Guardian of your health!

ЧУВАР на Вашето здравје!

Kujdestar i shëndetit tuaj!

CONTENT - СОДРЖИНА

PUBLIC HEALTH

EVALUATION AND RELATION OF DETERMINANTS OF RISK PERCEPTION IN THE RESIDENT POPULATION LIVING NEAR INDUSTRIALLY CONTAMINATED SITES

Sandra Kosteska[1], Marija Topuzovska-Latkovik[2]

[1] Institute for Accreditation of Republic of North Macedonia, Skopje, North Macedonia
[2] Institute for Sociological, Political and Juridical Research, Skopje, North Macedonia

Abstract

The association between industrial pollution and human health is of high importance for public health. Living near industrially contaminated sites (ICSs) and being exposed to increasing concentrations of environmental pollutants along with disadvantaged social and economic conditions result in an increased occurrence of diseases. There are 16 identified industrially contaminated sites in the Republic of North Macedonia, and of all of them, chemical industry AD OHIS - Skopje and lindane dump located near the plant, according to almost all categorizations, has been evaluated to pose the highest level of ecological and health risk, although there has been no recent evidence about these issues. The main aim of this study was to obtain general information about risk perception of resident population living around and near AD OHIS in the Skopje region. Methods: A standardized and modified questionnaire was sent to the participants in an electronic form by e-mail and was published on social networks and municipalities' web sites. The responses to the questionnaire were given anonymously and voluntarily. The results were analyzed using descriptive statistical methods with calculating central tendency parameters and analytical statistical methods with correlation and Pearson χ2 test and independent sample test. Results: During the observation period, 220 people responded to the survey, with female respondents being predominant (70%). Analysis showed that there was no significant difference between genders regarding risk perception. According to Likert scale, with 95% CI, among anthropogenic sources, respondents stated they were extremely worried about air pollution and very worried or worried about water pollution, noise, waste and dangerous industry. The diseases that trigger a response of greater concern were those related to allergies, respiratory diseases and cancer. Age, education and information related to ecological risks significantly influenced on judgement of the environmental conditions in the living area of resident population (p<0.05). Conclusions:Investigation results showed that resident population in the exposed Skopje region has a high level of perception and is susceptible to ecological risks by anthropogenic and natural influence. There is a direct relationship between exposure and environmental health impact. Also, socioeconomic characteristics (gender, age, education) and cognitive factors have influence on risk perception level.

ЈАВНО ЗДРАВЈЕ

ЕВАЛУАЦИЈА НА ФАКТОРИТЕ И НИВНАТА ПОВРЗАНОСТ ПРИ ПЕРЦЕПЦИЈА НА РИЗИКОТ КАЈ РЕЗИДЕНТНОТО НАСЕЛЕНИЕ ВО ОКОЛИНАТА НА ИНДУСТРИСКИ КОНТАМИНИРАНИ ТОЧКИ

Сандра Костеска[1], Марија Топузовска-Латковиќ[2]

[1] Институт за акредитација на Република Северна Македонија, Скопје, Северна Македонија
[2] Институт за социолошки и политичко-правни истражувања, Скопје, Северна Македонија

Извадок

Врската помеѓу загадувањето кое потекнува од индустријата и човековото здравје е од високо значење за јавното здравје. Животот во непосредна близина на индустриски контаминираните точки (ИКТ) и експонираноста на високи концентрации на еколошки полутанти, придружени со нарушени социјални и економски услови, резултира со зголемена појава на заболувања. Од вкупно 16 идентификувани контаминирани точки во Република Северна Македонија, хемиската индустрија во АД ОХИС - Скопје и депонијата со линдан која се наоѓа во непосредна близина, безмалку во сите категоризации правени досега е оценета со највисок еколошки но и јавноздравствен ризик иако никогаш не е правено опсежно истражување на тие аспекти.Главната цел на ова истражување беше да се добијат основни податоци за перцепцијата на ризик на резидентното население кое живее во околината или непосредна близина на ИКТ АД Скопје во скопскиот регион. Методи.Истражувањето беше спроведено со доставување на стандардизиран модифициран прашалник во електронска форма преку електронска пошта и објавен на социјалните мрежи и интернет страницата на скопските општини. Пополнувањето беше анонимно и доброволно.За анализа на резултатите беа користени дескриптивни статистички методи со одредување мерки на централна тенденција и аналитички статистички методи со корелација и Pearson χ2 (хи-квадрат тест) и т-тест за независни примероци и анализа на варијанса. Резултати.Вкупно 220 испитаници доброволно го пополнија прашалникот, од кои поголемиот дел (70%) беа испитаници од женски пол, меѓутоа анализата покажа дека нема сигнификантна разлика меѓу половите во однос на перцепција на ризикот. Според Likert-овата скала, со 95% CI од антропогените извори како најголем ризик со екстремна изложеност испитаниците го чувствуваат загадувањето на воздухот,а со значително до умерено загадувањето на водата, бучавата, отпадот и опасната индустрија.Како три најчести патолошки состојби кои може да се појават при изложеност на контаминирана средина си сметаат алергиите, респираторните заболувања и канцерот. Возраста, степенот на образование и информираноста за еколошките ризици сигнификантно влијаат на мислењето поврзано со условите на животната средина во која живее резидентното население (р<0,05). Заклучок.Резултатите покажаа дека резидентното население во експонираниот скопски регион има високо ниво на перцепција и е чувствително на влијанието на еколошките ризици од антропоген и природен карактер и смета дека постои директна поврзаност помеѓу експозицијата и влијанието на еколошката средина врз здравствената состојба. Исто така, може да се заклучи дека социоекономските карактеристики (пол, возраст, степен на образование) и когнитивните фактори имаат влијание врз нивото на перцепција на ризиците.

Introduction

The association between industrial pollution and human health is of high importance for public health. Living near Industrially Contaminated Sites (ICSs) and being exposed to increasing concentrations of environmental pollutants along with disadvantaged social and economic conditions result in an increased occurrence of diseases during both childhood and adulthood[1-3]. There are many identified ICSs in Europe, and in the Republic of North Macedonia 16 contaminated sites have been identified within National Plan and feasibility studies financed by EC[4].

In the past, industrially contaminated sites in North Macedonia have been investigated several times. Inappropriate treatment and waste handling (industrial and household waste) are considered as main sources of contamination. Stafilov has investigated dispersion of heavy metals in different regions of Macedonia[5-6]. According to these data, dispersion of waste substances, partially or in majority is done through the air that results in contamination of soil, surface and underground water, and by resuspension in dry soil is returning in the air again. This environmental pollution has impact on human population also, with serious health risks. Out of a total of 16 contaminated sites in North Macedonia, from health and ecological point, three are classified as the most dangerous[7]. Chemical industry AD OHIS –Skopje, according to almost all categorizations, has been evaluated with the highest level of ecological and health risk, although there has been no recent evidence about these issues. This company is no longer active, but there is lindane dump near the company (which is left there for more than 30 years), chlor-alkali dump and HCH dump (also left) as an extremely toxic organic compound. There is a treat that hazardous compounds from this locality could be dispersed in all near or wider environmental media, but the risk is much higher if we take into consideration that this factory is located in the middle of a populated area of the city of Skopje[8].

The European Environment Agency[9] has also confirmed that air pollution, noise, bad smells, and traffic have a severe impact on a population's health, and that human activities (mainly in the sectors of industry, energy, and transport) produce relevant environmental pressures[10-11].

Although environmental problems caused by industrial activities in the area have been subject of attention by governments and the industrial sector, many interested parties are still concerned and believe that the risks associated with industrial activities still exist. One of the critical issues is failed risk communication among residents, governments, and the industrial sector. This failure has impacted the decision-making process which cannot be carried out if there is no agreement among all parties involved. Governments mostly make decisions regarding the development of industrial activities based on experts' scientifically estimated risks; however, local residents' risk judgments are not well understood or considered. As a result, industries have been grow-

ing despite public objections. Thus, the differences in risk judgments among residents, governments, and the industrial sector are a major cause of the problems in risk communication[12].

