotrophin (hCG)

Kasum et al. in 2016 examined the combination of a GnRH agonist along with a human chorionic gonadotrophin (hCG) trigger in order to achieve oocyte maturation and retrieval (58). This dual trigger could be a possible treatment for empty follicle syndrome and PORs, since it is associated with increased live births and a better quality of preserved embryos (58). Double triggering was also studied by Haas et al. in 2019 (59). Thirty-three PORs were allocated to three random groups with different protocols regarding the addition of GnRH agonist in combination with hCG. The group that was administered the double trigger protocol showed a higher number of top-quality embryos than the other two. However, the small sample size of the study requires further evidence to validate its clinical implementation (59). Mak et al. in 2017 conducted a double-blinded study to examine the effects of urinary human chorionic gonadotrophin (uhCG) compared with the supplementation of mid-follicular phase recombinant luteinizing hormone (rLH) (60). Unfortunately, clinical birth rates and other parameters of the IVF cycle were similar, except for the live birth rate per cycle, which was higher for the uhCG group. Further RCTs are required to verify these results (60).

Letrozole

Letrozole is a highly selective, non-steroidal aromatase inhibitor. It prevents estrogen syntheses by inhibiting the aromatase enzyme activity, thus increasing the expression of FSH receptors on the follicle (61). Letrozole administration could improve pregnancy rates in conventional GnRH antagonist protocols, as demonstrated in a recent meta-analysis by Qin et al. in 2021 (62). Bastu et al. in 2016 carried out a RCT to examine the impact of adding or not adding letrozole to a standard ovulation stimulation protocol, which included POR patients who received three different gonadotropin doses during ovulation stimulation (63). Mild stimulation with the addition of letrozole was as effective as stimulation with higher doses of gonadotropins alone in this patient population (63). Conversely, Kamath et al. in 2017 did not provide sufficient evidence regarding the beneficial supplementation of

conventional GnRH agonist or antagonist protocols concerning live-birth or pregnancy rates (21).

Recombinant Luteinizing Hormone (rLH)

While the need for FSH in ovarian stimulation is evident, a question remains regarding the role of rLH in different IVF population groups (64). Gizzo et al. in 2015 attempted to determine the optimal timing to administer rLH during an in vitro fertilization cycle (65). Although increased endometrial thickness appeared when rLH was administered at the beginning of the follicular phase, the highest ovarian response occured when rLH was administered in the mid-to-late phase. The study's limited size and lack of information regarding the differences in intra-follicular growth factors suggest that further large-scale clinical trials should be conducted (65). Moreover, the definition of the LH threshold in GnRH analogue treated cycles, as well as identification of which subgroups of women could benefit from adjuvant rLH treatment, have not been clearly answered (66). Humaidan et al. in 2017 evaluated the effectiveness of COS comparing a fixed-ratio combination of recombinant human FSH plus recombinant human LH (r-hFSH/r-hLH) with that of r-hFSH monotherapy (67). The incidence of pregnancy outcome failure was significantly lower in the r-hFSH/r-hLH group than in the r-hFSH group, but live birth rates were similar in both groups (67).

Luteal Phase Ovarian Stimulation (LPOS)

Llácer et al. in 2020, attempting to assess the efficacy of LPOS compared with follicular phase ovarian stimulation (FPOS), achieved comparable results (68), but they are probably not conclusive, similar to those of Chen et al. in 2021 (69), since the number of oocytes collected was similar with both luteal and follicular stimulation (68).

Myo-Inositol (MI)

Inositols are a family of carbocyclic polyalcohols, with nine possible stereoisomers, including MI (70). MI has proven useful in issues related to female infertility and in sustaining physiological pregnancy (70). Caprio et al. in 2015 performed a prospective controlled observational trial to examine the effectiveness of myoinositol on ovarian function in PORs (71). The patients were divided into two groups. In the treatment group, 38 patients were enrolled who had been taking MI (4 g) + folic acid (FA) (400 μg) for the previous 3 months, while the control group included 38 patients taking FA (400 μg) alone for the same study period (71). MI supplementation in poor responders resulted in an increased number of oocytes retrieved (71), as well as in implantation and pregnancy rates (72). Similar positive results regarding ovarian response were shown after the double-blinded randomized controlled study by Mohammadi et al. in 2021 (73) who allocated the two study groups according to Caprio et al. in 2015 (71).

Progesterone

In an attempt to develop new stimulation regimens, the administration of endogenous and exogenous progesterones was used in order to block the LH surge during ovarian stimulation (74). Massin et al. in 2017 found that this technique does not affect the number of oocytes collected or the quality of the embryos obtained (74). However, the main disadvantage observed was that it requires total freezing and delayed transfer (74). Advancing our knowledge in this direction, progesterone administration was found to inhibit granular cell proliferation and antral follicle growth during ovarian stimulation via phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) pathways (75).

Progestin (P)

Since the efficacy of progestin on poor responders had not yet been extensively examined, Chen et al. in 2017 performed a clinical trial in order to ascertain the outcomes of progestin-primed minimal stimulation on PORs (76). The study provided evidence showing that stimulation through progestin

is able to control the ovulation of the dominant follicle, while not affecting oocyte quality (76). So, progestine could be used as a possible means to prevent premature ovulation (76). Specifically, progestin-primed ovarian stimulation using 4 versus 10 mg of medroxyprogesterone acetate per day is comparable, and did not differ in terms of the number of oocytes retrieved and pregnancy outcomes (77). Therefore, progestin-primed ovarian stimulation could be the first choice for ovarian stimulation due to its better control of LH concentrations, lower costs, and easier oral and not intravenous administration (78). Finally, the most representative strategies and pharmaceutical stimulation protocols proposed in the current literature for POR are summarized in Table 2. It should also be mentioned that controlled ovarian stimulation for fertility preservation in patients with malignancy could be also challenging. Although the type of cancer has not been proven to significantly affect ovarian reserve and ovarian response (79), patients with high-grade cancer have a decreased number of retrieved mature oocytes and cryopreserved embryos (80).

Conclusion

POR management represents a great challenge for assisted reproduction technology specialists. Due to the lack of a standard definition, as well as the heterogeneity of the factors associated with POR cases, no consensus has been reached on the most beneficial therapeutic intervention to overcome poor oocyte retrieval. Although many strategies and pharmaceutical treatments have been suggested to manage POR, none has been proven superior to the others, in terms of the number of oocytes retrieved per ovarian cycle, the number of competent embryos, or live birth rates. Further large-scale randomized studies are needed to validate the experimental techniques in the search for successful individualized treatment regimens.

What Is Already Known on This Topic:

Patients defined as " poor responders" show minimal response to controlled ovarian hyperstimulation, although there is no distinct/standard definition of poor ovarian response (POR). Although infertility specialists are endeavoring to improve cycle outcomes in poor responders by adopting multiple management strategies, the estimated risk of cycle cancellation is still about 20%.

What This Study Adds:

This review summarizes all the latest studies published between 2015 and 2021 concerning the management protocols adopted for POR cases. None has been proven superior to the others, in terms of the number of oocytes retrieved per ovarian cycle, the number of competent embryos or live birth rates. Further large-scale randomized studies are needed to validate experimental techniques in the search for successful individualized treatment regimens.

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Text Neck Syndrome: Disentangling a New Epidemic

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Abstract
The aim of this report is to provide a brief review of the predisposing factors for Text Neck Syndrome, along with diagnostic and therapeutic approaches in young and adult populations. Text neck pain is a worldwide public health problem, largely reported nowadays. Currently, data have shown that the erroneous use of personal computers and cell phones might be correlated with the development of various clinical symptoms that are defined as "text neck syndrome". Modified radical changes in everyday life may ameliorate the powerful forces on the cervical spine that can lead to cervical degeneration, along with other developmental, medical, psychological, and social complications that are attributed to text neck syndrome. **Conclusion.** New technologies and the potentially harmful addiction to cell phones and computers while reading or texting are inducing an epidemic of text neck syndrome. By focusing on postural correction, both pain alleviation and a better quality of life can be achieved for the patient. The predisposing factors and therapeutic approaches for this syndrome that affects public health remain to be further elucidated.

Key Words: Text Neck ▪ Anatomy ▪ Syndrome ▪ Spinal Pain.

Introduction

Neck pain in general is a global cause of disability. It is a public health problem that has increased remarkably nowadays. The prevalence, independent of age, is high, and equal to low back pain. Epidemiological data indicate that 73% of university students and 64.7% of people who work from home have neck or back pain. 39.2% of them admit to being less productive due to neck or low back pain (1, 2). Indeed, the unavoidable addiction to personal computers and cell phones for texting has contributed to the increase in the prevalence of neck pain.

To understand better the anatomy and physiology of human posture the following definitions are provided:

- Posture is the structural framework of the human body intended to resist gravity while humans are standing, sitting or moving, by maintaining an upright position.
- In order to examine and locate the different distortion patterns better, four Posture Quadrants are used: PQ 1: includes the head and cervical spine ("craniocervical posture"), PQ 2 includes the upper limbs, the shoulders, scapulae, thoracic spine and ribcage, PQ 3: affects the lumbar spine and the pelvis, and PQ 4: extends from the femoral head of each leg to the distal phalanges of each foot.
- Forward Head Posture (FHP) is defined as a postural distortion pattern involving the head and neck, the 1st PQ, characterized by protrusion of the head in a sagittal plane so that it is placed anterior to the trunk. It is found in 2 types: the 1st is characterized by the flexion of the cervical spine (as when looking down at a cell phone), and the 2nd type is when the lower cervical spine remains in flexion while the upper cervical spine is extended to keep the gaze at the horizontal level (as when someone is looking at a computer screen in front of them).

There is growing evidence that, compared to neutral standing, adults, or even children, display more FHP when viewing a cell phone (3). Moreover, FHP leads to mechanical strain forces on the joints and ligaments of the cervical spine, and as a result there is increased gravitational force on the posterior neck musculature. Altogether, these factors play a crucial role and support a biomechanically based hypothesis. Inappropriate neck posture while reading or texting on personal computers and cell phones leads to the manifestation of a complex cluster of clinical symptoms commonly defined as "Text Neck Syndrome" (TNS). TNS can be diagnosed and screened by physiotherapists, or even by self-perception, with estimation of the frequency of neck pain. The core of the early diagnosis and screening of the high risk population to present TNS is based on history, clinical examination and posture imaging. The cervical spine seems to be more vulnerable than the lumbar. Undoubtedly, use of many new technological devices involves an unnatural neck position, and this implies an association with neck pain. However, the etiopathogenesis of neck posture and neck pain remains to be elucidated further.

The aim of this study is to provide a brief review of the predisposing factors of Text Neck Syndrome, along with diagnostic and therapeutic approaches in young and adult populations.

The Etiopathogenesis and Symptomatology of TNS

The main factor which exacerbates this clinical condition is looking down for prolonged periods of time with the cervical spine in flexion, as when using a smartphone or tablet, reading on a laptop, or when protruding the head forward while viewing a computer screen. Mechanical stress in the cervical spine due to erroneous cell phone use induces poor posture and incorrect body alignment, which is associated with dysfunctional movement patterns, weak balance-ability and distorted function of the respiratory, circulatory, digestive, and nervous systems**.** In addition, sleeping in an improper posture, in which the normal curves of the spine are not "respected", e.g. sleeping in prone position, with the lumbar spine in extension and the cervical spine rotated and extended, or sleeping on the side forming a "C" – shape, can also result in FHP.

Furthermore, FHP can be associated with cervical spatial change, and a decreased range of motion (ROM), respiratory dysfunction, decreased vital capacity, dysfunction of the temporomandibular joint, carpal tunnel syndrome, and impaired proprioception. The visual system is of paramount importance to the neurology of the posture system, as it controls 70% of postural activities. Working with decreased visual acuity or eye movement dysfunction, patients will develop postural distortion patterns to compensate for this deficit. In order to correct the posture in this situation, correction of the patient's visual system is first required.

On the other hand, many individuals experience eye discomfort and vision problems when viewing digital screens for extended periods. The level of discomfort appears to increase with the amount of digital screen use. This results in so-called computer vision syndrome. Three major mechanisms that lead to this syndrome are the extraocular mechanism, accommodative mechanism, and ocular surface mechanism. In addition, the visual effects of the computer, such as brightness, resolution, glare and quality, are all known factors that contribute to computer vision syndrome. Prevention is the most important strategy in management. Modification of the ergonomics of the working environment, as well as controlling the lighting and glare on the device screen, taking frequent breaks, and education in proper posture and proper eye care are all crucial (4).

Other symptoms that accompany FHP are neck pain, shoulder tightness, headaches and migraines, jaw pain, and pain down the arm and forearm. As the head protrudes forward into a forward head posture, the tragus of the ear shifts forward in relation to the shoulders' coronal level. This is observable on posture imaging, i.e. by picturing the patient's posture to gain an accurate clinical finding which helps the patient's education, and

provides us with a precise way to measure clinical outcomes. The angle between the tragus of the ear to the C7 vertebrae and the horizontal line of C7 is called the "craniovertebral angle", which is one of the most reliable methods to assess the degree of FHP (5). A smaller craniovertebral angle indicates a greater degree of FHP (5).

The Anatomy and Biomechanics of TNS

FHP has been associated with increased load on the cervical spine (6) and changes in the length and strength of cervical muscles (7). FHP disturbs the delicate and complex mechanisms of balance as the Center of Gravity (COG) of the head gets dislocated in an anterosuperior direction. In addition, the COG of the whole body will be modified and will affect the postural control of all joints and the torso. By positioning the head forward, the distance between the sternum and the mandible increases, and as a result the infra-supra hyoid muscles are stretched and weakened by pulling back and down the mental protuberance (8).

In FHP the mastication muscles pull the mandible so to maintain the mouth closed, while the infrahyoid muscles contract in order to depress the mandible and retract it towards a posterior direction. The muscles of the thoracic wall (the intercostal muscles, pectoralis major and minor, serratus anterior) will present impaired mobility, as well as the muscles of the cervical spine and head (i.e. the levator scapulae, sternocleidomastoid, upper trapezius, the scalene and suboccipital muscles, the rectus capitis posterior major and minor, as well as the obliquus capitis inferior and superior). As for the rhomboids, middle trapezius, and supra- and infrahyoid muscles, they will appear stretched and will become weak in prolonged FHP (9). Generally, the muscular imbalances associated with FHP result from the combination of the elongation and weakening of the anterior neck muscles, and the contraction and stiffness of the posterior neck muscles (10).

Another aspect of TNP syndrome is the alignment of the neck with the centers of the shoulder, diaphragm and pelvic rings on a vertical line. Any deviation from this structural relationship means that the deep fascia, known as the myofascia, is of paramount importance regarding the musculoskeletal system-of the upper pole, which is disturbed. A dysfunctional position of the head can lead to occlusal problems and vice versa, or tooth gearing problems, that can affect the balance of the head on the neck.

The sternocleidomastoid muscles (SCM), acting unilaterally, cause ipsilateral-lateral flexion, contralateral rotation and lift the chin. Acting bilaterally, they help in head stabilization by flexion of the lower cervical spine and extension of the upper cervical spine (11). In chronic FHP, the SCM muscles exert strenuous force to hold the head upwards, and become short, tight and weak. The hyoid bone floats below the tongue, providing attachment points for both the delicate supra and infrahyoid muscles.

Incorrect posture for a long period of time, without respecting the normal kinematics of the spine and the position of the major joints, such as the shoulder joint (glenohumeral) and the hip joint, may create disorder in all four posture quadrants of the body. A swayback posture leads to FHP. A craniocervical and thoracopelvic posture (i.e. the position of the pelvis related to the thoracic and lumbar spine) is therefore a relatively important aspect in the treatment of the temporomandibular joint. A domino effect will occur when bending the head forward, as the COG is transferred forward. For compensation, the upper thorax increases the kyphotic curvature of the thoracic spine, that will result in the raising of the lordosis of the lumbar vertebrae. The shift of the COG will affect the biomechanics of the entire spine, as well as the slope of the pelvis.