The causes determining residents's risk judgments and perceptions need to be thoroughly studied in order to create effective risk communication between governments and the public[13]. Comprehending resident's fundamental understanding of risk-related judgment can help risk communicators achieve the following: effectively establish communication efforts, properly select pieces of information and their formats and foster information sharing among relevant parties. Risk perception is filtered differently by people according to their attitudes and moral values. Crawford-Brown[14] noted that residents' perceived risks might depend on the evidence they possess regarding the frequency, severity, and variability of effects. Resident's risk judgments also involve judgments of probability, severity of catastrophic consequences[15], and perceived control.

Currently, a range of previous, relevant researches has mostly explained risk perception based on the assumption that residential people have limited scientific knowledge and capability to cope with the risks they face; thus, their perceptions are significantly influenced by a wide spectrum of social and psychological factors such as fear, familiarity with the risk, ability to control the risk. For example, Slovic[16] in his book mentioned that Americans' perceptions of the dangers of nuclear waste storage were significantly affected by psychological factors such as fear, distrust, and uncertainty. However, at present, the enhanced quality in people's education, an increase in public environmental awareness, the strength of residents' social networks with other organizations, and various public media, people's easier access to risk-related information, possibly increase resident's capabilities to assess the risks they face. Psychological factors might therefore be less influential. On the other side, resident's risk perceptions might be processed based on their analytical way of thinking. Factors related to the nature of risks such as perceived probability of occurrence and severity of facing risks might be more powerful in predicting resident's perceived risks[17, 18].

Risk perception is a judgment of the adverse consequences of a particular hazard and can be made by an individual, a group of people, or society[19]. The term "risk perception" generally refers to natural hazards and threats to the environment or health and can be formed based on both belief and self-appraisal[20]. Until now, four approaches have been used to study how risks are perceived. The first approach is the sociocultural model, whereas the risk perception is constructed from beliefs influenced by social forces in society[21]. The second approach is the psychometric paradigm (basic model) which describes how risk perception is influenced by physical properties of the risk, psychological and cognitive factors[22,23]. The third approach is the interdisciplinary paradigm that applies several concepts to explain risk perception. The most distinct concept is

Kasperson'smodel which amplifies psychological, social, institutional, and cultural processes[24]. The last approach is the axiomatic measurement paradigm that focuses on how average people subjectively transform objective risk information. It is believed that risk perception is influenced by possible catastrophic consequences (fatal outcomes, mortality rates, etc.) and likelihood of occurrence[25].

Risk perception can be processed based on a rational system or an experimental system. The experimental evaluation includes psychological and cognitive factors. The studies showed that controllability and previous experience with the occurrence are factors that have significant influence on risk perception. Paolo et al.[26] in their study demonstrated that people smelling unfamiliar odors may exhibit a high-risk perception due to their concerns about respiratory diseases such as asthma and lung cancer. Gregory and Mendelsohn[27] stated that individual risk assessment was included in the person's perceived benefits (positive and negative). The family concern is a very important factor which shouldn't be neglected and has high influence on perceived risk perception[28].

Given the complex relationship that exists among perception, behavior, and socioeconomic characteristics of local populations, discussions in the field of risk perception and communication are increasing and are subject of interest of many scientific debates.

The principal aim of this study was to obtain general information related to risk perception of residents living around and near industrially contaminated site AD OHIS in the Skopje region. To achieve this aim, the following sub-aims were set: to obtain information of risk perception related to healthy condition of resident population; to obtain information about level and confidence of available information regarding environmental risks; to determine the relation between risk perception factors, age, and education of resident population and impact of cognitive factors.

Materials and methods
Study design

This was a cross-sectional study which was conducted in the period of December 2021-January 2022.

Sample included in the study

In this study, a total of 220 respondents were involved, all of them living in the city of Skopje, from several municipalities, taking into the consideration that industrially contaminated site AD OHIS plant is located in Skopje. This ICS, in almost all categorizations has been evaluated with the highest level of ecological and health risk with a high emotional impact on the resident population. The sampling method was chosen to provide comprehensive data about the population surveyed.

Survey tool

The main tool of the survey was the questionnaire. To achieve the aim of the survey, the modified standardised questionnaire for multi-

purpose investigation of the population living near high-risk ICS[29], was developed. The questionnaire was composed of four sections, which aimed to investigate: a) the characteristics of individuals; b)risk perception; c) availability of ecological information; and d) home conditions and healthy status. The form of the questionnaire was structured to obtain answers to the questions with a purpose to present variables. The questionnaire contains closed questions, for which respondents should answer the questions according to Likert scale[30], grading with scores from 1 to 4, where score 1 indicates "extremely exposed/ most probably", and score 4 indicates "not at all/ impossible". The other questions are open, for which quantitative and qualitative information was required, as well as partially closed questions where an alternative answer "other" could be chosen. The factors, variables and types of the

Table 1. Description of factors, variables and content of questionnaire

Fact Factors	Variables	Questionss
Risk perception	Exposure level to natural and anthropogenic ecological risks	To which level do you feel you are exposed to floods, noise, dangerous materials transport, hazardous waste, air pollution, extreme weather conditions, fires, water pollution, hazardous industry, earthquakes?
	Perception of environmental condition	How do you estimate the environmental condition in your living area?
	Healthy issues	To which level do you consider that it is possible for someone who lives near contaminated site to have: allergies, temporary/ permanent respiratory damage, temporary/ permanent damage of different organs, liver damage, cancer, leukemia, congenital malformations in infants delivered by parents being exposed to pollution?
Level of knowledge	Knowledge regarding information related to ecological risks	How much are you satisfied with the information about ecological risks in your living area?
	Media/ source of information	Which media/ source do you prefer and usually use to receive more information? Which kind of media would you like to inform you about particular risks? Which source/media do you believe the most regarding information about the risks that you are exposed to?

	Satisfaction regarding living conditions	Would you like to leave the present living place?
Physiological and cognitive factors	Health condition	In the past year, did you receive medications for treatment of some disease related to the respiratory system (not related to COVID-19)? At this moment are you receiving medications for treatment of heart and vascular diseases?

questions used are given in Table 1.

A large set of the questionnaire included items related to biological data, sociodemographic information, and state of health (sex, age, place of birth, level of education, self-perceived health status).Some of this information was used in the analyses of risk perception.The questionnaire was sent by e-mail and was published on the official web sites of municipalities in the city of Skopje. The completion of the questionnaire was anonymous and voluntarily.

Statistical analysis

Descriptive statistical methods

The structure of statistical series with attributive characteristics was calculated by determining relationship coefficients, proportions and rates. The structure of statistical series with numerical characteristics was calculatedby determining measures of central tendency (mean values – arithmetical mean) and dispersion measures (standard deviation). A p-value less than 0.05 was considered statistically significant, 95% confidence interval (CI) was calculated.

Analytical statistical methods

Significance in differences between distribution of responses of risk perception were tested applying correlation and Pearson χ^2 (chi-square test).The differences between gender responses were tested applying Independent Sample Test (t-test) and analyses of variance. The results are presented using tables and figures.

Results

Of a total of 220 respondents, more than a half, about 70% (n=154) were female and the other 30% (n=66) were male respondents who voluntarily answered the questions. The average age of respondents was 40 years, where the youngest was 23 years old and the oldest 70 years. As regards the education, the majority of respondents had a high-school diploma. Distribution of living places in the city of Skopje showed that half of the respondents who voluntarily completed the questionnaire were living in settlements near OHIS plant, as presented in Picture 1.