The Anatomical Complications of TNS and Other Correlated Consequences

Adults with neck pain show increased FHP when compared to asymptomatic adults, so FHP is significantly corrected by neck pain measures in adults and older people (12). FHP and thoracic shape have been reported to impact respiratory

function. Research has shown that forward head posture causes expansion of the upper thorax and contraction of the lower thorax, and these morphological changes cause decreased respiratory function. FHP is also associated with reduced proprioception (kinesthesia), which subsequently leads to cervical sensorimotor control disturbances. The delicate proprioceptive system of the cervical spine controls posture and balance. The change in muscle strength caused by FHP decreases the joint position sense (5, 13). It was also found that the more severe FHP becomes, the worse the proprioception will become (12, 14).

Concerning university students, it has been found that FHP is not significantly associated with disability, but does affect stress levels. Following research, it has been determined that participants with FHP exhibited abnormal sensorimotor control and autonomic nervous system dysfunction, in comparison with those presenting with simple normal head alignment (6, 15).

Treatment Plans and Exercise Ameliorate TNS Symptomatology

Therapeutic approaches for TNS include a 10-week home-based targeted exercise program that can improve postural alignment related to forward head posture. The aim of these exercises is to reverse the posture. By focusing on postural correction, both pain alleviation and a better quality of life can be achieved for the patient.

Other effective treatments for FHP may include posture exercises, oculomotor tasks, posture tape, respiratory exercises, and ergonomic corrections for the professional, as well as the home environment. Therapeutic exercises may result in major changes to the craniovertebral angle and moderate improvement in neck pain in participants with FHP. Modified cervical exercises that were performed for only a relatively short duration (four weeks) showed an improvement in forward head posture induced by using a smartphone (7, 15).

Finally, TNS may impact the normal development of children. Using tablets and cell phones most of the day with abnormal posture may impact neurological development during childhood and later in adulthood, which leads to pain and other dysfunctions. Incorrect posture patterns can be associated with poor respiration, poor balance, and neck and lumbar pain headaches, because of the body movements forward and down, that may cause hyperventilation, stress, and anxiety. Just by changing posture, ventilation is improved, and the better overall function of the nervous system and body in general is achieved.

Conclusion

Proper posture while using hand-held mobile technological devices at any age must be taught by physiotherapists with personalized exercise and related programs. The dangers of prolonged use of hand-held devices that lead to Text Neck Syndrome may have multifactorial consequences in the development of young and adolescents.

What Is Already Known on This Topic:

Text Neck Syndrome is a modern epidemic affecting children and adolescents. It is a complex syndrome that is exacerbated by erroneous use of personal computers and cell phones in a poor position. Numerous potentially modifiable risk factors contribute to the development of the syndrome.

What This Study Adds:

This article provides an up-to-date summary of the key points regarding text neck syndrome, and highlights its significance as it is a globally growing problem. To investigate the exact mechanisms and the etiopathogenesis responsible for this condition further, more studies are needed. Progressive studies could provide more data about the etiopathogenesis of the syndrome, and screening of the high-risk population could both help the early diagnosis and treatment, and show the incidence of the syndrome.

Authors' Contributions: Conception and design: RAT and TT; Acquisition, analysis and interpretation of data: RAT and DC; Drafting the article: RAT, DC and TT; Revising it critically for important intellectual content: TT; Approved final version of the manuscript: RAT, DC and TT.

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Case Report
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Pseudohyponatremia Secondary to Hypercholesterolemia in the Setting of Intrahepatic Cholestasis due to Metastatic Liver Disease: A Case Report and Review of the Literature

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Abstract

Objective. We describe a rare case of pseudohyponatremia in the setting of hypercholesterolemia caused by cholestasis due to metastatic liver disease and provide a review of the published cases in the literature. **Case Report.** We report a case of pseudohyponatremia in a 60-year-old man with rectal cancer with extensive metastasis to the liver. While assessing the patient for hyponatremia, extremely elevated serum cholesterol with normal serum osmolality was detected leading to the diagnosis of pseudohyponatremia. This is one of very few reports of pseudohyponatremia in patients with elevated cholesterol in cholestatic liver disease. **Conclusion.** Hypercholesterolemia is an exceedingly rare cause for pseudohyponatremia. Although pseudohyponatremia per se does not carry a risk to the patient, the delay in diagnosis and treatment plans may pose additional risks. Pseudohyponatremia needs to be considered in patients with low sodium and co-existing cholestasis from metastatic liver disease.

Key Words: Hyponatremia ▪ Cholestasis ▪ Osmolality ▪ Pseudohyponatremia.

Introduction

Hyponatremia is one of the most common electrolyte disturbances in an acute hospital setting (1). However, it is prudent for clinicians to be aware of the pseudohyponatremia phenomenon that indicates a spurious low sodium level resulting from decreased relative proportion of water from elevation of either lipids or proteins in plasma (2). Accordingly, the initial step in evaluating a patient with a low sodium level is exploring the possibility of pseudohyponatremia by checking serum osmolality. Pseudohyponatremia is an artifactual sodium level of less than 135 mmol/L when measured with the indirect ion-selective electrode method in conjunction with a normal serum osmolarity (3). Pseudohyponatremia is typically associated with hyperlipidemia and hyperproteinemia, where the water component is replaced by non-aqueous materials. The condition is rarely reported in patients with hypercholesterolemia, particularly in conjunction with cholestasis (4).

In this report, we describe a patient with pseudohyponatremia in the presence of intrahepatic cholestasis, which was caused by extensive metastatic liver involvement.

Case Presentation

A 60-year-old African American man was admitted to the hospital with worsening abdominal pain and declining functional status. He had become progressively weaker after receiving the first round of chemotherapy for advanced rectal adenocarcinoma with extensive metastasis to the liver, lungs and spine. On the day of admission, the hematologic analysis was significant for hemoglobin of 11.6 g/dL, platelets of 555/nL, and a white blood cell count of 7.4/nL. Blood work was significant for random glucose of 114 mg/dL, creatinine of 1.1

mg/dL, blood urea nitrogen of 20 mg/dL, potassium of 4.9 mmol/L, alanine aminotransferase of 215 U/L, aspartate aminotransferase of 180 U/L, alkaline phosphatase of 1,178 U/L, total bilirubin of 10.8 mg/dL, and serum sodium of 127 mmol/L. His serum sodium concentration 4 months earlier had been 137 mmol/L.

Magnetic resonance cholangiopancreatography was performed to evaluate the possibility of palliative stenting for his progressive cholestasis and showed liver enlargement with numerous mildly T2 hyperintense/T1 hypointense metastatic lesions throughout the liver. There was intrahepatic biliary ductal dilation, which was not amenable to palliative stenting.

Hypovolemic hyponatremia was suspected because of the patient's serum sodium of 127 mmol/L, his poor oral intake, and the presence of dry mucosal membranes on exam. Serum was sent to an outside laboratory to assess osmolality. Intravenous fluid boluses were given, and maintenance fluid was started. Despite adequate fluid resuscitation, on the third hospital day, the patient's serum sodium level further decreased to 125 mmol/L; thus, syndrome of inappropriate secretion of antidiuretic hormone (SIADH) was suspected. Fluid intake was restricted to 1.2 L/day, and the patient's serum sodium was 126 mEq/L on the following day. As a result of this improvement, therapy for SIADH was escalated with salt tablets. However, the serum sodium decreased to 121 mmol/L by the sixth day. At this time the result of serum osmolality became available revealing a normal level of 288 mOsm/kg, leading to a diagnosis of pseudohyponatremia.

Repeated laboratory tests showed worsening alkaline phosphatase levels at 1,698 U/L and total bilirubin at 15.3 mg/dL; random urine sodium was 63 mmol/L. A serum lipid profile workup revealed a total serum cholesterol of 816 mg/dL with triglyceride levels of 382 mg/dL and high-density lipoprotein of 9 mg/dL; the low-density lipoprotein levels were unmeasurable because the total cholesterol was >500 mg/dL. Interestingly, the patient's lipid profile 4 weeks earlier showed total cholesterol of only 213 mg/dL, triglycerides at 115 mg/dL, high-density lipoprotein at 36 mg/dL, and low-density lipoproteins at 153 mg/dL. No further treatment for hyponatremia was further offered as it was determined a laboratory artifact secondary to severe hypercholesterolemia.

Discussion

It is important to note that the commonly used laboratory measure reports plasma sodium level. Plasma typically contains 7% lipids and proteins, but elevated lipids/proteins reduce the amount of electrolytes per unit volume of plasma. Hence, the measured plasma sodium decreases when lipid/protein levels increase, but the concentration of sodium in plasma water, which is the physiologically important value, remains the same. Sodium levels measured using an indirect ion-selective electrode can result in a laboratory artifact, called pseudohyponatremia, because this method uses a dilution step before sodium is measured and estimates serum sodium on the basis of the presumed typical proportion of water in plasma. However, measurements with an ion-selective direct electrode do not require a dilution step and will report accurate levels in the setting of elevated lipid or proteins (4). This method is commonly used in point-of-care devices (5), whereas central laboratories use the indirect method because of its substantially larger capacity.

One of the most common causes of pseudohyponatremia is hypertriglyceridemia (>1,500 mg/dL) but it can occur as a result of hyperproteinemia in patients with plasma cell dyscrasias, intravenous immunoglobulin therapy, or, very rarely, elevated cholesterol (6). Pseudohyponatremia due to hypercholesterolemia is highly uncommon, with a few reported cases (6). The patient described in this report had hypercholesterolemia-induced pseudohyponatremia as a result of cholestatic obstruction from metastatic liver disease. A review of the literature for reported adult patients with pseudohyponatremia in the setting of hypercholesterolemia revealed 14 additional cases, summarized in Table 1.

The reported underlying mechanisms included cholestasis secondary to graft-versus-host disease

Table 1. Literature Review of Pseudohyponatremia Cases Secondary to Hypercholesterolemia

Author/ Reference number	Age (Year), Sex	Underlying condition	Sodium indirect method*	Sodium direct method*	Serum osmolality†	Total cholesterol‡	Publication year
Hickma et al. (9)	62, F	Primary biliary cirrhosis	115	134	NR§	3,011	1989
Ko et al. (11)	27, M	Primary biliary cirrhosis	116	132	304	1,830	1997
Turchin et al. (8)	64, F	Cholestasis due to chronic graft-versus-host disease	124	135	NR§	1836	2005
Turchin et al. (8)	37, M	Cholestasis due to chronic graft-versus-host disease	129	135	NR§	977	2005
Le Riche et al. (14)	29, F	Drug-induced intra-hepatic cholestasis	116	NR‡	NR§	2,815	2005
Inamoto et al. (7)	55, F	Cholestasis due to chronic graft-versus-host disease	101	139	273	4,091	2005
Klinke et al. (13)	36, M	Quetiapine-associated cholestasis	119	NR§	NR§	1,691	2010
Sivakumar et al. (18)	61, F	Obstructive biliary cholestasis secondary to pancreatic cancer	108	127	296	1,713	2011
Vo et al. (15)	41, F	Acute hepatitis C	120	135	297	2,621	2013
Ravella et al. (17)	40, M	Lymphoplasmacytic sclerosing cholangitis	121	138	297	2,109	2015
Hussain et al. (10)	43, F	Primary biliary cirrhosis	121	141	296	2,415	2015
Igbinedion et al. (16)	44, M	Obstructive Biliary Cholestasis in setting of chronic pancreatitis	110	132	302	2247	2017
Song et al. (6)	41, M	Cholestasis due to chronic graft-versus-host disease	121	144	309	1449	2018
El Hage et al. (12)	69, M	Drug-induced cholestatic hepatitis	119	132	283	1,340	2019
Pourafkari et al.‖	60, M	Cholestasis in setting of metastatic liver disease	127	NR§	288	816	2022

*mmol/L; †mOsm/kg; ‡mg/dL; §Not reported; ‖Current case.

after bone marrow transplantation in four patients (6-8), primary biliary cirrhosis in three patients (9-11), medication-induced obstructive jaundice in three patients (12-14), hepatitis C infection (15), chronic pancreatitis (16), autoimmune pancreatitis and lymphoplasmacytic sclerosing cholangitis (17), and pancreatic cancer (18) in one patient each.

Interestingly, all these patients presented with extreme cholestasis. Cholestasis can cause hyperlipidemia by increasing serum Lipoprotein X. Lipoprotein X, a major cholesterol transporter in the plasma, is formed when serum incubates with bile lipoproteins from unesterified cholesterol and phospholipids released from the bile ducts into the bloodstream (19). Lipoprotein X is the common factor in patients with cholestasis-induced hypercholesterolemia. The high level of this lipoprotein, which has a high phospholipid and free cholesterol content, contributes to the pathophysiology of pseudohyponatremia (6). Cholesterol levels are inversely correlated with serum sodium levels; therefore, severe hypercholesterolemia can exacerbate pseudohyponatremia (6).

Conclusion

We present a rare case of hypercholesterolemia-induced pseudohyponatremia in the setting of metastatic liver disease with subsequent cholestatic obstruction. This case highlights the importance of checking serum osmolality before addressing the hyponatremia. Although patients with cholestasis disorder and hyponatremia can have hypovolemic hyponatremia because of volume depletion secondary to vomiting and poor oral intake, it is essential to establish the presence of a

true hypo-osmolar state. In our case, technical issues delayed the serum osmolality results and thus recognition of pseudohyponatremia. Attempts at correcting sodium levels in patients with pseudohyponatremia may lead to unnecessary treatment with dreadful consequences.

What Is Already Known on This Topic:

Pseudohyponatremia is a laboratory artifact in which low measured serum sodium level is accompanied with a normal serum osmolality. Hypertriglyceridemia and hyperproteinemia are two common causes of pseudohyponatremia. Hypercholesterolemia is an exceedingly rare cause of pseudohyponatremia and has been reported in the setting of cholestatic liver disease and subsequent elevation in lipoprotein X.

What This Case Adds:

A literature review identified 14 cases of pseudohyponatremia in adult patients with hypercholesterolemia. Pseudohyponatremia needs to be considered in patients with low sodium levels and cholestasis in the setting of extensive liver metastases.

Authors' Contributions: Conception and design: LP, SKY and CC; Acquisition and interpretation of data: LP, SKY, CC and NN; Literature review: LP, SKY, CC, and NN; Drafting the manuscript: LP, SKY and CC; Critical revision: NN; Approval of final version: LP, SKY, CC and NN.

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Images in Clinical Medicine
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A Giant Polypoid Gastric Heterotopia of the Ileum as a Cause of Intussusception in an Adolescent

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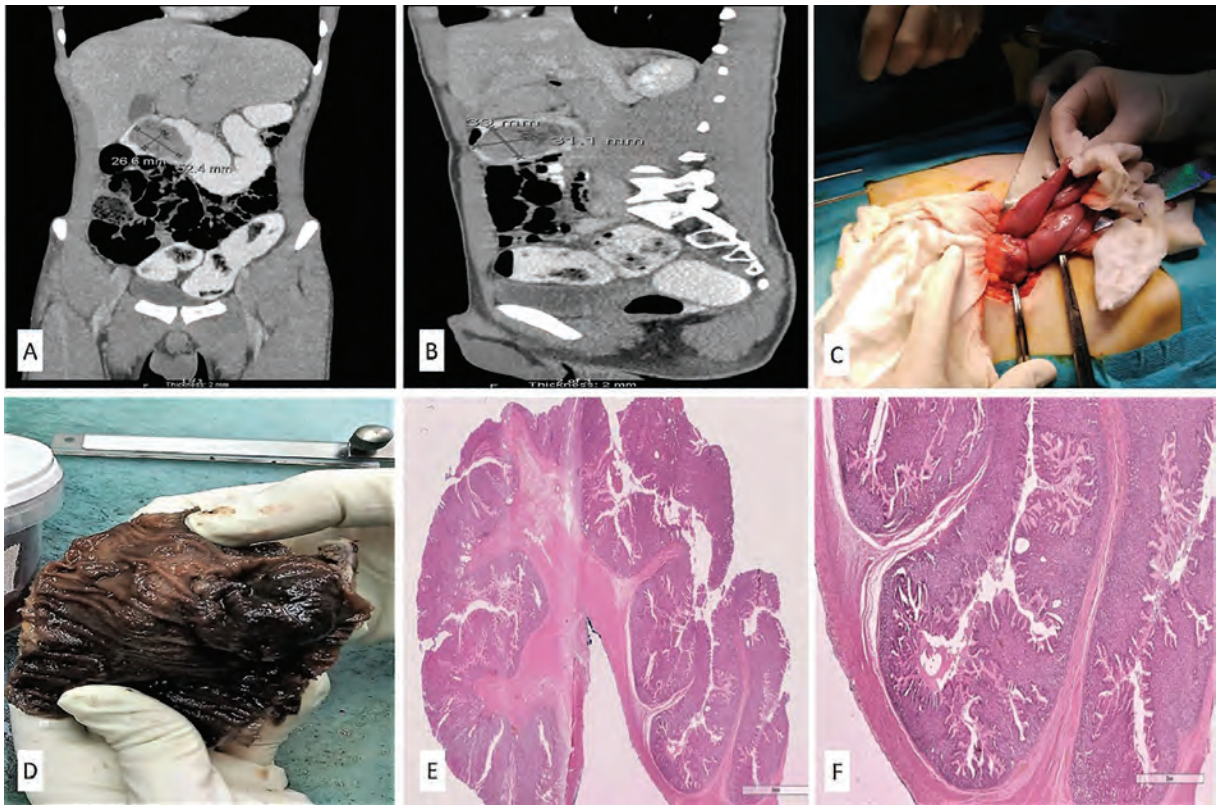
Key Words: Small Intestine ▪ Intussusception ▪ Gastric Heterotopia ▪ Polyps.



A 16-year-old boy presented to the emergency department with a 10-day history of abdominal pain and vomiting. Since childhood he had experienced occasional stomach pain and vomiting

related to his food intake. He denied diarrhea and other symptoms. An abdominal ultrasound was performed, revealing intussusception in the right hemiabdomen. It also showed a thickened and hypervascularized central intestinal gyrus within the cecum and ascending colon. Abdominal CT was also performed, revealing an invagination in the area of the ileocecum, with its head in the middle of the transverse colon. The presence of a mass was suspected at the head of the invagination, measuring approximately 5 × 3 cm (Panel A and B). Emergency exploratory surgery confirmed the radiologically verified intussusception (Panel C). Intraoperatively, a ~5 cm soft mass was found, occupying ~2/3 of the intestinal lumen. The mass was located approximately 50 cm proximal from the Bauchini valve. Partial ileal resection was performed with end-to-end hand-sewn ileal anastomosis. Gross examination of the resected specimen revealed a polypoid lesion that measured 50 × 30 × 25 mm (Panel D). Histopathological examination confirmed a well-demarcated polyp, lined by a foveolar type epithelium, with all the elements found in the gastric mucosa, and well-developed gastric glands (Panel E and F). No dysplasia or malignancy was identified within the gastric heterotopia. On the basis of the radiological, intraoperative and histopathological features, a giant polypoid gastric heterotopia was diagnosed. A second opinion was sought from an expert gastrointestinal pathologist who concurred with the final diagnosis.

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Narrative Review
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On Measuring Vaccine Effectiveness with Observational Study Designs

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Abstract
Herein, we present a bird's eye view of common observational study designs utilized for measurement of vaccine effectiveness. Assessing vaccines effectiveness is an integral part of vaccine research, particularly for the newly developed vaccines. A cohort study is prospective, directing from an exposure to one or more outcomes. The design is the best method to ascertain the attack rate of an infectious disease. A traditional case-control study is retrospective, directing from a given outcome to one or more exposures. The design cannot provide the relative risk, but it can provide the odds ratio, which is a good estimation of the relative risk when the attack rate is low. Critically depending on laboratory test results and performance, the test-negative case-control study design is another type of observational study commonly used nowadays for the evaluation of the vaccine effectiveness. Comparing to cohort and traditional case-control designs, conducting a test-negative case-control study is relatively cheaper and faster. Herein, we describe each of the above-mentioned study designs through examples generated by a Monte-Carlo simulation program assuming real-world conditions. **Conclusion.** The simulation shows that regardless of the study design employed, the diagnostic test specificity is of utmost importance in providing a valid estimate of the vaccine effectiveness.

Key Words: Vaccines ▪ Research Design ▪ Cohort Studies ▪ Case-Control Studies ▪ SARS-CoV-2.

Introduction

For every new vaccine, the vaccine effectiveness (*VE*), an index reflecting the measure of infection or disease risk reduction attributable to vaccination among vaccinated individuals compared with unvaccinated people under real-world situations, should be determined to figure out the future vaccination policy and strategy to be implemented. The *VE* is defined as:

$$VE = \frac{AR_{unvac} - AR_{vac}}{AR_{unvac}} = 1 - \frac{AR_{vac}}{AR_{unvac}} = 1 - RR \quad \text{(Eq 1)}$$

where AR_{unvac} and AR_{vac} represent the attack rates of the infection in the unvaccinated and vaccinated individuals, respectively, and *RR* is the relative risk (1, 2). The gold standard study designs to determine the *VE* are the randomized clinical trial and cohort studies (3). In such studies, the *AR* of the infection is evaluated after a period of time, say 3 months after vaccination, in the two groups of vaccinated and unvaccinated individuals who had been either randomized into two groups (in a randomized clinical trial) to abolish the effect of confounding variables, or at least matched for known important confounders (in a cohort study).

Although clinical trials are the best study design to measure *VE*, under certain circumstances, for ethical concerns, we are not able to conduct clinical trials; for instance, it is unethical not to vaccinate a susceptible person with exposure to

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an infectious agent for which an approved vaccine is available. Therefore, we need to conduct observational studies to determine the *VE* under more realistic situations in wider population groups in whom the *VE* may differ from that observed in clinical trials for several reasons including difference in the geographic parameters, sub-populations not included or under-represented in the original trials (*e.g.*, children and pregnant women), sub-optimal consideration of the cold-chain necessary for optimal effectiveness of the vaccine, incomplete vaccination, incorrect time spacing between the vaccine doses administered, emergence of new variants of the infectious agent, and many other factors (4, 5).

In this review, we will discuss the *pros* and *cons* of common observational studies used to determine *VE*. To better understand the issue, we first overview the study designs, present examples through using a simulation program, and finally describe a new type of case-control study, the so-called test-negative case-control (TNCC) study that has become common to use for the assessment of *VE*.

Setting

Suppose we want to study an arbitrarily chosen population of 2 000 000 individuals (for example, the population of a city like Shiraz) and that we have vaccinated a hypothetical fraction of 40% of the people against an infectious agent. Let the true *VE* after 3 months of vaccination be 0.70 and that we use a diagnostic test with a test sensitivity (*Se*) of 60% and a specificity (*Sp*) of 100% for the detection of the infection. Furthermore, suppose that the infected people present with signs and symptoms similar to a flu and that there is another flu-like illness that might also affect people living in the study community, independent of whether they have already been affected by the infection of interest or not. Also, assume that the infection has an *AR* of 15% in unvaccinated individuals (consistent with the *AR* of influenza) (6); and that the *AR* of the flu-like illness is 30% (consistent with the attack rates of non-influenza flu-like illness seen during a cold season) (7, 8). To make things simple, let us assume that the *AR* of the flu-like illness is independent of vaccination status against the infection of interest, duration since vaccination, age of people, and other variables. Now, suppose we want to estimate the *VE* of an influenza vaccine using various observational study designs.

Observational Studies

Clinical research studies can be classified into two broad categories — observational and interventional studies (9). In observational studies, the researcher just observes; no intervention occurs. Observational studies can further be categorized into longitudinal and cross-sectional studies, based on how the observations are made over the study period. Two important longitudinal observational studies are cohort and case-control studies (9).

Cohort

In a cohort study, two groups of individuals with and without exposure to a certain agent (*e.g.*, a vaccine) are followed for a certain period of time. The two groups are similar in (theoretically, perfectly matched for) all other variables but their exposure (10). In its simplest form, we compare the experience of the exposed group with that of the unexposed group and measure the incidence of a certain outcome in the two groups. If the incidence of the outcome in one group is significantly different from that in other group, then we conclude that there should be an association between the exposure and outcome of interest (10). Cohort studies are prospective — directing from an exposure to one or more outcomes.

Suppose that the disease of interest is influenza, and that a random sample of 50 vaccinated (exposed) and 50 unvaccinated (unexposed) individuals was taken from the above-mentioned population (Figure 1A). For the sake of simplicity, let us assume that the two groups were perfectly matched for other variables. The first five columns (Figure 1A) represent the exposed individuals; the remaining, unexposed. Let us define the outcome of interest as presence of influenza ascertained by

a diagnostic test (a positive test). Suppose that the 100 study participants were followed for 3 months and that 10 of whom were found test positive, which translated into an *AR* of 10% in 3 months. In other words, the risk of infection within 3 months, regardless of vaccination status, was 10%. Two (4%) out of 50 of the exposed (vaccinated) participants and 8 (16%) of 50 of unexposed (unvaccinated) individuals developed the outcome of interest (a positive test result). The *AR* of the infection was therefore 4% in the exposed and 16% in the unexposed group (Table 1B). The unexposed group carried a 4-fold (= 16%/4%) increase in the risk of infection as compared with the vaccinated group. In other words, vaccination decreased the risk of infection by 75%, the estimated *VE*.

Table 1. Test Results Stratified by Vaccination Status in Various Study Designs

A) General template	Disease*		
	Present	Absent	
Vaccinated	*a*	*b*	*a+b*
Unvaccinated	*c*	*d*	*c+d*
	a+c	*b+d*	*n*

B) Cohort	Disease*		
	Present	Absent	
Vaccinated	2	48	50
Unvaccinated	8	d	50
	10	90	100

C) Traditional Case-Control	Disease*		
	Present	Absent	
Vaccinated	8	23	
Unvaccinated	42	27	
	50	50	100

D) Test-Negative Case-Control	Test		
	Positive	Negative	
Vaccinated	2	32	
Unvaccinated	11	55	
	13	87	100

A) a 2×2 contingency table, the general template for various study designs of *n* participants, and examples for samples of 100 individuals using B) cohort; C) traditional case-control; D) test-negative case-control studies associated with Figure 1; *The status was determined by using a diagnostic test (or a battery of tests).

Let us examine the general parametric form of a cohort study of *n* participants (Table 1A). Then we can write:

$$AR = \frac{\text{number of infected people}}{\text{total number of participants at risk of infection}} = \frac{a+c}{n} = \frac{a+c}{a+b+c+d} \quad \text{(Eq 2)}$$

where *AR* is the marginal risk of infection in the whole study participants, regardless of vaccination status, and *a*, *b*, *c*, and *d* are Table cell values (Table 1A). In the same way, we can calculate the *AR* in the vaccinated and unvaccinated groups:

$$AR_{vac} = \frac{\text{number of vaccinated people with infection}}{\text{total number of vaccinated individuals at risk of infection}} = \frac{a}{a+b} \quad \text{(Eq 3)}$$

and

$$AR_{unvac} = \frac{\text{number of unvaccinated people with infection}}{\text{total number of unvaccinated individuals at risk of infection}} = \frac{c}{c+d} \quad \text{(Eq 4)}$$

The *RR* is:

$$RR = \frac{\text{risk in the exposed group}}{\text{risk in the unexposed group}} = \frac{AR_{vac}}{AR_{unvac}} = \frac{\frac{a}{(a+b)}}{\frac{c}{(c+d)}} = \frac{a(c+d)}{c(a+b)} \quad \text{(Eq 5)}$$

When the outcome is something like hospitalization or death, recognizing the outcome is easy. However, when the outcome is a variable like presence or absence of a certain disease, identifying the outcome is not always that easy. Normally, we rely on diagnostic test (or a battery of tests) results

to classify people into diseased and undiseased groups. Depending on the distribution of test results, test *Se* and *Sp*, and the prevalence of the disease, levels of uncertainty would be introduced. In our example, we missed 4 cases of influenza (3 gray and 1 red squares in Figure 1A) for having false-negative test results. This would affect the estimated risks in the two study groups and the *VE*. Having a test *Sp* of 100%, no false-positive result occurred.

The required minimum sample size for a cohort study being conducted for the measurement of *VE* is a function of the anticipated *VE* and the desired width of its confidence interval, the percentage of the vaccine coverage, and presumed *AR* in the unvaccinated group (5, 11). The minimum sample size can be calculated using a calculator available online (12).

A cohort study has several advantages. It is the best type of study design for the measurement of the incidence (*AR*, incidence, risk) of a given outcome (*e.g.*, a disease) under certain conditions. The temporal sequence of events is typically clear and one can usually make a cause-and-effect inference and ascertain the natural history of a disease. With this design, it is possible to measure risks of several outcomes (*e.g.*, infection, hospitalization, and death) and their association with a given exposure (*e.g.*, vaccination). The design is also very good to assess the risk of rare exposures (10). However, it

Figure 1. Examples generated by the simulation program based on the assumptions made in the Setting section of the article: 100-individual samples taken from a 2 000 000-individual population with a vaccination coverage of 40%. The test had a sensitivity of 60% and specificity of 100% for the detection of influenza (the outcome); attack rate of 15% in unvaccinated individuals for influenza, (6) and 30% for the flu-like illness (7, 8). A) Cohort study design: The left-most 5 columns are vaccinated; the remaining, unvaccinated. Those with a positive test are indicated. Others were test-negative. 4 individuals with influenza (3 gray and 1 red squares) had false-negative test results. B) Traditional case-control study design: The left-most 5 columns are test-positive individuals (considered cases); others were test-negative (controls). One of the test-negative individuals in the control group (1 red square) had really influenza (a false-negative test result). C) Test-negative case-control study design: Note that all 100 individuals have flu-like illness. 5 individuals with influenza (5 gray squares) had false-negative test results. The test specificity was 100%, hence, no false-positive result was obtained.

has some disadvantages. Following a large cohort for a long period of time is expensive. The intensity of follow-up should be equal in the two study groups, which is not always possible. Many of the study participants may be lost to follow up. Some of the participants in one group may decide to switch to another group (*e.g.*, an unvaccinated person may decide to receive the vaccine). Matching of the study groups for the important covariates and controlling of the known confounding variables are not always easy. And, the design is not appropriate for rare outcomes (10). Generally, cohort studies are more efficient in situations where the incidence of outcome is higher than the prevalence of exposure (13).

Case-Control

Case-control study design is retrospective. It begins with a certain outcome (*e.g.*, presence of an infection) and returns back in time to examine the level of exposure to one or more factors (*e.g.*, vaccination status) of those with the outcome (cases) and without the outcome (controls). The cases and controls are similar in (theoretically, perfectly matched for) all other variables but the status of the outcome (13). In a case-control study, because we are typically not aware of the real proportion of the cases and controls in the population, we are not able to estimate the risk (incidence and *AR*) in the two groups and the *RR*; instead, we compare odds of exposure in the two groups and calculate the odds ratio (*OR*), as follows (Table 1A):

$$\begin{aligned} OR &= \frac{\text{odds of exposure in cases}}{\text{odds of exposure in controls}} \\ &= \frac{a/c}{b/d} = \frac{a/b}{c/d} \\ &\approx \frac{a/(a+b)}{c/(c+d)}, \text{ if } a \ll b \wedge c \ll d \\ &\approx \frac{a(c+d)}{c(a+b)} \\ &\approx RR \end{aligned} \qquad \text{(Eq 6)}$$

If the *AR* is low (*i.e.*, $a \ll b \wedge c \ll d$), then $b \approx a+b$ and $d \approx c+d$, *OR* is an acceptable estimation for *RR* (Eq 6), and *VE* can be calculated as 1 – *OR* (2).