Picture 1. Distribution of respondents living in settlements near OHIS Plant
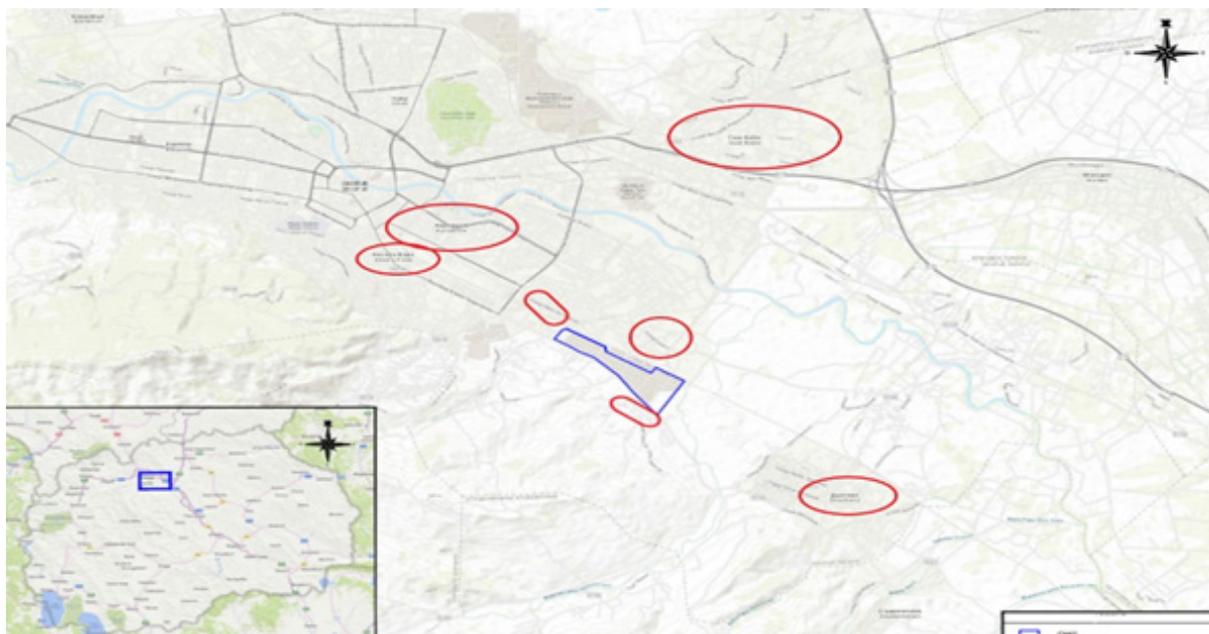
Source: The picture was created by the author. Note: The blue color represents borders of OHIS Plant, with the red color are marked settlements around the plant: Drachevo, Pintija, Lisiche, Gorno Lisiche, Aerodrom, KiselaVoda, Gazi Baba

Analysis of the questions and received answers for risk perception (Table 2) showed that residents, from anthropogenic sources (given in scale from 1 "extremely exposed" to 4 "not at all") perceived air pollution as the highest risk with extreme exposure level (*m=1.47*), followed by water pollution (*m=2.58*) and noise (*m=2.51*) which were perceived as great to moderate risks, hazardous industry (*m=2.73*) and hazardous waste (*m=2.95*) as a moderate risk, and as a minimum risk, they felt exposure to dangerous materials transport (*m=3.11*). Regarding the natural disasters, residents perceived extreme weather conditions (*m=2.39*), earthquakes and fires (*m=2.5* to *2.7*) be the highest risks, and floods were perceived as minimal risk with great to moderate exposure level (*m=3.25*).

Table 2. Risk perception- exposure

To which level do you feel you are exposed to the following risks?	*Lowest*	*Highest*	*Arithmetical Mean (m)*	*Standard Deviation (SD)*
Floods	1	4	3.25	0.764
Noise	1	4	2.51	0.894
Dangerous materials transport	1	4	3.11	0.85
Hazardous waste (chemical, radioactive)	1	4	2.95	0.99
Air pollution	1	4	1.47	0.658

Extreme weather conditions (hot waves, overflows)	1	4	2.39	0.937
Fires	1	4	2.74	0.813
Water pollution (underground, surface)	1	4	2.58	0.945
Hazardous industry	1	4	2.73	1.055
Earthquakes	1	4	2.5	0.852

As regards to the perception of environmental impact on health status, majority of respondents considered that bad conditions for living near contaminated site were the reason for occurrence of various pathological health conditions. In scale from 1 "most probable" to 4 "impossible", respondents answered that a very probable option for someone who lives near contaminated site is to develop some type of allergy (m=1.74), temporary and permanent respiratory damage (m=1.72, m=1.98), temporary and permanent damage to various tissues (m=1.95, m=2.13), liver disease (m=2.23), cancer (m=1.83), leukemia (m=2.12), congenital malformations in children delivered by parents living near contaminated site (m=2.01). The results are given in Table 3.

Table 3.   Risk perception- Probability of diseases onset

To which level do you consider that it is possible for someone who lives near contaminated site to develop:	Lowest	Highest	Arithmetical Mean (m)	Standard Deviation (SD)
Allergies	1	3	1.74	0.622
Temporary respiratory damage	1	3	1.72	0.609
Permanent respiratory damage	1	4	1.98	0.694
Temporary damage of various-tissues	1	4	1.95	0.647
Permanent damage to various tissues	1	4	2.13	0.747
Liver disease	1	4	2.22	0.772
Cancer	1	4	1.83	0.674
Leukemia	1	4	2.12	0.825
Congenital malformations in infants delivered by parents being exposed to pollution	1	4	2.01	0.796

Analysis given in Table 4 shows a positive correlation between age, education, and perception regarding environment. Older respondents and respondents with higher level of education perceived that environmental conditions in their living area are more serious ($r<.275$, $p<0.01$, $r<.170$, $p<0.05$, respectively).

Table 4. Impact of level of knowledge regarding awareness and cognitive factors on risk perception

		Age	*Education*	**How do you estimate the environmental conditions in your living area?**	**How much are you satisfied with the information about ecological risks in your living area?**	**Would you like to leave the present place of living?**
Age	Pearson Correlation	1	.306**	.275**	0.038	0.1
	Sig. (2-tailed)		0	0	0.574	0.142
Education	Pearson correlation	.306**	1	.170*	.139*	.146*
	Sig. (2-tailed)	0		0.012	0.041	0.032
How do you estimate the environmental conditions in your living area?	Pearson correlation	.275**	.170*	1	.274**	-.253**
	Sig. (2-tailed)	0	0,012		0	0
How much are you satisfied with the information about ecological risks in your living area?	Pearson Correlation	0.038	.139*	.274**	1	-.221**
	Sig. (2-tailed)	0.574	0.041	0		0,001
Would you like to leave the present place of living?	Pearson Correlation	0.1	.146*	-.253**	-.221**	1
	Sig. (2-tailed)	0.142	0.032	0	0.001	

Note: **$p<0.01$, *$p<0.05$

A positive correlation was considered between awareness of environmental risks and perception for environmental conditions. Respondents who were more aware of environmental risks evaluated environmental conditions to be poor (r<.274, p<0.01). For the other variables, a negative correlation was found between estimation of environmental conditions and willingness to leave the place of living (r<-.253, p<0.01), perception of having poor environmental conditions increased the wish to leave the current living place. Respondents who wanted to leave the current living place very much showed greater awareness of environmental risks (r<-.221, p<0.01).

As regards the gender, for these three issues, evaluation by independent sample test (t-test), given in Table 5, showed that there was no significant difference between female and male respondents (p>0.05).

Table 5. Summary difference between genders regarding perception of environmental condition

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
How much are you satisfied regarding information of ecological risks in your living area?	Equal variances assumed	2.437	.120	.276	215	.783	.024	.086	-.146	.193
	Equal variances not assumed			.257	106.409	.798	.024	.092	-.159	.206
How do you estimate the environmental condition in your living area?	Equal variances assumed	.108	.743	.392	215	.695	.039	.098	-.155	.232
	Equal variances not assumed			.384	118.104	.702	.039	.100	-.160	.237
Would you like to leave the present living place?	Equal variances assumed	4.861	.029	-.683	215	.495	-.071	.104	-.276	.134
	Equal variances not assumed			-.650	110.885	.517	-.071	.109	-.288	.146

Data regarding preferred media for providing information related to risks (Figure 1), showed that the highest percentage of respondents preferred electronic communication via internet networks (90%) and national TV (70%).