Suppose a random sample of 50 diseased (defined as test-positive individuals) and 50 undiseased (test-negative) people from the above-mentioned population was taken (Figure 1B, Table 1C). The *OR* (Eq 6) is then:

$$\begin{aligned} OR &= \frac{a/b}{c/d} \\ &= \frac{8/23}{42/27} = \frac{0.35}{1.56} = 0.22 \end{aligned} \qquad \text{(Eq 7)}$$

The estimated *VE* after 3 months of vaccination was then 0.78 (= 1 – 0.22). Depending on the test results distribution and performance, and the prevalence of the disease of interest, false-positive and false-negative results may occur. In our example, we had one false-negative individual (Figure 1B).

The required minimum sample size for a case-control study being conducted for the measurement of *VE* is a function of the anticipated *VE* and the desired width of its confidence interval, and the presumed prevalence of vaccination in the control (undiseased) group (5, 11). The minimum sample size can be calculated using a calculator available online (12).

The case-control design has several advantages. It is the most efficient study design in terms of time and money spent, and efforts made (13). The design is especially appropriate for rare outcomes; this makes sense if we consider that for observing a rare outcome, a researcher conducting a cohort study should have normally follow a large group of people for a long period of time. In a case-control study we can begin with a given outcome and examine one or more exposures. However, the design is not appropriate when the exposure frequency is low. In measuring the *VE*, the *AR* is usually acceptably low and case-control studies would give satisfactory estimates. Although it is usually easier to conduct a case-control study compared to a cohort

study, conducting the investigation could still be labor-intensive — intense efforts are still needed to identify and recruit the controls from the population (13).

Test-Negative Case-Control (TNCC)

A TNCC study design technically has a case-control design. The only difference is the way the cases and controls are recruited. It seems that the first complete description of the method dates back to 1985, when Miettinen mentioned the methodology in his book (14). The method has widely been used for measuring influenza *VE*, first employed by Skowronski in Canada in 2004–2005 (15). The design has been frequently used thereafter, most commonly for assessing the *VE* (16-20).

In a TNCC study, a group of people referred to a healthcare center for a reason, say complaining of flu-like illness, is considered the study sample. All test-positive individuals are considered "cases;" test-negative individuals, "controls." The exposure (vaccination) status is then ascertained in the two groups (21).

Suppose a sample of 100 patients complaining of a flu-like illness referred to a healthcare center was taken (Figure 1C, Table 1D). A diagnostic test was then performed for each of the study participants to ascertain whether they had the infection of interest or not. The 13 test-positive individuals (2 orange and 11 gray squares in Figure 1C) were considered cases; the remaining 87 (= 100 – 13), controls. The individuals were then asked about their vaccination status (exposure, Table 1D). As in traditional case-control study design, the *OR* can be calculated as:

$$OR = \frac{a/b}{c/d} = \frac{2/32}{11/55} = \frac{0.06}{0.20} = 0.31 \quad \text{(Eq 8)}$$

The *VE* after 3 months of vaccination was then 0.69 (= 1 – 0.31).

The TNCC design has all the advantages and disadvantages of traditional case-controls (*e.g.*, outcome misclassification, recall bias). We observed 5 diseased individuals with false-negative test results, misclassified to the control group (Figure 1C). The study design, however, has the advantage of reducing the effect of difference in health-care seeking behavior between the exposed and unexposed groups (3-5, 16). TNCC study is relatively cheaper and faster to conduct in comparison with cohort and traditional case-control studies (16).

Monte-Carlo Simulation

R software version 4.1.0 (R Project for Statistical Computing) was used for simulation. The pseudocode of the program is presented in Table 2 (see Appendix for the R codes). The simulation parameters were initially set to the values described earlier (see Setting).

The estimated *VE* derived from a study with a sample size of 100, although more appropriate for presentation as a graph (Figure 1), would be associated with high degrees of uncertainties due to sampling error. The simulation program was therefore run with a sample size of 10 000 individuals, keeping other parameters the same (Table 2), which resulted in estimated *VE* values of 0.686, 0.705, and 0.710 for cohort, traditional case-control, and TNCC designs, respectively (Table 3).

To obtain more accurate results, we repeated the above *in silico* experiments of 10 000 individuals for different combinations of the test *Se* (varying from 60% to 100%) and *Sp* (varying from 80% to 100%) for 1000 times, and reported the mean of *VE* (Figure 2). The simulation was performed for *AR* of 15% for unvaccinated individuals, compatible with the *AR* of influenza (6); and an *AR* of 5% for SARS-CoV-2 infection (5).

As expected, cohort design gave the most accurate estimates provided using a highly specific diagnostic test. Results obtained from traditional case-control design and TNCC design were very similar (Figure 2). The test *Sp* was more important than the *Se*.

Table 2. The Pseudocode of the Simulation Program*

```
Begin
  Make a population of 2 000 000 individuals, 40% of whom were vaccinated;
  15%, had the disease; and 30%, had a flu-like illness
  Loop for various values of Se and Sp
    Determine the test status of each individual in the population based on Se and Sp
    # Cohort
    Loop for 1000 times
      Choose at random from the population, 5000 vaccinated and 5000 unvaccinated individuals
      Calculate the RR based on the test status
      VE = 1 – RR
    End Loop
    # Traditional Case-Control
    Loop for 1000 times
      Choose at random from the population, 5000 diseased and 5000 undiseaded individuals
      Calculate the OR based on the test status
      VE = 1 – OR
    End Loop
    # Test-Negative Case-Control
    Loop for 1000 times
      Choose at random from the population, 10 000 individuals with flu-like illness
      Calculate the OR based on the test status
      VE = 1 – OR
    End Loop
  End Loop
  Draw the results as a graph
End
```

*See Appendix for the R codes.

Table 3. Test Results Stratified by Vaccination Status in 10 000-individual Samples Using Different Study Designs

A) Cohort	Disease*		
	Present	Absent	
Vaccinated	136	4864	5000
Unvaccinated	433	4567	5000
	569	9431	10 000

B) Traditional Case-Control	Disease*		
	Present	Absent	
Vaccinated	874	2089	
Unvaccinated	4126	2911	
			10 000

C) Test-Negative Case-Control	Test		
	Positive	Negative	
Vaccinated	113	3901	
Unvaccinated	543	5443	
			10 000

A) cohort; B) traditional case-control; C) test-negative case-control; *The status was determined by using a diagnostic test (or a battery of tests).

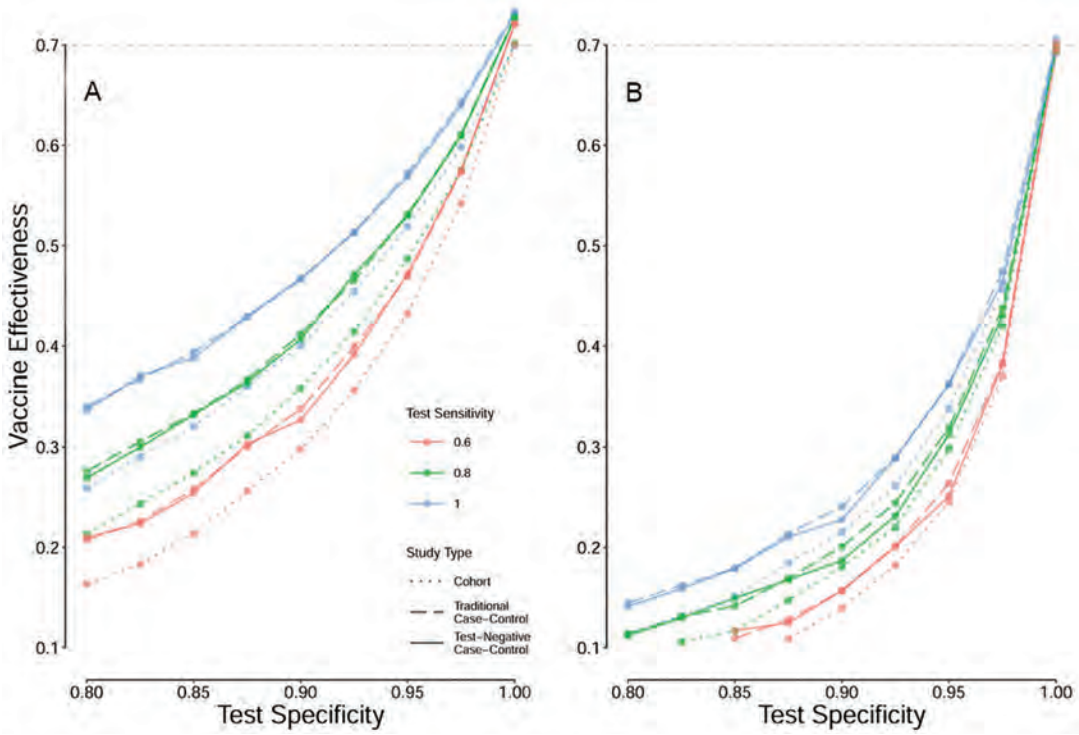

Figure 2. Vaccine effectiveness derived from *in silico* studies simulating various study types under different conditions. The horizontal dash-dotted gray line represents the true vaccine effectiveness. The results are mean values of the vaccine effectiveness derived from 1000 repetitions of a Monte-Carlo simulation (Table 2). Each time, 10 000 individuals were examined under combinations of the test sensitivity (varying from 0.6 to 1 [*i.e.*, 60% to 100%]) and specificity (varying from 0.8 to 1 [*i.e.*, 80% to 100%]). The simulation was performed for attack rates of A) 15% for unvaccinated individuals, compatible with the that of influenza (6); and B) 5%, for SARS-CoV-2 infection (5). In both situations, the results obtained are satisfactory when the test specificity is almost 1 (*i.e.*, 100%).

Discussion

It was found that cohort studies are superior to other observational designs for the evaluation of *VE*. This is because only a cohort study can accurately determine the *AR* of the disease in the vaccinated and unvaccinated groups. A case-control study can only provide *OR* as an estimate for *RR*. However, this assumption is only true when the *AR* is considerably low (Eq 6). Given the low *AR* for many infectious disease (*e.g.*, influenza and SARS-CoV-2), the calculated *OR* is not much different from the *RR* and thus a case-control study can also give a satisfactory estimate for the *VE*. Furthermore, using logistic regression analysis, the *OR* can be adjusted for various confounding variables to give an adjusted *VE* (22). This is in keeping with our results; for an *AR* of 15% (Figure 2, left panel), the *VE* estimates derived from the two studied case-control designs overestimated the true *VE*; with a lower *AR* of 5%, these studies gave estimates closer to the real *VE*.

The TNCC design has the advantage over cohort and traditional case-control studies for being less expensive and faster to conduct (16). In keeping with our simulation results, numerous *in silico* studies have also shown that the design can provide estimates of *VE* in good agreement with those of cohort and traditional case-control studies provided that a highly specific diagnostic test is used (2, 6). Fortunately, the tests commonly used for the diagnosis of influenza and SARS-CoV-2 infections have a *Sp* of almost 100% (23, 24); most of the published studies using the TNCC design have used highly specific diagnostic tests (25, 26). Considering the cardinal importance of the test *Sp* in the validity of the *VE* obtained from TNCC

design, it is prudent to use higher cut-off values in the interpretation of tests with quantitative results used to classify the cases and controls (27).

Limitations of the Study

Our study has several limitations. The simulation was limited by its simplicity; it overlooked many important factors. As an example, it was assumed that the *AR* of flu-like illness was independent of vaccination and disease status, age of people, and other variables. This was of course not the case in real life. In this way, selection of cases and controls from the subpopulation of people with flu-like illness, as it was done in the TNCC study, was practically equivalent to selecting cases and controls from the source population. That is why the traditional case-control and the TNCC designs provided almost similar results (Figure 2). If a test with a *Sp* of nearly 100% is utilized, almost no false-positive result would occur. However, if a person had had the infection of interest before vaccination, the test might remain positive for a long time, even when there is no active infection (28). Another situation that makes things complicated is usage of different vaccines (as in case of vaccination in some people against SARS-CoV-2). More complex simulations are necessary to address these limitations and provide more realistic results. Nevertheless, in this article we just intended to provide a bird's eye view of the designs commonly used in assessing the *VE*, not to provide an in-depth review of these methods.

In a nutshell, the TNCC design, which has recently been frequently used in assessing the *VE*, may reduce but not eliminate the effect of all confounding variables and selection bias due to differential recall of the exposure compared with traditional case-control design (16, 29). The chief advantage of TNCC over cohort and traditional case-control studies is the fact that it does not require much resources and it can be conducted during a relatively short period of time; it can be nested in routine surveillance without any concerns regarding the validity of the estimates derived. The design is not only commonly used in assessing the *VE*, but can also be utilized to measure risks in other settings such as antibiotic resistance (30), and venous thrombosis (31), to name only a few other applications. Using logistic regression analysis or stratification, the obtained estimated *OR* can be adjusted for important confounding variables (22). Although the method seems to be easy to do, it should be used with caution as the design suffers from all the limitations mentioned for observational studies (9, 10, 13, 32, 33). The TNCC design provides a unique opportunity for the interdisciplinary collaboration between laboratory sciences and epidemiology considering the important caveats in both areas of investigation.