Figure 1. Information sources
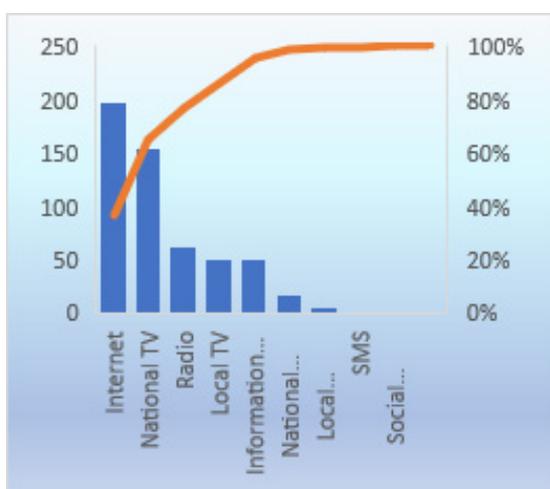
Figure 2. Level of confidence in information sources
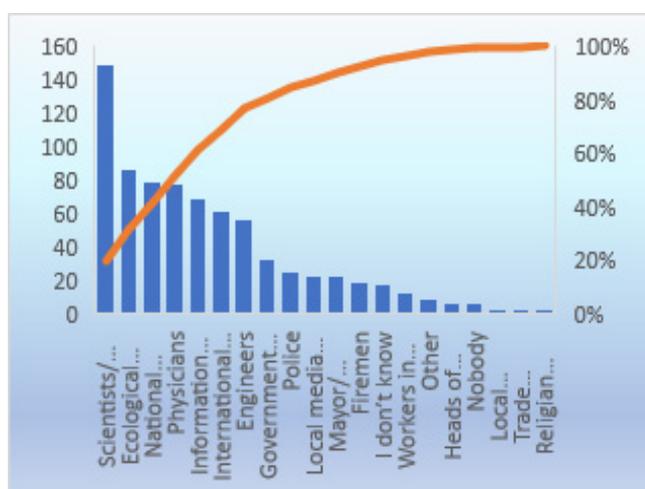
The distribution of respondents' results as regards to confidence level in information sources related to environmental risks is given in Figure 2. However, it can be concluded that the largest percentage of respondents believed the scientists (approximately 68%), followed by ecological societies (39%) and physicians (35%).

Discussion

In this study we evaluated the perception of environmental risks by resident population living in the Skopje region, in the area where one of the 16 identified environmental hot spots in North Macedonia is located and has a high emotional impact and presents a huge health concern.

During the observation period, 220 people responded voluntarily to the survey, with female respondents being predominant. As it is known, the female population is more sensitive than the male to environmental issues and similar results with females more frequently responding is reported in other studies[31]. According to Flynn et al.[32], gender has a powerful impact on risk perception and in majority of studies, females are respondents who overestimate the risk. Nevertheless, there are studies[33] that reported opposite results or results with no differences in responses between genders, which is in agreement with our study.

As far as self-selected sample's general information is concerned, the respondents were aged between 23 and 70 years old, with an average age of 40 years. Although the survey was distributed in a way favored by people more inclined towards the use of IT tools, our respondents were represented by all age groups.

Another note worthy result was the correlation between education qualifications and perception of environmental risks. This has also been presented in other surveys such as those of Carducci et al. and Ozdemir et al.[34, 35]. Their respondents with a higher level of education perceived environmental risks to be high.

The correlation between respondents' concern regarding environmental impact and respondents' risk perception of health condition was also clearly seen in our study. The results of respondents' perception confirmed that there was a cause-related link between respondents' judgment of environmental conditions and health status. Most of the respondents in our study estimated environmental conditions in the living area to be severe and more concern was registered among older respondents. According to the literature data, there is no doubt that population exposed to contaminated environment has higher risk perception regarding development of diseases. In our study, diseases that triggered a response of greater concern were those related to allergies, respiratory problems and cancer. The concern about congenital defects was also high, although these pathological conditions were much rarer than other diseases. These points out toward one of the major problems of risk perception: the relationship between the sources of anxiety for the community and the actual existence of a health risk, couldn't always be clearly described.

Furthermore, the literature indicates limited evidence of increased risk for certain types of cancer[36] and for congenital defects as a whole[37]. These studies, however, mainly refer to old plants and have several methodological weaknesses which restrict the validity of the results (exposure assessment is often poor, the analysis is based on ecological level, reference to few individuals). Other studies indicated the effects of air pollution on respiratory diseases[38]. To summarize, the order of magnitude of the risk perception described in the literature could differ from what people perceive.

In the present study, respondents perceived polluted air, waste and hazardous industry as a three major risk factors for environmental exposure. This is similar to the results of a survey conducted by Bena et al.[39] who investigated the perception of local population living near the incineration plant for solid waste in Turin, Italy. In this study, anthropogenic hazards generated more concern than natural hazards. In other studies[40] it has also been found that people in Italy feel more vulnerable to anthropogenic than natural risk. Public acceptance of anthropogenic risk is influenced by trust and local experience. Furthermore, it is conditioned and constantly revised by information from multiple sources, including the media, and by the influence of peers and others so that communication plans must have reliable tools to support such elements. In many studies, including the previous mentioned survey by Bena et al.[39], there is an inverse proportion between greater risk perception and greater distance from the pollution source. This was not the case in the study of Cavazza et al.[41], who found no relationship between residence near the incineration plant that had been operational for more than 30 years, and citizens' attitudes towards it. This is very similar to the judgements of respondents in our survey where we found that perception was identical and irrespective of the distance of contaminated source, lindane dump, active for more than 30 years. The other finding in our study was the significant correlation between judgement of environmental conditions and level of education. This result corresponds with literature data which confirm that perception level, education, degree of involvement, cognitive factors and uncertainty are important factors which influence on local community judgment regarding potential source of environmental risk. As it has been described in the study of Janmaimol and Watanbe[42], respondents in high-risk communities judged risks based on their perceived probability of environmental contamination. Respondents in moderate-risk communities assessed risks by considering the probability of being impacted by the contamination, as well as the potential adverse impacts they might face; the perceptions of residents in low-risk communities were not processed based on the rational system but were created on the basis of their beliefs, which were affected by previous experiences.

In regard to the availability of information related to environmental risks, respondents expressed more trust in scientists and experts than in physicians, but nevertheless that is the time changing process. A high percentage of respondents

had more confidence in ecological societies, and this might have been expected given that in the period of conducting the survey there was an ongoing campaign for remediation of one of three lindane dump. A very low percentage of trust in the local institutions (only 10%) is a worrisome problem, and it must be emphasized in order to initiate actions for increasing public trust. The lack of trust could have a positive effect since it can stimulate critical thinking, which would result with more careful analysis of the problems and possible solutions.

Conclusions

In the present study we evaluated the determinants of environmental risk perception perceived by voluntary participants in the survey who are living near the ICS OHIS plant Skopje and surrounding settlements in the Skopje region. The results found that resident population has a high level of perception and vulnerability to anthropogenic and natural environmental risks and perceived direct correlation between exposure and environmental impact on health status (CI of 95%, $p<0.05$). The older population and population with a higher education perceived that environmental conditions in their living area are more serious ($r<.275$, $p<0.01$, $r<.170$, $p<0.05$, respectively), and it can be concluded that socio-economic characteristics (gender, age, education level) have impact on risk perception. On the other side, we determined a significant correlation between cognitive factors and perception levels. The availability and trust in information sources, the wish for leaving the present place of living have had an impact on respondents' estimation of environmental conditions, which resulted in a negative significant correlation ($p<0.05$).

References

1. Barker DJP. The origins of the developmental origins theory. J Intern Med 2007; 261: 412–417.

2. Prüss-Üstün A, Wolf J, Corvalán C, Bos R, Neira M. Preventing disease through healthy environments: A global assessment of the burden of disease from environmental risks, 2nd ed.; World Health Organization: Geneva, Switzerland, 2016; ISBN 978-92-4- 156519-6.

3. Fleming TP, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM et al. Origins of lifetime health around the time of conception: Causes and consequences. Lancet 2018; 391: 1842–1852.

4. MOEPP. National plan for waste management 2009-2015 of the Republic of Macedonia. 2008.

5. Stafilov T, Peeva L, Nikov B, De Koning A. Industrial hazardous waste in the Republic of Macedonia. Applied Environmental Geochemistry – Anthropogenic İmpact on Human Environment in the SE Europe, Ljubljana, Proceedings Book (ŠAJN, R., ŽİBERT, G. & ALİJAGİĆ, J., (Eds.)), 2009, ISBN 978-961-6498-18-0, 108-112.

6. Stafilov T. Environmental pollution with heavy metals in the Republic of Macedonia.– Con-

tributions, Section of Natural, Mathematical and Biotechnical Sciences MASA 2014;35(2): 81–119.

7. Bloemen JThH. Biomonitoring of Macedonia. Report on the Twining project MK 12 IB EN 01 – Further strengthening the capacities for effective implementation of the acquis in the field of air quality, Report no.1, 2016; p. 20.

8. Industrial Contaminated Sites ("hotspots") National Waste Management Plan and Feasibility Studies. Contract No.: 01/MAC05/05/002. Ref. No.: EUROPEAID/115138/D/SV/MK.

9. European Environment Agency, Human Activities, https://www.eea.europa.eu/publications/92-827-5122-8/page011.html.

10. WHO European Centre for Environment and Health. Healthy Environments for Healthier People. 2018, eceh-eng.pdf (who.int).

11. Dettori M, Pittaluga P, Busonera G, Gugliotta C, Azara A, Piana A, et al. Environmental risks perception among citizens living near industrial plants: A cross-sectional study. Int J Environ Res Public Health 2020; 17: 4870.

12. Fischhoff B, Ann B, Marilyn J Q. Risk perception and communication. Annu Rev Public Health 1993; 14: 183–203.

13. Ropeik D. Risk perception in toxicology-part I: Moving beyond scientific instincts to understand risk perception. Toxicol Sci 2011; 121: 1–6.

14. Crawford-Brown DJ. Risk-Based Environmental Decisions: Methods and Culture; Kluwer Academic Publishers: New York, NY, USA, 1999.

15. Slovic P. Perception of risk. Science 1987; 236: 280–285.

16. Slovic P. Perceived Risk, Trust and Democracy. In The Perception of Risk; Slovic, P., Ed.; Earthscan: London, UK, 2000; pp. 316–326.

17. Leiserowitz GF, Weber EU, Hsee CK, Welch N. Risk as feeling. Psychol.Bull 2001; 127: 267–286.

18. Epstein S. Integration of the cognitive and the psychodynamic unconscious. AmPsychol 1994; 49: 709–724.

19. Aven T, Renn O. Risk Management and Governance; Springer Verlag: New York, NY, USA, 2010.

20. Slovic P, Weber EU. Perception of risk posed by extreme events. In Proceedings of the Conference on Risk Management Strategies in an Uncertain World, New York, NY, USA, 12–13 April 2002.

21. Grendstand G. Grid-group theory and political orientations: Effects of cultural biases in Norway in the 1990s. Scand Polit Stud 2000; 23: 217–244.

22. Fischhoff B, Watsan S, Hope C. Defining risk. Policy Sci 1984; 77: 123–139.

23. Sjoberg L, Drottz-Sjoberg BM. Risk Perception of nuclear waste: Experts and the Public; Risk Perception Report 16; Center for Risk Research: Stockholm School of Economics, Stockholm, Sweden, 1994.

24. Kasperson RE, Renn O, Slovic P, Brown HS, Emel J, Goble R, Kasperson JX, Ratick S. The social amplification of risk: A conceptual framework. Risk Anal 1988; 8: 177–188.

25. Weber EU. Decision and Choice: Risk, Empirical Studies. In International Encyclopedia of the Social and Behavioral Sciences; Smelser, N.J., Baltes, P.B., Eds.; Elsevier Science Limited: Oxfords, UK, 2001; pp.13347–13351.

26. Paolo L, Perkins C, Lyons M. Health risk perception and environmental problem: Findings from ten case studies in the North West of England; Summary Report; Faculty of Health and Applied Social Sciences: Liverpool John Moores University, Liverpool, UK, 2009.

27. Gregory R, MendelsohnR. Perceived risk, dread, and benefits. Risk Anal 1993; 1: 259–264.

28. Dosman DM, Admowicz WL, Hrudey SE. Socioeconomic determinants of health and food safety-related risk perceptions. Risk Anal 2001; 21: 307–317.

29. Mudu P, Terracini B, Martuzzi M. Human health in areas with industrial contamination.WHO Regional Office for Europe, Copenhagen, 2014.

30. Likert R. A technique for the measurement of attitudes; Columbia University: New York, NY, USA, 1932.

31. Johnson T. Environmentalism and NIMBYism in China: Promoting a rules-based approach to public participation. Environ Politics 2010; 19: 430–448.

32. Flynn J, Slovic P, Mertz C. Gender, race, and perception of environmental health risks. Risk Analysis. 1994; 14(6):1101–1108.

33. Signorino G, Beck E. Risk perception survey in two high-risk areas. Pierpaolo Mudu; Benedetto Terracini; Marco Martuzzi. Human health in areas with industrial contamination, World Health Organization – Europe. 2014; pp.232-245.

34. Carducci AL, Fiore M, Azara A, Bonaccorsi G, Bortoletto M, Caggiano G, et al. Environment and health: Risk perception and its determinants among Italian university students. Sci Total Environ 2019.

35. Ozdemir ED, Sener S. The impact of higher education on environmental risk perceptions. China USA Bus Rev Sep. 2016; 15: 459–471.

36. Porta D, Milani S, Lazzarino AI, Perucci CA, Forastiere F. Systematic review of epidemiological studies on health effects associated with management of solid waste. Environ Health 2009;8:60.

37. Ashworth DC, Elliott P, Toledano MB. Waste incineration and adverse birth and neonatal outcomes: a systematic review. Environ Int 2014;69:120 –32.

38. Golini MN, Ancona C, Badaloni C, Forastiere F. Morbidity in a population living close to urban waste incinerator plants in Lazio region (Central Italy): a retrospective cohort study using a before-after design. Epidemiol Prev 2014;38(5):323 –34.

39. Bena A, Gandini M, Cadum E, Procopio E, Salamina G, Orengia M, Farina E. Risk perception in the population living near the Turin municipal solid waste incineration plant: survey results before start-up communication strategies. BMC Public Health2019; 19:483.

40. Salvati P, Bianchi C, Fiorucci F, Giostrella P, Marchesini I, Guzzetti F. Perception of flood and landslide risk in Italy: a preliminary analysis. Nat Hazards Earth Syst Sci 2014;14:2589 –603.

41. Cavazza N, Rubichi S. Ways of thinking about the incinerator: a typology of citizens' mindsets. Soc Sci J. 2014;51:422 –430.

42. Janmaimool P, Watanabe T. Evaluating determinants of environmental risk perception for risk management in contaminated sites. IntJ Environ Res Public Health 2014; 11: 6291-6313.