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

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APPENDIX

The Simulation Program in R

```
library("ggplot2")
library("ggh4x")

N <- 2000000       # Population size
v_coverage <- 0.4  # Vaccine coverage
n <- 5000          # Sample size (in each group)
rep <- 1000        # Number of repetitions
AR <- 0.15         # Attack Rate in unvaccinated
AR_ill <- 0.30     # Attack Rate of flu-like illness
se <- 0.60         # Test sensitivity
sp <- 1.00         # Test specificity
VE <- 0.70         # Vaccine effectiveness

set.seed(123)
dat <- data.frame(dis = rep(0, N), Vac = rep(0, N), ill = rep(0, N))

dat$Vac[1:round(v_coverage*N)] <- 1
dat <- dat[sample(nrow(dat)),]       # Shuffle dat
dat$ill[1:round(AR_ill*N)] <- 1

len <- length(dat[dat$Vac == 0,]$dis)
dat[dat$Vac == 0,]$dis <- ifelse(runif(len, 0, 1) < AR, 1, 0)

len <- length(dat[dat$Vac == 1,]$dis)
dat[dat$Vac == 1,]$dis <- ifelse(runif(len, 0, 1) < AR*(1-VE), 1, 0)

dat$Vac = factor(dat$Vac, labels=c("Unvaccinated", "Vaccinated"))
dat$dis = factor(dat$dis, labels=c("Undiseased", "Diseased"))
dat$ill = factor(dat$ill, labels=c("Not ill", "Ill"))

dat_ve <- data.frame(AR = NA, se = NA, sp = NA, VE = NA, CI_lo = NA, CI_hi = NA, study = NA)

ve <- rep(NA, rep)
for (se in seq(1.0, 0.6, by = -0.2)){      #-- for test sensitivity from 0.6 to 1.0
  for (sp in seq(1.0, 0.8, by = -0.025)){  #-- for test specificity from 0.8 to 1.0
    dat_dis <- dat[dat$dis == "Diseased",]
    dat_dis$test <- ifelse(runif(nrow(dat_dis), 0, 1) < se, 1, 0)

    dat_undis <- dat[dat$dis == "Undiseased",]
    dat_undis$test <- ifelse(runif(nrow(dat_undis), 0, 1) < sp, 0, 1)

    dat <- rbind(dat_dis, dat_undis)
    dat$test = factor(dat$test, labels=c("Negative", "Positive"))

#-------------------------------------------------------------------------------------------

#--- Cohort
    dat_vac <- dat[dat$Vac == "Vaccinated",]
    dat_unvac <- dat[dat$Vac == "Unvaccinated",]
    if (nrow(dat_vac) < n | nrow(dat_unvac) < n){
      print("Cohort: Sample size too high!")
    }

    for (i in 1:rep){
      #-----------         Exposed      --------------------       Unexposed
      d <- rbind(dat_vac[sample(nrow(dat_vac), n), ], dat_unvac[sample(nrow(dat_unvac), n), ])
```

```
      t <- table(d$Vac, d$test)
      #-- 1-RR
      ve[i] <- 1-(t[2, 2]/(t[2, 1] + t[2, 2])) / (t[1, 2]/(t[1, 1] + t[1, 2]))
    }
    dat_ve <- rbind(dat_ve, data.frame(AR = AR, se = se, sp = sp,
            VE = mean(ve, na.rm=TRUE), CI_lo = as.numeric(quantile(ve, 0.025, na.rm=TRUE)),
            CI_hi = as.numeric(quantile(ve, 0.975, na.rm=TRUE)), study = "Coh"))

#-------------------------------------------------------------------------------------------

#--- Traditional Case-Control
    dat_dis <- dat[dat$test == "Positive",]      #-- Cases
    dat_undis <- dat[dat$test == "Negative",]    #-- Controls
    if (nrow(dat_dis) < n | nrow(dat_undis) < n){
      print("Case-Control: Sample size too high!")
    }

    for (i in 1:rep){
      #----------         Diseased     --------------------       Undiseased
      d <- rbind(dat_dis[sample(nrow(dat_dis), n), ], dat_undis[sample(nrow(dat_undis), n), ])
      t <- table(d$Vac, d$test)
      #-- 1-OR
      ve[i] <- 1-(t[2, 2]/t[1, 2]) / (t[2, 1]/t[1, 1])
    }
    dat_ve <- rbind(dat_ve, data.frame(AR = AR, se = se, sp = sp,
            VE = mean(ve, na.rm=TRUE), CI_lo = as.numeric(quantile(ve, 0.025, na.rm=TRUE)),
            CI_hi = as.numeric(quantile(ve, 0.975, na.rm=TRUE)), study = "CC"))

#-------------------------------------------------------------------------------------------

#--- Test-Negative Case-Control
    d_ill <- dat[dat$ill == "Ill",]             # Include only ill people
    OR <- rep(NA, rep)

    for (i in 1:rep){
      d <- d_ill[sample(nrow(d_ill), 2*n),]
      t <- table(d$Vac, d$test)
      #-- 1-OR
      ve[i] <- 1-(t[2, 2]/t[1, 2]) / (t[2, 1]/t[1, 1])
    }
    dat_ve <- rbind(dat_ve, data.frame(AR = AR, se = se, sp = sp,
            VE = mean(ve, na.rm=TRUE), CI_lo = as.numeric(quantile(ve, 0.025, na.rm=TRUE)),
            CI_hi = as.numeric(quantile(ve, 0.975, na.rm=TRUE)), study = "TNCC"))
  }
}
dat_ve <- dat_ve[-1, ]
write.csv(dat_ve, file = "VE.csv", row.names = FALSE)

#----------------- Graphs Figure 2
#dat_ve <- read.csv("VE.csv")

dat_ve$study <- factor(dat_ve$study, levels = c("Coh", "CC", "TNCC"),
                               labels = c("Cohort", "Traditional\nCase-Control",
                                                    "Test-Negative\nCase-Control"))

ggplot(dat_ve, aes(x = sp, color = as.factor(se), fill = as.factor(se),
         linetype = as.factor(study))) +
  geom_hline(yintercept = 0.70, color = "gray70", linetype="dotdash") +
  geom_line(aes(y = VE), size = 0.9, alpha = 0.6) +
  geom_point(aes(y = VE), size = 2.5, alpha=0.6) +
  xlab("Test Specificity") +
  theme_classic() +
  guides(x = "axis_truncated", y = "axis_truncated") +
```

```
scale_linetype_manual(values = c("dotted", "longdash", "solid")) +
scale_y_continuous(name="Vaccine Effectiveness",
       breaks=seq(0.2, 0.8, by = 0.1)) +
theme(aspect.ratio = 6/4,
legend.position = c(0.97, 0.03), legend.key.width=unit(1.2,"cm"),
legend.key.height=unit(0.9,"cm"),
legend.justification = c("right", "bottom"),
legend.text = element_text(size = rel(0.9)),
axis.text = element_text(size = rel(1.3), color = "black"),
axis.title = element_text(size = rel(1.9))) +
guides(color=guide_legend(title="Test Sensitivity", order = 1),
       fill=guide_legend(title="Test Sensitivity", order = 1),
       linetype=guide_legend(title="Study Type", order = 2))
```

Historical Article

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Medical Biography of Isak Samokovlija: The Famous Bosnian-Herzegovinian Writer

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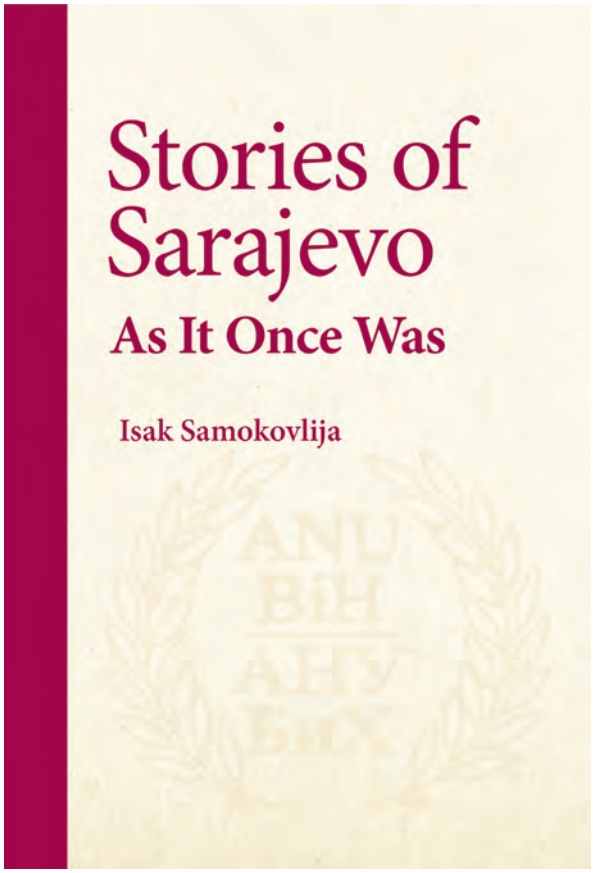
Abstract

The purpose of this paper is to shed light on the biographical, professional, and health-educational works of Dr. Isak Samakovlija, who was better known as a writer than a doctor in the country where he was born. He was born in 1889 in Goražde, the easternmost province in the Austrian-Hungarian monarchy, into a modest Jewish merchant family. He attended high school in Sarajevo and completed his studies in medicine in Vienna in 1917. During the First World War, he served twice in the Austro-Hungarian army. After the end of the First World War in 1918, he completed a medical internship at the National Hospital in Sarajevo. He began his service as a doctor, first in Goražde and then in Fojnica and Sarajevo. After the establishment of the Independent State of Croatia in May 1941, he was dismissed from his duties in the service without the right to pension or support, and without the right to appeal. In the Independent State of Croatia, he was twice mobilized into the Home Guard and was manager of the clinic in the Alipašin Most refugee camp. After World War II, he was the head of the Health Education Department of the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina in Sarajevo. Together with a group of enthusiastic doctors, he founded and edited the first Bosnian medical journal *Život i Zdravlje* (Life and Health). In that journal, Dr. Samokovlija published 29 articles of health and educational content. In 1949, Dr. Samokovlija left the Ministry of Public Health and continued to edit the literature and art journal *Brazda*, but he still had a private practice until the end of his life. He died in Sarajevo on January 15, 1955. He was buried with the highest state honors at the Jewish cemetery in Sarajevo. **Conclusion.** Isak Samakovlija (1889-1955) was one of the first medical doctors born in Bosnia and Herzegovina. He made a significant contribution to the improvement of people's health after the First and Second World Wars in the places where he worked. His special contribution are his articles on health education.

Key Words: Doctors and Writers ▪ Isak Samokovlija ▪ Sarajevo ▪ Bosnia and Herzegovina.

Introduction

Isak Samokovlija was a doctor and writer. Both of these occupations were inextricably linked in his life. His medical work, from which his family and he lived, did not give him enough time for literary work. In 1954, in a conversation with the journalist Siniša Paunović[1], he said: "I mostly worked (wrote) at night. When I finished my medical work, I would throw myself into literature" (1). He published his first short stories in 1926, first in *Jevrejski život* (Jewish life)[2] (2) and the magazine *Gajret*[3] (3), and already in 1927, his short story *Rafina avlija* was published in *Srpski književni glasnik*[4] (4). Just two years later, his first collection of short stories, "From Spring to Spring," was published by the Sarajevo Writers' Group. From then, with the interruption of the war years, 1941–1945, until the end of his life, Dr. Samokovlija continued to write literary texts with varying intensity, that is, as much as his medical affairs and life circumstances allowed (1). Many writers and literary critics wrote about his literary work and also his literary creativity (5). After the Second World War, alongside his medical work, he continued his literary work, and since 1954, his short stories have been translated into many foreign languages (6-13) (Picture 1). Nobel laureate Ivo Andrić[5],



Picture 1. The front page of Isak Samokovlija's book in English.

his contemporary, in an introduction to his book, Salamon's Letter, wrote: ... "The deeply human Isak Samokovlija is one of the best writers that Bosnia and Herzegovina has given to our literature" (14), and Meša Selimović[6], in his memories on Samokovlija, said that Samokovlija, after Andrić and Kočić[7], was "the best Bosnian storyteller."

However, only as much was written about his medical work as was necessary to indicate the connection between his profession and his writing. He worked as a doctor until the end of his life, and never "escaped" from medicine to literature. Working for many years in various medical jobs, he gained the reputation of a "respected doctor" who, with the same zeal and enthusiasm, treated the people in the hills and mountains of Goražde and Fojnica, in the slums of Sarajevo, and the children, women, and elderly people exiled from eastern Bosnia in the Alipašin refugee camp. After the Second World War he made a significant contribution to the improvement of people's health, especially in the field of health education. This is best illustrated by his excellent medical texts, which at the time of their creation had a significant role in the eradication of infectious diseases. For him, medical and literary affairs were, together, his primary motivation. Due to its importance and his approach it, medical work had a certain advantage. It cannot be claimed that he thought about literature while working as a doctor, but it is known that certain events from his medical practice were repeatedly an inspiration for his literary creativity, which in its own way strengthened the connection between medicine and literature.

The purpose of this paper is to shed light on the biographical details, professional and health-educational work of Dr. Isak Samokovlija.

Samokovlija's Short Biography

Isak Samokovlija was born on September 3, 1889 in Goražde[8] on the River Drina, a small Bosnian town about seventy kilometers east of Sarajevo, into a merchant family of Sephardic (Spanish) Jews. His father, Moša, was engaged in trade, which enabled him to earn enough to support his large family only modestly. Despite this, he tried to provide his children with the widest possible education. Isak's mother, Rifka, also born in Samokovlija, was a housewife and a caring mother who lived for her children. Among those who knew her, she was remembered as an honorable person, with soft and warm speech and elegant behavior (16). Isaac's ancestors traced their origins to the Baruch family, which at the end of the 15th century was on its way to Turkey with other Jews exiled from Spain to the Ottoman Empire. They found refuge in Samokovo, a small town in the southwestern part of Bulgaria, after which they became known as the Samokovlija (17) in Bosnia, where they settled in 1860.

Isak grew up in his hometown and began his many years of education. He later referred frequently to that time in his life, remembering it fondly, and wrote about it. In his short autobiography, he

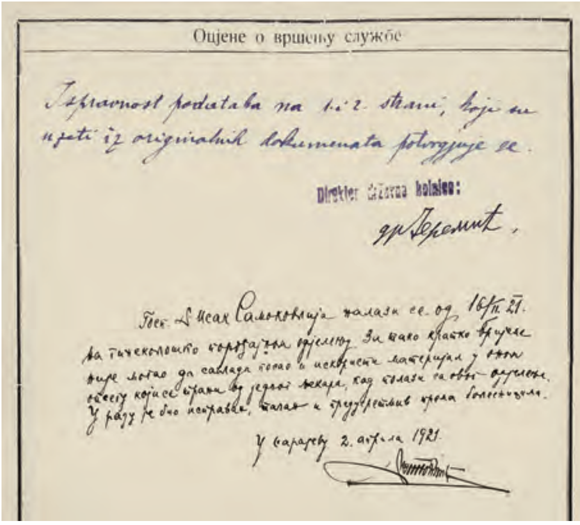
wrote: "I climbed all the hills, screaming together with all the children, Muslim and Christian, and grew up with them. We fought, broke each other's heads, but in everything it was as if we were from one mother and one father. We ate cakes for Pesach, gourabies for Eid, and pretzels for Easter" (18). He finished elementary school in Goražde in 1902, high school in Sarajevo in 1910, and then went to Vienna to study medicine in the same year, as a scholarship holder of the Jewish cultural and educational society *La Benevolencija*[9]. After the outbreak of the First World War in 1914, he was mobilized into the Austro-Hungarian army with other students from the Viennese Medical Faculty and sent to the front. He served as a medic, performing medical services as a medical lieutenant in Galicia on the Russian-Polish border, in the vicinity of Pest, and on the Romanian front. He continued his studies with other mobilized students in 1916 at the Faculty of Medicine, Vienna University, and the following year he successfully completed his studies, when he became a doctor of general medicine. He was then mobilized again and sent to the front in Belgrade, then Niš, and the border with Albania. Having learned, during a short leave in Vienna in 1918, that his father had died, he went to Sarajevo, where he received the order to be transferred from the front to Sarajevo Military Hospital (19). In Vienna in the same year he married Miss Hedda Brunner (20) in a military synagogue. Isak had three children: a son Mišo[10], and two daughters, Mirjam[11] and Rikica[12].

The Peaceful Period between the Two World Wars

Health conditions in Bosnia and Herzegovina immediately after the First World War were characterized by the poor general and health education of the people, their poor health and hygiene conditions and habits, especially of the rural population, the frequent occurrence of various infectious diseases that had the characteristics of epidemics, the insufficient number of primary health care institutions, and a shortage of health personnel. However, from the 1920s, there was a significant improvement in health conditions in Bosnia and Herzegovina, thanks to the implementation of public health policies in the new state, the Kingdom of Serbs, Croats, and Slovenes/Yugoslavia, as a result of the work of Dr. Andrija Šampar[13], head of the Hygiene Department at the Ministry of Public Health in Belgrade (1919-1930). For the first time, preventive medical institutions in the form of public health centers or hygiene institutes with epidemiological stations, biochemical, bacteriological, and parasitological laboratories, children's, school, anti-tuberculosis, anti-venereal, anti-traumatic and other dispensaries were opened, depending on the current pathology in a certain area, and institutes for the health care of mothers, children, and youth. The opening of new health institutions throughout Bosnia and Herzegovina significantly contributed to the improvement of primary health care, and enabled young doctors and other medical personnel to gain experience more quickly, and to progress in the service through hard and diligent work.

On February 5, 1919, after the end of the First World War, Dr. Isak Samokovlija began his civilian medical service as a secondary physician at the National Hospital in Sarajevo. From February 16, 1921, he worked at the Obstetrics and Gynecology Department of the State Hospital in Sarajevo. Dr. Jovan Bokonjić[14], the head of that department, wrote: "He is correct in his work, punctual and accommodating to patients" (Picture 2). He took the oath of allegiance to King Peter I on February 26, 1919, in Sarajevo (Picture 3).