PUBLIC HEALTH

KNOWLEDGE ABOUT HUMAN PAPILLOMAVIRUS AND ASSOCIATED FACTORS AMONG POPULATION IN THE REPUBLIC OF KOSOVA

Ernad Kosumi[1], Viktor Isjanovski[2], Mome Spasovski[3]

[1] University Clinical Center of Kosova, Department of Gynecology and Obstetrics, Pristina, Kosova
[2] Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Republic of North Macedonia
[3] Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Institute of Social Medicine, Skopje, Republic of North Macedonia

Abstract

Above 70% of sexually active women and men will be infected with human papilloma irus at some point in their lives, and several may yet be infected in more than one circumstance. Around 80% of sexually active women acquire HPV infection, while for the most part, they are asymptomatic with the immune system-mediated clearance of contagion within 6–12 months. High-risk papillomavirus is accountable for causing cancer associated with the cervix, vulva, vagina, anus, penis, and oropharynx. This survey aims to investigate the level of knowledge among residents in the Republic of Kosova regarding human papillomavirus (HPV) infections and HPV-associated diseases and to discover the relationship between these factors. Material and methods: A cross-sectional survey was performed by investigating the knowledge concerning HPV infection in the population aged 18 to 35+ years. The study was conducted in the interval from June 2021 - August 2021. The sample included 500 participants. The questionnaire was anonymous, and participants were free to end the participation at any time, without finishing the questionnaire. Results: More than half of the respondents knew about HPV - 70.0%, and 29.6% did not know. Respondents with secondary and higher education showed 18 times more knowledge for HPV (OR = 18.1311 95% CI: 8.7465-37.5852) than respondents with primary education. To the question Can HPV cause cancer of the cervix, 37.2% (n=186) of the respondents gave a correct answer. The results presented that most participants knew what HPV was (70%), but they also revealed that most participants had low or moderate knowledge about HPV, which was a comparable result with earlier studies. A small number of participants in this survey knew that HPV could heal by itself (12.8%), which was similar to results presented in other surveys. Conclusions: It is important to improve the inadequate knowledge about HPV among the population in the Republic of Kosova. In order to do that, it could be significant to assess which factors affect the knowledge so that young women and all adolescents will get pushed to use protective measures against cervical cancer and have protected sexual behaviour.

JAVNO ZDRAVJE

ЗНАЕЊА ЗА ХУМАН ПАПИЛОМАВИРУС И ФАКТОРИ ПОВРЗАНИ СО НЕГО КАЈ НАСЕЛЕНИЕТО НА КОСОВО

Ернад Косуми[1], Виктор Исјановски[2], Моме Спасовски[3]

[1] Универзитетски клинички центар на Косово, Оддел за гинекологија и акушество, Приштина, Косово
[2] Универзитет „Св.Кирил и Методиј" во Скопје, Медицински факултет, Република Северна Македонија
[3] Универзитет „Св.Кирил и Методиј" во Скопје, Медицински факултет, Институт по социјална медицина, Скопје, Република Северна Македонија

Извадок

Над 70% од сексуално активните жени и мажи ќе бидат заразенисо хуман папиломавирус во некоја фаза од нивниот живот, а неколку сепак може да бидат заразени во повеќе од една околност. Околу 80% од сексуално активните жени добиваат ХПВ инфекција, додека во најголем дел, тие се асимптоматски со отстранување на заразата посредувано од имунолошкиот систем во рок од 6-12 месеци. Високоризичниот папиломавирус е одговорен за предизвикување рак поврзан со грлото на матката, вулвата, вагината, анусот, пенисот и орофаринксот.Ова истражување има за цел да го провери нивото на знаење кај жителите во Република Косово во врска со инфекциите со хуман папиломавирус (ХПВ) и болестите поврзани со ХПВ и да ја открие врската помеѓу овие фактори. Материјал и методи: Спроведовме студија на пресек за знаењето на ХПВ инфекцијата кај популацијата на возраст од 18 до 35+ години. Студијата беше спроведена во интервалот од јуни 2021 до август 2021 година. Примерокот вклучи 500 ученици. Прашалникот беше анонимен, а учениците можеа слободно да го прекинат учеството во секое време, без да го завршат прашалникот.Резултати: Повеќе од половина од испитаните знаеја за ХПВ - 70,0%, а 29,6% од нив не знаеја. Испитаниците со средно и високо образование покажаа 18 пати повеќе знаење за ХПВ (OR= 18,1311 95% CI: 8,7465-37,5852) од испитаниците со основно образование. На тврдењето ХПВ може да предизвика рак на грлото на матката, 37,2% (n=186) од испитаниците дале точен одговор.Резултатите покажаа дека повеќето ученици знаеја што е ХПВ (70%), но тие исто така покажаадека повеќето ученици имаа ниско или умерено познавање за ХПВ, што е споредлив резултат со претходните студии. Мал број од учениците во ова истражување знаеја дека ХПВ може сам да се лекува (12,8%), што е слично со резултатите од други истражувања.Заклучок: Од клучно значење е подобрување на несоодветното знаење за ХПВ кај луѓето во Република Косово. За да се направи тоа, би можело да биде значајно да се процени кои фактори влијаат на знаењето, така што младите жени и сите адолесценти ќе бидат принудени да користат заштитни мерки против рак на грлото на матката и да имаат заштитено сексуално однесување.

Introduction

Human papillomaviruses (HPVs) are an essential group of viruses affecting the cutaneous and mucosal epithelia. HPVs trigger diseases related to high morbidity and mortality rates, involving benign lesions and cancer[1].

Above 70% of sexually active women and men will be infectedat some pointin their lives, and several may yet be infected in more than one circumstance[2].

The projected incidence of HPV contagion is high, with 14 million people affected yearly and 79 million people with predominant infection[3]. HPV is linked with numerous cancer types in both men and women. Amongst women, in 2012, HPV was related with 74% of cancer cases, 70% of which were cervical cancer[4].

There is a need for evidence about the HPV registry and cervical cancer in Kosova; however, a survey led by Zejnullahu V. in 2016 in Kosova presented a high HPV frequency, estimated up to 50.93% (109 samples) out of 214 cervical samples[5].

Papillomaviridae family consists of more than 200 varieties of HPV, which are categorized into five classes: Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus Mupapapillomavirus, and Nupapapillomavirus[6]. All the classes are responsible for the numerous kinds of HPV-associated cancers.

Moreover, based on oncogenicity, the mucosal type (alpha) is divided into two subtypes which are low risk (LR), HPV 6, and HPV 11, which are recognized to cause benign genital warts, and high-risk (HR) cervical cancer[7]. HPV 6 and HPV 11 are also recognized to cause respiratory papillomatosis, predominantly in children[8].

High-risk papillomavirus is responsiblefor causing cancer associated with the cervix, vulva, vagina, anus, penis, and oropharynx[9]. Most infections are benign, leading to lesions such as cutaneous warts on the hands, feet, and anogenital zones. Only a small number of diseases with specific types of HPV can last and progress to cancer, such as oropharyngeal, cervical, vulvar, vaginal, and penile cancers[10].

Around 80% of sexually active women acquire HPV infection, while most of them are asymptomatic with the immune system-mediated clearance of the infection within 6-12 months[11].

Cervical cancer is undoubtedly the most widespread HPV-associated disease[10]. Almost all cases of cervical cancer are a result of a continued or chronic HPV infection. The fourth most recurrent cancer in women globally is cervical cancer, and it accounts for approximately 528,000 new cases per year[2].

Nearly 85% of the worldwide burden happens in the less developed countries, accounting for approximately 12% of all female malignancies. In 2012, an estimated 266,000 deaths were credited to cervical cancer, accounting for 7.5% of all female cancer deaths, with closely 90% of these deaths happening in the less developed countries[2].

In the developing regions, cervical cancer may cover up to 25% of all female cancer cases12 and is only led by breast cancer as the most frequent cause of cancer deaths in women globally[13].

Statistics on cancer percentages in Kosovo are unavailable as a national cancer office is in the procedure of re-launching, and recording procedures have yet to become official. However, incidence information presented for 2013 reported 224 breast and 27 cervical cancer cases. A survey led by Knowles and Packer in 2008 evaluated that at least 50% of female genital cancers in Kosova were not formally documented[14]. The 2015 annual report of the National Institute of Public Health of Kosova identified 68 new cases of cervical cancer in Kosova in 2015.