On March 1, 1921, he began working as a district doctor in his native Goražde (Picture 4), where he remained until November of the same year. After the death of King Petar I Karađorđević on July 11, 1921, he took the official Oath of Allegiance to the new ruler, King Aleksandar, in Goražde on September 9, 1921. Official records of his medical work in his native Goražde have not been found. While searching for those documents, I came across a note about Dr. Isak Samokovlija, written by his younger daughter Rikica (21): "My Dad's first job was in his native Goražde. I remember his stories, his desire to give the best he could

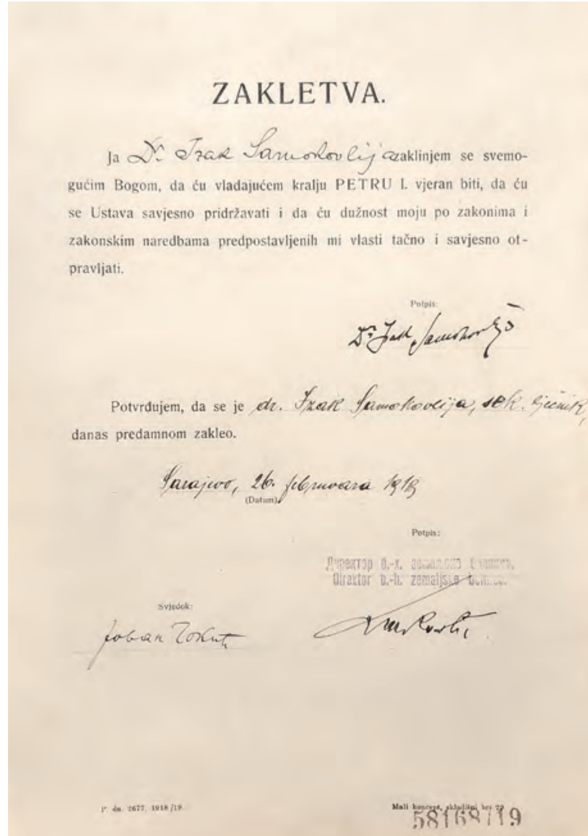


Оцјене о вршењу службе

Ispravnost podataka na 1. i 2. strani, koje su uzeti iz originalnih dokumenata potvrđuje se.

Direktor državne bolnice:

Picture 2. Official evaluation of the work of Dr. Isak Samokovlija at the State Hospital in Sarajevo in 1921 (ABiH. Isak Samokovlija's file).



ZAKLETVA.

Ja Dr. Izak Samokovlija zaklinjem se svemogućim Bogom, da ću vladajućem kralju PETRU I. vjeran biti, da ću se Ustava savjesno pridržavati i da ću dužnost moju po zakonima i zakonskim naredbama predpostavljenih mi vlasti tačno i savjesno otpravljati.

Potpis:

Potvrđujem, da se je dr. Izak Samokovlija, sek. liječnik, danas predamnom zakleo.

Sarajevo, 26. februara 1919
(Datum)

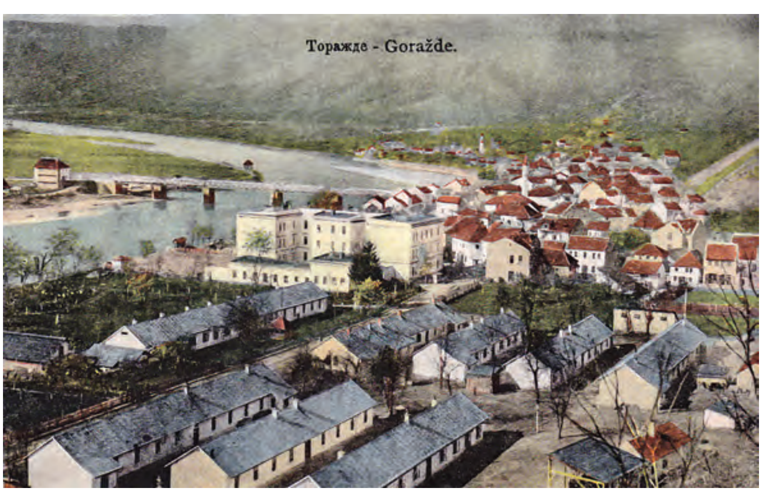
Potpis:

Svjedok:

Picture 3. Oath of allegiance to King Petar I Karađorđević, Sarajevo, February 26, 1919 (ABiH. Isak Samokovlija's file).

to his hometown. It was not difficult for him to visit very distant villages carrying his doctor's bag.

That was why he also learned to ride a horse, because many villages were inaccessible by other means, especially in winter. He often came home wet, frostbitten but happy, and shouted from the gate, "– Man, he and his mother will be fine!" Once, however, there was no escape, and then he was sad, sullen, silent, he just came in quietly, undressed, muttering, "If only they had called me earlier..." His greatest concern was the people's lack of education, as they often called herbalists, quacks and witch doctors when a doctor was needed. At every opportunity, he explained and convinced people not to believe in witchcraft and not to treat themselves without the advice of a doctor. He was often angry when he heard, "Well, we call you when nothing else helps!"



Picture 4. Goražde in the 1920's.

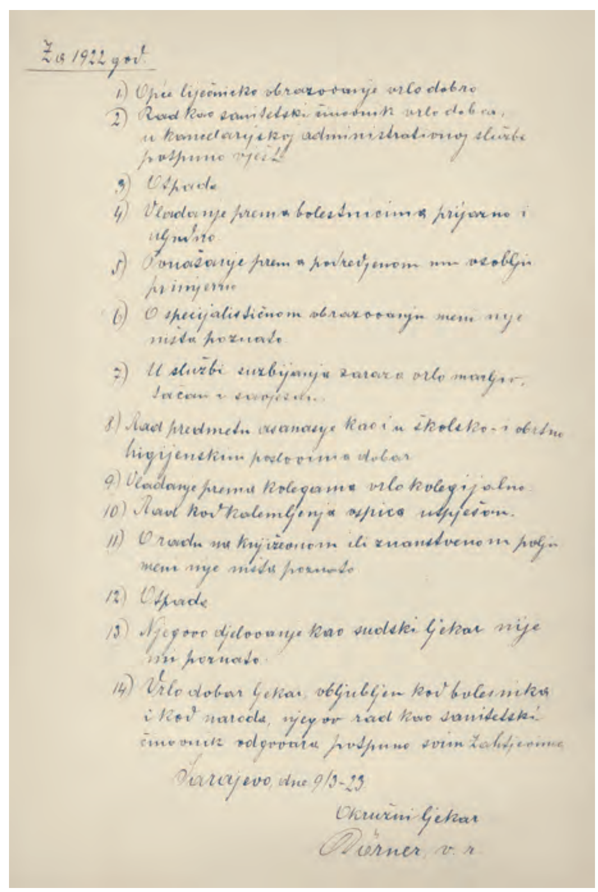
At the end of October 1923, he was officially transferred as a district doctor to Fojnica (Picture 5) (22), a small Bosnian town, where he stayed until November 1925. Samuel Elazar[15] learned from his wife, Mrs. Hedda, about the days Dr. Isaac Samokovlija spent in Fojnica, during a chance meeting and informal conversation in Zenica in 1941.

Many years later, he wrote about it: "Isak went to work before seven o'clock and until ten he did administrative work for the county, involving many reports and statistics. After that, he immediately went to the infirmary, where the waiting room was already full. He worked there until 2 p.m. and sometimes until 4 p.m., depending on how many home visits there were, and whether he had to provide first aid at work sites in the forest or at the sawmill. He would come home exhausted. He would hardly eat any lunch, simply longing to lie down and rest. But after a few hours of rest, calls for home visits would follow. The worst was when they took him to the village at night, often in rain and snow, without a horse. He would take his doctor's bag in one hand and a stick in the other and go, as his predecessor, Dr. Isak Israel would say, on "medical tourism' (23).

Picture 5. Fojnica in the mid 1920's. Courtesy of Nevres Jemendžić, a collector from Fojnica.

In the official annual review of Dr. Samokovlija's work in 1922, his superior, district physician Dr. Bürner, wrote, among other things, the following: "A very good doctor, loved by patients and the people, his work as a medical officer fully meets all requirements" (Picture 6) (24).

Despite the fact that he was a favorite doctor among patients and the people, in 1924 he experienced awkwardness as a doctor in Fojnica simply because he did not succumb to the Bosnian market mentality, that is, because he did his medical work professionally, adhering to the code of medical ethics. The unpleasantness must have moved him, considering that he was a conscientious and self-sacrificing doctor, and a man with an uncompromising attitude towards injustice. Marko Marković[16] wrote about this in the daily newspaper *Oslobođenje* (25). A problem arose after a certain Fr. Šumanović filed a complaint with the Fojnica Catholic parish office against Dr. Isak Samokovlija, to be sent to the Archbishop's Vrhbosna Ordinance in Sarajevo because of his alleged "arrogance and intolerance". In the further



Za 1922 god.

1) Opće liječničko obrazovanje vrlo dobro
2) Rad kao sanitetski činovnik vrlo dobar, u kancelarijskoj administrativnoj službi potpuno vješt
3) Otpada
4) Vladanje prema bolesnicima prijazno i uljudno
5) Ponašanje prema podredjenom mu osoblju primjerno
6) O specijalističnom obrazovanju meni nije ništa poznato
7) U službi suzbijanja zaraza vrlo marljiv, tačan i savjestan.
8) Rad predmetu asanacije kao i u školsko- i obćim higijenskim poslovima dobar
9) Vladanje prema kolegama vrlo kolegijalno.
10) Rad kod kalemljenja ospica uspješan.
11) O radu na književnom ili znanstvenom polju meni nije ništa poznato
12) Otpada
13) Njegovo djelovanje kao sudski liječnik nije mi poznato.
14) Vrlo dobar liječnik, obljubljen kod bolesnika i kod naroda, njegov rad kao sanitetski činovnik odgovara potpuno svim zahtjevima

Sarajevo, dne 9/3-23

Okružni liječnik
Bürner v. r.

Picture 6. Official evaluation of the work of Dr. Isak Samokovlija, Fojnica, 1922 (ABiH. Isak Samokovlija's file).

procedure, the application was forwarded to the Grand Prefect of the Sarajevo Region (health department) for inspection and further procedure, and then it was officially forwarded to the head of the Fojnica section. After a ten-day investigation, Fr. Šumanović's claims were refuted, and Dr. Isak Samokovlija's personality and medical work received a positive evaluation.

At the beginning of November 1925, Dr. Samokovlija was transferred to Sarajevo as a doctor in the Sarajevo district, Sarajevo region, with headquarters in Sarajevo (Picture 7) (26). Later, he worked as a doctor in various professional, administrative and medical posts. By a decision of the Minister of Social Policy and Public Health of the Kingdom of Yugoslavia, on February 18, 1930, he was appointed as a regular member of the Banovina Sanitary Council of the Royal Administration of the Drina Banovina in Sarajevo (27). From May 1939, he held the position of senior advisor to the head of Sarajevo County. From July 1939, he served as senior advisor to the head of Brčko County, based in Bosanski Šamac (28). In the Drina Banovina, he was appointed head of the Department's Health Department for social policy and public health (29).

During that period, he also had a private practice. In her memories of his father, his younger daughter, Dr. Rikica Najdanović Samokovlija, wrote about it: "When he returned to Sarajevo, he had a small private practice on the ground floor of his house where he worked in the afternoons. The prices of the examinations were very moderate, but still too high for the poor. As a result, on Thursdays my father admitted the sick poor without charge. It was free. He received them with the same care and dedication as the others. People knew this and greatly appreciated his charitable work. He later turned many stories about the poor from Bjelave and other Sarajevo mahals into his "stories" (21).

Dr. Samokovlija was a member of the Medical Chamber for Bosnia, Herzegovina, Dalmatia and Montenegro until 1930, and later the Medical Chamber of Drina Banovina until 1941. As an active and conscientious member, he was often elected to working bodies, and improved their work by his energy and knowledge.

The Period of the Second World War, 1941–1945

The Second World War found Dr. Isak Samokovlija in Sarajevo (Picture 8) in the position of head of the Health Department of the Department for Social Policy and Public Health of the Royal Administration of Drina Banovina in Sarajevo (29).

On May 7, 1941, after the establishment of the Independent State of Croatia (*Nezavisna Država Hrvatska*)[17] and on the basis of the Legislative Decree of April 22, 1941, and according to the authority obtained from the *Poglavnik* (the Chief), the Chief's Commissioner dismissed him from his duties without the right to pension or support, and without the right to appeal (30). He was forced to wear a yellow ribbon with a Star of David and a round sign designating him as a Jew, in the form of a yellow badge with the letter Ž painted black in the middle, by which the Nazis marked all Jews at that time.

From December 19, 1941 to April 20, 1942, he was mobilized for a home defense exercise in order to "become acquainted with the work

Picture 7. Sarajevo in the mid 1920's.

of doctors in combat units of the home defense". He started military exercises in December 1941, staying for a short time in Ugljevik, where he worked as a doctor for the 6th Infantry Regiment (31).

At the beginning of 1942, he was reassigned as a doctor to the 3rd Battalion, 5th Infantry Regiment in Ševarlije[18] near Doboj, where he remained until March 20, 1942, when he returned to Sarajevo (32). There are few written traces of how he endured the military discipline of the ruling regime during home defense exercises in isolated Bosnian towns. He probably told family members and trusted friends about it. The only things that were found in his personal legacy, which is stored in the Museum of the Literature and Performing Arts of Bosnia and Herzegovina in Sarajevo, are texts written on two correspondence cards that Dr. Isak Samokovlija sent to his friend Hamid Dizdar[19]. Both were sent from Ševarlije. They were written in ink and in easily legible letters (33, 34). Given that Dr. Isak Samokovlija knew that letters and stationery were censored, it is hard to believe that the accuracy of the written text was preserved.

Picture 8. Postcard of Sarajevo from the Second World War. Courtesy of the Historical Museum of Bosnia and Herzegovina in Sarajevo (Collection of photographs, Fund Radnički pokret, inventory number FRP 2354).

Picture 9. Refugees on the streets of Sarajevo, around 1942. Courtesy of the Historical Museum of Bosnia and Herzegovina in Sarajevo (Collection of photographs, Fund Radnički pokret, inventory number FRP 2184).

At the end of March 1942, Dr. Isak Samokovlija returned to Sarajevo. At that time, there were many uncared for refugees in Sarajevo (Picture 9), mostly from eastern Bosnia. At the same time, a massive typhoid and typhoid epidemic broke out (over 3,000 cases), and there were also many people suffering from dysentery (35).

With the aim of stopping the further spread of these diseases, the competent health services considered it necessary urgently to increase the number of professional staff, and take the necessary anti-epidemic and general hygiene measures. The health service at the City Polyclinic in Sarajevo was managed by Dr. Asim Musakadić[20] and Dr. Ekrem Idrizbegović[21]. They believed that, first of all, they should hire doctors who were out of work in Sarajevo. At their suggestion, the Great Prefect, Dr. Ismet-bey Gavrankapetanović[22] rendered a decision by which Dr. Isak Samokovlija was assigned to the Office for Refugees of the Greater Vrhbosna Parish in Sarajevo, where he assisted sick Muslim refugees from eastern Bosnia at the City Health

Department (36). The decision also stated that the amount of the fee for his work would be determined by the Office for Refugees of the Greater Vrhbosna Parish in Sarajevo. The German occupation authorities, fearing the spread of infectious diseases among their army, decided to move refugees suffering from typhus outside the city center (37). For this reason, in April 1942, the construction of a refugee settlement began, that is, a camp for refugees on Alipašin Most[23] (37). The resettlement of refugees from Sarajevo to the refugee camp at Alipašina Most began, under the orders of the German military authorities, on May 20, 1942 (37). The camp accommodated children of both sexes, women and elderly men (38).

From an epidemiological point of view, the resettlement of refugees was carried out with the aim of preventing the spread of these diseases in the metropolitan area of Sarajevo. However, the spread of the typhus epidemic still continued among the refugee population because refugees were sent to the camp regardless of whether they were sick, infected or healthy.