A further cross-sectional study in 2016 conductedby Romejko-Wolniewicz *et al*., presented a high incidence degree of cervical cancer in Kosova ASR(W)=23.8, and a high mortality degree.

HPV may be transmitted throughout perinatal (during birth from mother to child)[16], genital infections (genitals, anus, or mouth of an affected sexual partner)[17], hands, shared object, blood, surgery (during laser ablation of a condyloma (wart) or electrocautery)[18] HPV does not transmit through ordinary stuff like toilet seats[16], although the types that cause warts may transmit through surfaces such as floors[19].

This survey aimed to investigate the level of knowledge regarding human papillomavirus (HPV) infections and HPV-associated diseases among residents in the Republic of Kosova and to discover the relationship between these factors.

Material and methods

A cross-sectional survey was performed by investigating the knowledge concerning HPV infection in the population aged 18 to 35+ years. The study was conducted in the period from June 2021 - August 2021.

The sample was obtained based on convenient selection, and included 500 participants.

Information from participants in the study group was gathered through interviews, using a pre-designed questionnaire, online version: distribution via social media, paper version: distribution during student conferences, lectures, and distribution in libraries. Survey clarification was available whenever it was necessary. Each part of the questionnaire offers the objectives of the survey and the measures for protecting anonymity. Participation was voluntary, and all information was handled confidentially.

Criterion for inclusion was the age of the participants (from 18 to 35+ years). This age group is selected because it is the part of the population that is most vulnerable to HPV infection and HPV-associated diseases and a crucial part of the society that decides and will decide to vaccinate their children against HPV. The latter comes up from the fact that people subject to mandatoryHPV vaccination are 12-year-old girls who do not decide for themselves and whose vaccination needs their parents' permission.

Given that a validated questionnaire is not available in the Republic of Kosova, we remodelled a series of questions based on Knowledge, Attitude and Practice (KAP) survey in settings with a related socio-cultural situation to the Republic of Kosova to investigate KAP about HPV. We also conducted a pilot study to

test the validity and trustworthiness of the questionnaire. As reported by World Health Organization, KAP survey data can recognize how knowledge differences, cultural beliefs, or behavioral patterns may influence understanding and action and cause struggles or create obstacles for HPV vaccination.

The design of the questionnaire was guided by the survey and the literature review of the knowledge regarding HPV.

The questionnaire was composed of 2 groups. The first group of questions gives information on the sociodemographic characteristics of the respondents, including age, gender, location (urban or rural), level of education, occupation, marital status, and sexual activity of respondents. The second group refers to the respondents' knowledge about HPV and the source of transmission of HPV.

The pilot questionnaire was sent to 50 respondents, and according to the perceived response, corrections and adjustments were made to the target population and the survey's objectives.

The participation was voluntary; all information was handled in confidence. The participants were provided a detailed clarification of the reasons for the survey and were notified of the actions being taken to protect their anonymity. The questionnaire was anonymous, and participants were free to end the participation at any time, without finishing the questionnaire.

A password-protected computer was used to keep the excel sheets, and a secure lockable cabinet was also used to save filled questionnaires.

Results

The study for knowledge on human papillomavirus and associated factors included 500 respondents, citizens of the Republic of Kosova.

Sociodemographic data of the respondents

Prevalence of adult groups in the study ranged from 21.0% (age group from 18 to 21 years) to 18.8% (age group from 34+ years) (Table 1 and Figure1a). The percentage difference wasstatistically insignificantforp<0.05.

A larger percentage of respondents were from females 60.0%, and 40.0% were males (Table 1b).

The respondents surveyed mostly have completed secondary education - n=301 (60.2%), followed by those with university degree - n=108 (21.6%), and the lowest number were without education - n=24 (4.8%) (Table 1).

N=324 (64.8%) were from the city (urban environment) and n=176 (35.2%) were from the village (Table 1).

26.0% of the respondents were married, 40.4% were singles, 31.4% were in a relationship, other 0.4% and 1.8% were divorced (Table 1).

Approximately half of the respondents - 44.8% were employed, 33.4% were students, and 14.6% were unemployed, 3.0% were still in school and 4.2% had other kind of occupational status (Table1).

Table 1. Overview of the sociodemographic characteristics of the respondents

Age - Years	Count	Percentage
18 - 21	105	21.0
22 -25	101	20.2
26 – 29	100	20.0
30 – 33	100	20.0
34+	98	18.8
Gender		
Male	200	40.0
Female	300	60.0
Education		
No education	24	4.8
Elementary	67	13.4
High School	301	60.2
University	108	21.6
Place of Residence		
Urban	324	64.8
Rural	176	35.2
Marital Status		
Single	202	40.4
Married	130	26.0
Other	2	0.4
In a relationship	157	31.4
Divorced	9	1.8
Occupational Status		
Student	167	33.4
Employed	224	44.8
Unemployed	73	14.6
School	15	3.0
Other	21	4.2

Sexual activity

69.4% of the respondents were sexually active, most of them (51.0%) had only one sexual intercourse in the last year, and 22.8% of them used a condom (Table 2).

Knowledge about Human Papillomavirus

More than half of the respondents knew about HPV - 70.0%, and 29.6% of them did not know; the percentage difference was statistically significant for p<0.05 (Difference test, p=0.0000).

Table 2. Overview of the sociodemographic characteristics of the respondents

Are you sexually active?	Count	Percentage
Yes	347	69.4
No	144	28.8
Missing	9	1.8
How many sexual partners have you had in the past 12 months?		
0	150	30.0
1	255	51.0
2-3	59	11.8
4-5	14	2.8
5+	13	2.6
Missing	9	1.8
Did you use a condom at your last sexual intercourse?		
No	377	75.4
Yes	114	22.8
Missing	9	1.8

No relationship (for p>0.05) was registered between demographic characteristics (age, gender, marital status, sexual activity, number of partners) versus whether HPV was known (Pearson Chi-square: 9.11173,df=4,p=.058374; Pearson Chi-square: 3.63155, df=1, p=.056697; Pearson Chi-square: 6.31422, df=4, p=.176888; Pearson Chi-square: .005119, df=1, p= .942961; Pearson Chi-square: 7.40833, df=4, p=.115829).

There was a correlation(for p<0.05) between sociodemographic characteristics (place of living, level of education, employment status, condom use) and HPV knowledge (Pearson Chi-square: 7.98593, df=1, p=.004715; Pearson Chi-square: 97.5601, df=3, p=0.00000; Pearson Chi-square: 73.8518, df=4, p=.000000; Pearson Chi-square: 8.29637, df=1, p=.003973).

Respondents from urban areas showed 1.7 times (OR = 1.7037 95% CI: 1.1757-2.4688) greater knowledge for HPV than respondents from rural areas.

Respondents with secondary and higher education showed 18 times more knowledge for HPV (OR = 18.1311

95% CI: 8.7465-37.5852) than respondents with primary education.

The employed respondents showed 7 times more knowledge for HPV (OR= 6.8182 95% CI: 3.5464-13.1082) than non-employed respondents.

Students showed 8 times (OR = 7.9895% CI: 4.1057-15.5296) more knowledge than unemployed respondents.

Respondents who used a condom showed 2 times (OR = 1.9015 95% CI: 1.2233-2.9557) more knowledge for HPV than respondents that did not use a condom.

Table 3. Presentation of the knowledge of respondents about HPV

Do you know what human papillomavirus (HPV) is?	Count	Percentage
Yes	350	70.0
No	148	29.6
Missing	2	0.4

In the multiple answers, 42.0% of respondents did not know the way of HPV transmission. 48.6% of respondents knew about transmission through vaginal sex, 34.6% through anal and 30.6% through oral sex; 1.4% (7) of respondents did not answer.

More than half of the respondents, 54.2%, know about the way HPV is transmitted.