At the beginning of the work in the Alipašin Most Camp, 19 employees were employed. Among them was Dr. Isak Samokovlija (Picture 10). Thanks to his professional and organizational work, in the following few months the incidence of infectious diseases gradually decreased, and they eventually disappeared. His most significant activity in that period was his active involvement in medical and social work. In cooperation with the representatives of "Merhamet"[24] from Sarajevo, he successfully cared for and placed orphaned children staying in the camp in various facilities and Sarajevo families (23).

At the end of September 1942, the status of Dr. Isak Samokovlija under employment law changed. He became an employee of the Institute for Combating Endemic Syphilis in Banja Luka (the Institute)[25], although he never stayed in that city during the Second World War, nor did he work in the field of suppression of syphilis at that time. This title was only formal and legal, since he continued to work as the manager of the infirmary at the Refugee Camp on Alipašin Most.

In fact, the administrative headquarters were in Banja Luka, and consisted of administrative officers and the manager of the Institute, Dr. Stanko Sielski[26]. The direct work of the doctors employed there took place in the hygiene institutes, public health centers, and other hygiene institutions in Bosnia and Herzegovina, often in improvised field clinics in remote Bosnian villages where there were no permanent or even occasional doctors. The institute's doctors treated the population not only for syphilis but also for other diseases (39), which was of great importance for the Bosnian people because, immediately before the Second World War, or at its very beginning, a significant number of doctors of Serbian nationality fled from the Ustaša authorities and the war, leaving Bosnia for Serbia. The difficult war period and the dangers to which well-intentioned citizens of Sarajevo who protected the lives of vulnerable citizens, regardless of their national, religious or professional affiliation, could be exposed meant that, among others, Dr. Isak

Picture 10. Dr. Isak Samokovlija, fourth from the left, in the Alipašin Most Refugee Camp with refugees and coworkers, 1943. Courtesy of Dr. Jelice Najdanović Bokonjić.

Samokovlija and his family managed to survive and avoid as much as possible the horrors that Sarajevo's Jews and others experienced during the Second World War. It is certain that his fellow doctors made a significant contribution to this, especially at the beginning of the war, but also in the later war years. This is best seen in the fact that, in critical periods, Dr. Samokovlija was temporarily "sheltered" outside of Sarajevo in small Bosnian towns under the guise of home defense military exercises, or in his later employment at the Institute. The idea of his employment at the Institute was most likely planned by his colleagues in Sarajevo, who, in some sense, "took care of him" from the beginning of the Second World War. Today, one can only guess at their names because it was all done in great secrecy. However, given his employment at the Institute, it may be assumed with high probability that this idea was supported and implemented in Zagreb by Dr. Stanko Sielski and Dr. Ante Vuletić[27].

The contract[28] signed on September 19, 1942 with the Ministry of Health of the National People's Republic of Croatia on establishing the employment relationship of Dr. Isak Samokovlija with the Institute was made possible by Dr. Isak Samokovlija's acquisition of the status of civil servant, which for him as a Jew and his family meant above all a certain level of safety and material security, but also a number of other "privileges". Dr. Samokovlija was employed for an indefinite period of time as a contract employee to perform work on the suppression of endemic syphilis in Bosnia and Herzegovina, and other tasks prescribed by the legal provisions on the establishment of the Institute in the area assigned to him by the Institute, with a monthly salary of HRK 5,600. In addition, he was entitled to a monthly allowance of HRK 300 for his wife and HRK 200 for each child. The monthly reward for work was often increased, so that in January 1945 it amounted to HRK 16,500.[29]

However, in order to be employed at the Institute and exercise his contractual rights, he had to submit, in addition to proof of professional training, general personal documents, a certificate of impunity and good governance, and a certificate that he was not under guardianship. In addition, it was necessary to submit to the Home Office a baptismal certificate, a certificate of a sworn oath to the NDH, and a certificate that the marriage that he and his wife Hedda had established back in 1919 in the military synagogue in Vienna had been validated by the Catholic Church.

After Dr. Isak Samokovlija submitted all the required documents to the authorities, on November 13, 1942, he was appointed a contracted employee of the Institute.[30] On January 1, 1943, he officially took up the position of contracted physician of the Institute, based in the Institute of Hygiene in Sarajevo, and was assigned to the position of manager of the clinic in the Alipašin Most Camp, where he had been working since its opening in May 1942.[31]

In remembering her father, Dr. Rikica Najdanović, Samokovlija's daughter, wrote that his work in the Alipašin Most Camp during the Second World War brought him face to face not only with numerous illnesses but also with the tragic fate of the exiled population, stating that working with refugees exhausted him, in addition to his own illnesses and personal tragedies. However, he never allowed this to show, and often, according to the refugees' statements, he was the only bright spot in their lives during that difficult wartime (21).

One of his many troubles from the Second World War period, which happened in the Alipašin Most Refugee Camp, was recorded in official documents. A written complaint against him was submitted by a certain Olga Jablačkov, a nurse at the Alipašin Most Refugee Clinic, on June 23, 1944.[32] She accused him of working privately in the Refugee Clinic, of using medicines and bandages procured for refugees for private patients, of earning over HRK 10,000 a day, of eating from the hospital kitchen, although he received food like all other employees, of keeping food in his room and freely disposing of it, and paying more attention to private patients than to refugees. At the request of the competent ministry, Dr. Isak Samokovlija wrote a detailed answer. After the warden of the camp gave his opinion,[33] in the final file, T. 997/44

dated August 28, 1944, signed by deputy prefect T. Jurin, it was stated that the administration forbade doctors to receive private patients in the camp infirmary and, following the executive procedure, Dr. Samokovlija was found not guilty.

On March 25, 1945, Dr. Isak Samokovlija was again mobilized into the Home Guard.[34] This time he served as a doctor in the Ustaša units, which, together with the German units, retreated in panic towards the north from Hadžić via Zenica, Maglaj and Doboj. After several previous unsuccessful attempts to escape from the Ustaša forces, on April 13, 1945, he managed to escape and hide in Doboj until the liberation of the country. Dr. Samokovlija did not write later in more detail about how he managed to escape, where and how long he hid, who helped him, or how he felt then, and no notes about it have been found. However, it is possible to learn more about it from the notes by Siniša Paunović[35] as well as from the short "farewell" letter[36] that Samokovlija sent from Doboj to his family in Sarajevo, where it can be seen that those were difficult, uncertain days for him, full of suffering and pain, and that in his mind he was saying goodbye to his family and friends, thinking that he would never see them again.

The Post-War Period, 1945–1955

From April 25 to May 7, 1945, Isak Samokovlija, as a member of the Yugoslav Army, was the manager of Hospital No. 7 in Tuzla (40). Later, he continued working in the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina in Sarajevo. He was the head of the Health Education Department. With pen and written word, in his own way, Dr. Samokovlija worked in various fields, popularizing preventive medicine. He wrote educational medical texts that were comprehensible, interwoven with evidence and adapted to the wider masses, and which he shaped and published in the form of medical articles, popular brochures, leaflets, and slogans.[37] At the same time, he organized health-education lectures, with the aim of teaching the people about current health pathologies, which in those years mainly involved epidemics of various acute infectious diseases, or were a consequence of the people's deep-rooted beliefs in folk medicine for the prevention and treatment of various diseases.

Together with a group of enthusiastic doctors, Dr. Samokovlija founded and edited the first Bosnian medical journal, *Život i Zdravlje* (Life and Health)[38] (Picture 11), and became its first editor. The journal had two editorial boards. The wider committee included: Dr. Mića Branisavljević[39], Dr. Vladimir Čavka[40], Dr. Ante Čićić[41], Dr. Blagoje Kovačević[42], Dr Alija Karahasanović[43], Dr. Luka Šimović[44], Dr. Asaf Šarac[45], Dr. Mara Živanović[46]; and more narrowly: Dr. Miroslav Feldman[47], Dr. Teodor Ilić[48] and Bogdan Zimonjić[49].

The members of the Editorial Board also published their articles in the journal *Život i Zdravlje* (Life and Health) and thus significantly contributed to its quality and regular publication. The publisher of the journal was the State Publishing


ПОШТАРИНА ПЛАЋЕНА У ГОТОВУ

ЖИВОТ
и ЗДРАВЉЕ

ЧАСОПИС ЗА ЗДРАВСТВЕНО ПРОСВЈЕЋИВАЊЕ

ГОД. I АПРИЛ 1946 БРОЈ 1

САДРЖАЈ:

Уредништво: Наша ријеч — Др. Војислав Кецмановић. Једо: Наше перспективе — Др. Недо Зец: Наш задатак данас — Др. Павле Каунић: О заразним болестима — Др. Сергије Рамзин: Епидемије и борба против њих — Др. Ернест Грин: Цијепљење против заразних болести — Др. Асаф Шарац: О имунитету — Др. Лука Шимовић: Пјегавац — И. З.: Народне снаге у акцији за сузбијање пјегавца — Др. Мирослав Фелдман: О трбушном тифусу — Др. Лука Шимовић: Повратна грозница — Др. Маша Живановић: Дјечје заразне болести — Др. Исак Самоковлија: Баба Мара — Музур Атифа: Шта ме је руководило — Уредништво: Мртвим друговима — Бранко Чопић: Др. Младен Стојановић, нар. херој — И. С.: За наше пионире — Др. Мехмед Татлић: Задаци здравствене секције — Др. Миња Бранисављевић: Здравствена служба сеоца, осигурања — Теодор Нанић: Хасаново преображење — Др. Оскар Гинсберг: Херојство — Др. Богдан Чабак: Мајка Југовића — Др. Евгеније Шерстнев: Наши здравствени активисти — Др. Алија Томић: Подизање сред. мед. кадрова — Плин комора са сувим врућим ваздухом — О новим лијековима — Народна медицинска изрека — Медицински изрази који се често чују — Вијести — Поруке уредништва.

Цртежи од академских сликара Романа Петровића и Исмета Мујезиновића

УРЕЂУЈЕ: РЕДАКЦИОНИ ОДБОР МИНИСТАРСТВА НАРОДНОГ ЗДРАВЉА НАРОДНЕ РЕПУБЛИКЕ Б. и Х.
ИЗДАЈЕ: „СВЈЕТЛОСТ" ДРЖАВНО ИЗДАВАЧКО ПРЕДУЗЕЋЕ БОСНЕ И ХЕРЦЕГОВИНЕ, САРАЈЕВО

Picture 11. Cover of the first issue of the journal *Život i Zdravlje* (Life and Health).

Company *Svjetlost*, Sarajevo, and it was printed at the Sarajevo State Printing House.[50]

As editor, Dr. Isak Samokovlija designed the contents of the journal with texts on current health issues. He took care of the regularity of the publication of the journal, and invited his colleagues to write articles on given topics, and famous Bosnian painters[51] to illustrate the journal with their drawings. In addition to the usual medical texts written by prominent Bosnian doctors, Dr. Samokovlija also published his own articles on health and education in the journal. In total, he wrote twenty-nine texts with health and educational content. He did this in a planned and methodical manner, choosing topics to write about based on current pathologies. Almost all his health education texts were written in a literary and artistic style, which was quite unusual in medical circles at the time. However, the language in which the texts were written was simple, interesting and popular, making them easy to read and understand, even for the lay population. Today, when we read them, Dr. Samokovlija's health education texts not only reflect the state of health in Bosnia and Herzegovina of that time, but also provide the reader with an opportunity to learn about the life and customs of the population at the time of their creation.

Picture 12. Funeral procession and coffin with the body of Isak Samokovlija, Sarajevo, January 17, 1955. Courtesy of Dr. Jelice Najdanović Bokonjić.

Already in 1949, Dr. Isak Samokovlija left the Ministry of Health of the People's Republic of BH (the Ministry). That year also marked the end of his involvement with the journal *Život i Zdravlje* (Life and Health). Later, he continued to edit the literature and art journal *Brazda*, a job he had been working on since 1948. He was the editor of this Journal until 1951, and later was an editor at the publishing company *Svjetlost*. Leaving the Ministry did not mean leaving medicine because he had a private practice until the end of his life, remaining faithful to both medicine and literature.

He died in Sarajevo on January 15, 1955 and was buried with the highest state honors at the Jewish cemetery in the Sarajevo settlement Kovačići, on the southeastern slopes of Mount Trebević (Picture 12). On behalf of the Society of Physicians of Bosnia and Herzegovina, Dr. Mića Branisavljević said farewell to Dr. Isak Samokovlija. The only obituary written about him as a doctor was published in the journal *Život i Zdravlje* (Life and Health).[52]

Concluding Remarks

In Bosnia and Herzegovina, his native country, he was known more as a writer than as a doctor, most likely because his literary work overshadowed his professional medical work, or simply because there is insufficient interest in the history of medicine amongst the medical public of Bosnia and Herzegovina. In both fields, this is best illustrated by the fact that only one short obituary was written after his death. It seems that the "historians of medicine" in Bosnia and Herzegovina considered him more of a writer than a doctor. What was written about his medical work was only what was needed to show the connection between his profession and his writing, although he worked as a doctor for the rest of his life and never "escaped" from medicine to literature. Through his work, he made a significant contribution to the improvement of people's health after the First and Second World Wars wherever he worked, and working with children, women, and the elderly in the Alipašin Most Refugee Camp during the Second World War. His special contributions are the articles on public health published in the first Bosnian medical journal, *Život i Zdravlje* (Life and Health).

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

Notes

[1] Siniša Paunović (Čačak, Aug 25, 1903-Belgrade, Apr 9, 1995). Journalist, writer and collector.

[2] *Jevrejski* život (Jewish life), a weekly newspaper for cultural, political and economic issues; owner and publisher Albert D. Kajon, managing editor Albert Cohen. The paper was published from 1924 to 1928.

[3] Gajret was the newsletter of the society of the same name for supporting the education of Bosniak students. The magazine was published in Sarajevo from 1907 to 1941, with interruptions from 1914 to 1921 and 1923. It was edited by Edhem Mulabdić, Mustaj-beg Halibašić, Osman Đikić, Murat Sarić, Avdo Sumbul, Šukrija Kurtović, Abdurezak Hifzi Bjelevac, Hamza Humo and Hamid Kukić. The magazine cherished the traditional spiritual values of Bosniaks, but was also open to the positive cultural influences of Christian civilization. In the period from 1921 to 1941 it openly supported the political idea of the national identification of Bosniaks as Serbs. [cited 2022 September 4]. Available from: http://www.enciklopedija.hr/Natuknica.aspx?ID=21015.

[4] *Srpski književni glasnik* is one of the most important Serbian literary magazines. It was published from February 1901 to July 1914 and, renewed, from September 1920 to April 1941. It was launched by Bogdan Popović, who was also the first editor. Later editors were Pavle Popović (1905–06) and Jovan Skerlić (1905–1914), and it was restored in 1920 by Bogdan Popović and Slobodan Jovanović, and then had several editors. [cited 2022 March 9]. Available from: https://pretraziva.rs/pregled/srpski-knjizevni-glasnik.

[5] Ivo Andrić (Travnik, Oct 9, 1892-Belgrade, Mar 13, 1975). Writer of novels and short stories in the Bosnian, Croatian, and Serbian languages, who was awarded the Nobel Prize for Literature in 1961.

[6] Meša Selimović (Tuzla, Apr 26, 1910-Beograd, Jul 11, 1982). A novelist, storyteller, literary historian, memoirist, and critic.

[7] Petar Kočić (Stričići near Banja Luka, Jun 29, 1877-Belgrade, Aug 27, 1916) [cited 2022 March 9]. Available from: https://www.biografija.org/knjizevnost/petar-kocic/

[8] Goražde was at the time part of the Austro-Hungarian Empire.

[9] The Jewish cultural-educational and humanitarian society "*La Benevolentija*" was founded in January 1892 with the aim of providing scholarships to talented Sephardic students who were studying trades or attending higher schools throughout the Austro-Hungarian Monarchy. The society ceased to be a scholarship society for solely Sephardic young men in 1908 and became the Jewish Society for Education and Culture, which removed the distinction between Sephardi and Ashkenazim. La Benevolencia. Wikipedia [cited 2022 Jan 9]. Available from: https://hr.wikipedia.org/wiki/La_Benevolencija.

[10] Mišo Samokovlija (Goražde, Mar 30, 1920-Sarajevo, Feb 22, 1974). He finished elementary school and high school in Sarajevo in the 1940/41 school year, enrolled in medical studies, which he interrupted due to the start of the Second World War, and joined the Partisans. After liberation, he worked as a salesman in Sarajevo and Banja Luka.

[11] Mirjam Samokovlija Vujošević (Goražde, Jun 27, 1921-Sarajevo, Jan 23, 2003). She finished elementary school and the Trade Academy in Sarajevo. She worked as the head of the foreign exchange department of the National Bank in Sarajevo.

[12] Rikica Samokovlija Najdanović (Fojnica, Mar 19, 1923-Belgrade, Aug 29, 2014). She finished elementary school and high school in Sarajevo. She graduated in medical studies in Zagreb in 1952. She passed the specialist exam in paediatrics in 1958. She defended her doctoral dissertation in 1974. She began her university career in 1959 being selected as an assistant, and was finally a full professor at the Faculty of Medicine in Sarajevo.

[13] Andrija Štampar (Brodski Drenovac near Pleternica, Sep 1, 1888-Zagreb, Jun 26, 1958). Croatian encyclopedia, online

edition. Miroslav Krleža Lexicographic Institute, 2021 [cited 2022 Jul 9]. Available from: http://www.enciklopedija.hr/Natuknica.aspx?ID=59892.

[14] Jovan Bokonjić (Kostajnica, Dec 19, 1883-Sarajevo, Aug 20, 1970). He graduated on March 24, 1911 from the Faculty of Medicine of the German University in Prague. After returning to Bosnia, he was employed as a secondary physician at the National Hospital in Sarajevo on June 1, 1911. He worked in the gynecology, surgery, and psychiatry departments. At the beginning of the First World War, he was interned in a concentration camp in Arad (Romania). After liberation, he continued to work at the National Hospital. From July 1919, he worked independently as head of the Department of Gynecology and Obstetrics until his retirement.

[15] Samuel Elazar (Gračanica, Dec 17, 1902-Sarajevo, Nov 23, 1989), close friend of Isak Samokovlija, and a famous Sarajevo pharmacist, historian of Bosnian pharmacy and medicine, and a cultural and public worker.

[16] Marko Marković (Zvornik, Feb 27, 1896-Pale, Aug 12, 1961) was a Bosnian writer, cultural worker, and close friend of Isak Samokovlija.

[17] Commonly referred to as the NDH.

[18] A populated area on the railway line leading from Doboj to central Bosnia.

[19] Hamid Dizdar (Stolac, Feb 22, 1907-Sarajevo, Jul 17, 1967), a close friend of Isak Samokovlija, Bosnian poet, storyteller, bibliophile, journalist, publicist, ethnologist, cultural and public worker.

[20] Dr. Asim Musakadić (Sarajevo, Jan 15, 1894-Sarajevo, Aug 1, 1979). He graduated from the Faculty of Medicine in Zagreb in the 1926/1927 academic year. He worked as a doctor in Sarajevo. During the Second World War, he was the head of the City Polyclinic in Sarajevo. He collaborated illegally with the National Liberation Movement (commonly referred to as the NOP) in 1941–1945. After the liberation, he worked until his retirement in 1963 at the City Polyclinic in Sarajevo.

[21] Dr. Ekrem Idrizbegović (Bugojno, Oct 21, 1903-Sarajevo, Feb 3, 1996). He graduated from the Faculty of Medicine in Belgrade in 1930. He was a doctor in Prozor, Foča and Sarajevo. During the Second World War, he worked as a physician at the City Polyclinic in Sarajevo. From 1941 to 1945, he illegally collaborated with the NOP. From April 16, 1945, until the end of the war, he was a member of the NOP. From after the liberation until his retirement in 1964, he worked as the head of the Children's Department of the Military Hospital in Sarajevo.

[22] Dr. Ismet-bey Gavrankapetanović (Počitelj, Jan 1, 1877-Sarajevo, Oct 29, 1959), Grand Prefect of the Greater Vrhbosna Parish in Sarajevo (Sep ?, 1941-Oct 1, 1944).

[23] A Sarajevo settlement that today belongs to the municipality of Novi Grad.

[24] The Muslim charity "Merhamet" was founded in Sarajevo in 1913 with the aim of helping the poor. The activities of "Merhamet" depended on the goodwill and solidarity of wealthier individuals, mostly Bosniak merchants, artisans, landowners, scholars, and social activists. By a decision of the authorities, "Merhamet" ceased to operate in 1946. Work was resumed on February 2, 1991. During the Second World War, it played a significant role in caring for exiled and orphaned children from eastern Bosnia.

[25] The Institute for Combating Endemic Syphilis in Banja Luka was established by the NDH in 1941 with the aim of finally eradicating this disease from Bosnian villages as quickly as possible. This served as the conceptual foundation for the Institute's legal provisions. However, the main goal of the originator of the idea of founding the Institute was that the Jewish doctors in the NDH who had already been, or were about to be, deported to labor camps or death camps, should be taken as soon as possible to a health facility in Bosnia that would in some way protect them from being killed or deported to concentration camps, without any hope of return.

[26] Dr. Stanko Sielski (Gračanica, Jun 18, 1891-Zagreb, Oct 31, 1958). From July 19, 1941 to Aug 25, 1944 he was director of the Institute for Combating Endemic Syphilis in Banja Luka, and then from July 19, 1941 to Aug 25, 1944 he was Dean of Sarajevo's Faculty of Medicine, and from Aug 1, 1944 to May 13, 1945 director of Tuzla's Sanitary and Epidemiological Station (later the Institute of Hygiene) from Feb 1, 1946 to Oct 31, 1958. At the end of 2014, he was posthumously declared Righteous Among the Nations. Although he was proposed as a righteous man from Bosnia and Herzegovina, he was, by a mistake made at Yad Vashem, ranked among the Righteous from the State of Croatia.

[27] Dr. Ante Vuletić (Sarajevo, Oct 2, 1899-Zagreb, May 14, 1977). He was one of the initiators of rescuing Jewish doctors from death and the idea of founding the Institute for the Suppression of Endemic Syphilis in Banja Luka in 1941. During the Second World War, he was the director of the Croatian Institute of Hygiene and an associate of the Ministry of Health of the NDH in Zagreb. He received the Israeli award, Righteous Among the Nations.

[28] ARS. Isak Samokovlija's file. The service contract was concluded between NDH, represented by the Minister of Health, Dr. Iva Petrić and Dr. Isak Samokovlija on October 28, 1942.

[29] ARS. Isak Samokovlija's file. Accounts Court of the Independent State of Croatia Zagreb, January 4, 1945. Number: 6381-P-1944. Subject: Dr. Isak Samokovlija's salary increase.

[30] ARS. Isak Samokovlija's file. Document number: 72128-0-1942. Zagreb, November 13, 1942. Appointment of Dr. Isak Samokovlija from Goražde as a contract officer to perform work on the suppression of endemic syphilis at the Institute for Combating Endemic Syphilis in Banja Luka, signed by the Minister of Internal Affairs, Dr. Nikšić.

[31] ARS. Isak Samokovlija's file. A written statement by Dr. Isaka Samokovlija was sent on January 1, 1943 to the Institute about taking up the duties of contracted doctor.

[32] Historical Museum of Bosnia and Herzegovina. Application against Dr. Isaac Samokovlija. Number 5332. NDH Fund. Box 22.

[33] Ibid.

[34] Archives of Yugoslavia. Ministry of Social Policy and Public Health Collection (Fasc. No. 159). Personal and professional information about Samokovlija (Moše) dr. Isaac. Sarajevo, June 15, 1946.

[35] *Pisci izbliza* (Writers up close). Belgrade: Prosvjeta.

[36] Museum of Literature and Perform Arts of Bosnia and Herzegovina. Zbirka Isaka Samokovlije 1- 1027; pp 266; 945.

[37] The publishing house "Life and Health" of the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina published the magazine of the same name, as well as brochures and leaflets with health and educational content. The brochures were written by experienced and respected Bosnian doctors of the time, and depending on the title and current pathologies, were printed with a circulation of 2,000 to 10,000 copies..

[38] *Život i Zdravlje* (Life and Health) was the first medical journal in Bosnia and Herzegovina. It began to be published in April 1946 with a circulation of 6,000 copies. Its content was of a popular-health-educational nature. The journal was edited by the Editorial Board of the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina. It was published regularly four times a year.

[39] Mića Branisavljević (Višegrad, Feb 21, 1891 - Sarajevo, Mar 3, 1978). He graduated from the Faculty of Medicine of Charles University in Prague on March 8, 1920. He worked as a doctor in Jajce for more than 20 years. Later, in Sarajevo, he performed highly positioned administrative duties. Among other things, he was the head of the Ministry of Health of the People's Republic of Bosnia and Herzegovina and the manager of the Koševo Clinical Hospital.

[40] Vladimir Čavka (Orašje, Oct 31, 1900 - Belgrade, July 3, 1984). He completed his medical studies in Vienna in 1924. He was a university professor in Zagreb, Belgrade, and Sarajevo. He was the founder of the journal *Medicinski arhiv* and its first editor-in-chief. He was the first president of the Scientific Society of Bosnia and Herzegovina (BH) and a member of the Academy of Sciences and Arts of BH. His activity in the organization of the ophthalmology service and the eradication of trachoma in BH was significant.

[41] Anto Čičić (Kreševo, Apr 1, 1893–Sarajevo, Dec 24, 1958). He graduated from the Faculty of Medicine in Lviv (Ukraine) in 1925. He was the first director of the High School of Dentistry in Sarajevo, which was founded in 1946.

[42] Blagoje Kovačević (Grahovo, Montenegro, Mar 25, 1900-Zagreb, Dec 1, 1959). He was buried at the Lav cemetery in Sarajevo. He graduated from the Faculty of Medicine in Belgrade in 1926. He specialized in surgery in Paris and Berlin. Before World War II, he was the head of the Surgical Department of the State Hospital in Sarajevo. From the end of 1944 to the middle of 1945, he was the manager of the hospital of the 5th Corps of the NOB in Jajce. After the Second World War, he was the head of the Surgical Department of the General State Hospital in Sarajevo. He was one of the founders of the Faculty of Medicine in Sarajevo. He was Dean of the same faculty, president of the Society of Physicians of Bosnia and Herzegovina, and a long-time president of the Red Cross of Bosnia and Herzegovina. He was elected a regular member of the Scientific Society of Bosnia and Herzegovina in 1952..

[43] Alija Karahasanović (Foča, Sep 27, 1902-Sarajevo, Oct 8, 1978). He graduated from the Faculty of Medicine in Belgrade in 1931. He specialized in surgery at the Surgical Clinic in Belgrade from 1933 to 1937. During World War II, he was a prisoner of war in Germany. After the liberation, he worked as a surgeon in the General State Hospitals in Sarajevo (1945–1952), Banja Luka (1952–1953), and Tuzla (1954–1961), where he was head of the Surgical Department and manager of the General Hospital. From 1962 until his retirement, he worked at the Health Center in Sarajevo.

[44] Luka Šimović (Zvirovići, Čapljina municipality, Oct 17, 1895-Sarajevo, Mar 28, 1979). He graduated from the Faculty of Medicine in Vienna in 1924. Before the beginning of the Second World War, he was appointed manager of the National Health Center in Tuzla, and he held that position until October 1943. After the liberation, in 1945, he was appointed director of the then Hygiene Institute in Sarajevo, and he held that position until the end of 1951. He retired in 1960.

[45] Asaf Šarac (Sarajevo, Sep 30, 1896-Sarajevo, Dec 15, 1968). He graduated from the Faculty of Medicine in Vienna in 1924. From 1937 until the establishment of the NDH, he was the manager of the State Hospital in Sarajevo. During the Second World War, he worked as the head of the Infectious Diseases Department of the Military Hospital in Sarajevo, then as a specialist doctor and head of the Infectious Diseases Department of the General State Hospital in Sarajevo. After the liberation in 1945, he was the head of the Infectious Diseases Department of the General State Hospital in Sarajevo, and after the opening of the Faculty of Medicine in Sarajevo in 1946, he was also the first head of the Clinic for Infectious Diseases.

[46] Maša Živanović (Delnice, Croatia, Dec 14, 1890-Belgrade, Aug 12, 1960). She graduated from the Faculty of Medicine in Vienna in 1916. From 1919 she worked as a children's doctor at the City Infirmary in Sarajevo, and from 1924 to 1941 in the Children's Dispensary, that is, at the Institute for Health for Mothers, Children and Youth in Sarajevo, where she was doctor and director of the institution. During World War II, she lived in Belgrade, where she had a private practice. After liberation in June 1945, she returned to Sarajevo and continued working at the Institute for Health for Mothers, Children and Youth. At the same time, she worked for the Ministry of Public Health of Bosnia and Herzegovina. She was the president of the Women's Movement in Sarajevo from 1924 to 1934.

[47] Miroslav Feldman (Virovitica, Dec 28, 1899–Zagreb, May 30, 1976). A Yugoslav writer, doctor, poet, and participant in the National Liberation War. He began his medical studies in Zagreb in 1919, and in 1921 he continued to study at the Faculty of Medicine in Vienna, from where he graduated in 1924. After the Second World War, from 1945 to 1947, he was head of the health service in the Ministry of Public Health of Bosnia and Herzegovina.

[48] Teodor Ilić (Batkuša, Bosanski Šamac, Feb 28, 1893-Dubrovnik, Dec 20, 1974). In his high school days, before the First World War, he was a member of "Young Bosnia" and one of its leaders in the Tuzla region. He finished high school in Tuzla in 1914 and medical school in Prague. Between the two wars, he served as a county doctor in Serbia and Bosnia and Herzegovina. From 1945 to 1949, he was the assistant minister of health and social policy in the Government of the People's Republic of Bosnia and Herzegovina, and from 1949 to 1957, when he retired, he was the director of the Institute for Health Education and the head of the Health Education Department of the Central Institute of Hygiene in Sarajevo. After the departure of Dr. Isaka Samokovlija from the Ministry of Public Health, he was the editor-in-chief of the magazine *Život i Zdravlje* (Life and Health).

[49] Bogdan Zimonjić (Sarajevo Feb 2, 1899-Sarajevo Jan 8, 1966). He studied medicine in Vienna. He specialized in internal medicine and radiology in Vienna and Prague. In the period between the two world wars and after the liberation until 1947, he was the head of the Internal Diseases Department of the State Hospital in Sarajevo. During the Second World War, he worked with the NOP. He hid and treated the wounded and sick. He was one of the founders and professors of the Faculty of Medicine and head of the First Internal Diseases Clinic in Sarajevo. He published a significant number of medical articles in the country and abroad. According to the wishes of Dr. Zimonjić, his sister Ljubica Zimonjić, a former Sarajevo teacher, founded the "Dr. Bogdan Zimonjić" Foundation fund. The foundation stopped working in 1992.

[50] *Život i Zdravlje* (Life and Health) - journal for health education. 1946; 1(1). Imprint.

[51] Roman Petrović (Donji Vakuf, Oct 1, 1896-Sarajevo, May 25, 1947); Ismet Mujezinović (Tuzla, Dec 2, 1907-Tuzla, Jan 7, 1984).

[52] Ilić T. In memoriam: Dr. Isak Samokovlija, the first editor of our magazine [in Bosnian]. *Život i Zdravlje* (Life and Health)1955;10(1):16-17.

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