Table 4. Presentation of the knowledge of respondents for transmission of HPV

Do you know what human papillomavirus (HPV) is?	Count	Percentage
I don't know	210	42.0
Through vaginal sex	243	48.6
By touching the genitals	37	7.4
Anal sex	173	34.6
Oral sex	153	30.6
Sitting on the WC	56	11.2
During delivery from mother to child	72	14.4
Other	2	0.4
Missing	7	1.4
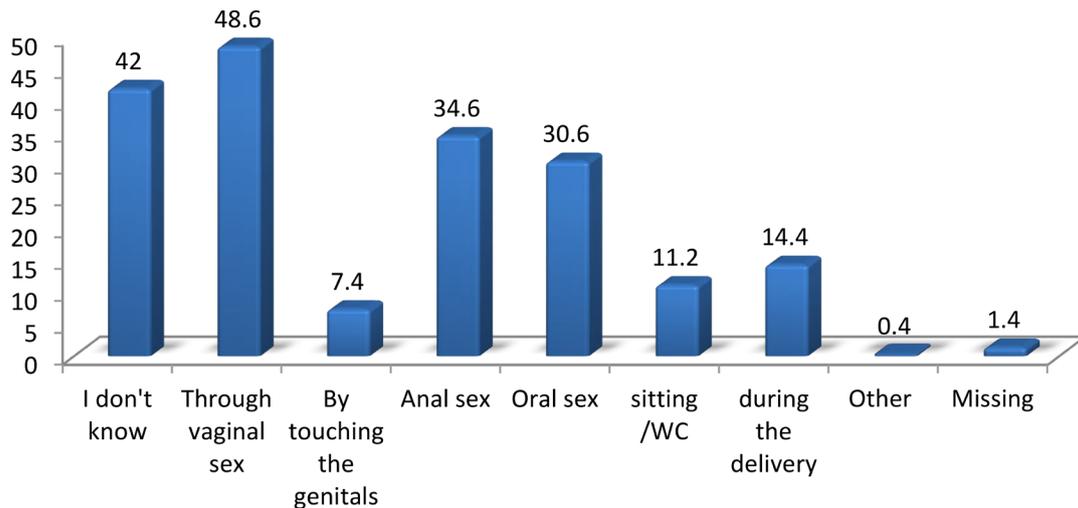
Figure 1. Presentation of the knowledge of respondents for HPV transmission in percentage

No correlation was registered (for p>0.05)between sociodemographic characteristics (gender, marital status, sexual activity, number of partners) and the mode of HPV transmission (Pearson Chi-square: .164483, df=1, p=.685062;Pearson Chi-square: 7.38793, df=4, p=.116754;Pearson Chi-square: .150191, df=1, p=.698353; Pearson Chi-square: 5.20195, df=4, p=.267197).

To the question *Can HPV heal by itself*, the largest number of respondents, 60.8% (n=304), responded I do not know, 25.4% (n=127) of the respondents gave false answer, 12.8% (n= 64) gave true answer and 1.0% (n=5) of the respondents did not answer. The percentage difference between the unknown answer versus the other modes of choice was statistically significant for p<0.05 (Differential test P= 0.0000) (Table 5).

Table 5. Answers of respondents about healing of HPV

Can HPV heal by itself [TRUE]		
I don't know	304	60.8
False	127	25.4
True	64	12.8
Missing	5	1.0

To the question *Can HPV be cured by taking antibiotics*, 59.8% (n=299) responded I do not know, 15.6% (n=78) of respondents chose true option, 23.4% (n=117) false option and 1.2% (n=6) of respondents did not answer. Percentage difference between unknown answer and other options was statistically significant for p<0.05(Difference test P=0.0000) (Table 6).

Table 6. Answers of respondents on the question *Can HPV be cured by taking antibiotics*

Can HPV be cured by taking antibiotics [FALSE]		
True	78	15.6
I don't know	299	59.8
False	117	23.4
Missing	6	1.2

To the question *Can HPV can cause cancer of the cervix/mouth of the womb*, most of the respondents, 58.4% (n=292), did not know. 37.2% (n=186) of respondents gave a correct answer, 2.4% (n=12) incorrect and 2.0% (n=10) did not answer. The percentage difference between the unknown and the options was statistically significant for p<0.05 (Difference test P=0.0000) (Table 7).

Table 7. Answers of respondents about HPV consequences

Can HPV cause cancer of the cervix/mouth of the womb [TRUE]		
I don't know	292	58.4
True	186	37.2
False	12	2.4
Missing	10	2.0

Discussion

To the best of our information, the current survey is the most extensive and more comprehensive research conducted to gather information on the level of knowledge regarding human papillomavirus (HPV) infections and HPV-associated diseases in the Republic of Kosova and to discover the relationship between these factors. Seventy percent of the participants knew what HPV was.

More than half of the respondents, 54.2%, knew how HPV was transmitted. Of these, the most common answer of the respondents, 48.6% was transmission through vaginal sex. 69.4% of the respondents were sexually active. Most of them (51.0%) had only one sexual intercourse in the last year, and 22.8% used a condom during the previous intercourse.

The results showed that most participants knew what HPV was (70%),

but they also revealed that most participants had low or moderate knowledge about HPV, which is a comparable result with earlier studies[20-26]. Numerous studies[20,26-27] have confirmed that if the level of education is higher, then the knowledge of HPV is significantly greater. A Swedish survey[22] has demonstrated that not only educational level but also income is a cause related to knowledge. It would be interesting to explore if income correlates with knowledge. Lacking information could also be a reason for poor knowledge about HPV[21]. Thus, it could be interesting to explore what kind the information is given. The results indicate the extreme necessity for public education that tackles such lack of information amongst this group of topics. A small number of participants in this survey knew that HPV could heal by itself (12.8%), which is similar to other surveys[20,26]. More than half of the participants in this survey knew that HPV was a sexually transmitted infection, which is in agreement with the results from other surveys[20-21,26]. 37.2% of participants in this survey knew that HPV could cause cervical cancer, but 58.4% did not know this. This outcome is similar to numerous other studies[20-21,26]. The deficit knowledge of HPV and the fact that 75.4% of participants did not use a condom at their last intercourse is considered to be severe as HPV is the most common sexually transmitted infection[28], and cervical cancer is caused by HPV[28]. It is fundamental to broaden the knowledge of HPV so that people will understand how to avoid the infection from ever happening and so that young men and women will have protected sexual behavior.

This survey has some possible limitations. Firstly, the survey questions were explored by a cross-sectional study design. Such a strategy prevents the determination of fundamental associations among various factors and results. Secondly, evidence was collected by self-reported questionnaire, and hence, several answers might have described incorrect data. Apart from this criticism, the privacy of the survey might have lowered the deviation in the answers. This research, though, presents significant strengths: firstly, it delivers evidence from a vast number of participants, and this permits investigation of extremely weak relations among variables; secondly, the information was complete; thirdly, the participation percentage was very high, perhaps indicating increased curiosity for this study.

Conclusion

A significant number of the population contributing to this survey had a poor knowledge about HPV. It is crucial to improve the inadequate knowledge about HPV among the people in the Republic of Kosova to change behavior in order to be vaccinated against HPV. As a footstep towards getting a lower rate of cervical cancer and reducing the number of deaths because of the disease, serious efforts in health education are required. The preventive work needs upgrading. To do so, it could be significant to assess which factors affect the knowledge so that young women and all adolescents will get pushed to use protective measures against cervical cancer and have protected sexual behavior.

References

1. zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology. 2009;384(2):260-5.

2. WHO: International Agency for Research on Cancer. Cervical cancer: Estimated incidence, mortality and prevalence worldwide in 2012. In: The GLOBOCAN 2012 Database. Lyon, France: International Agency for Research on Cancer; [Accessed: July 15, 2018]

3. Satterwhite CL, Torrone E, Meites E, et al. Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis 2013; 40:187–93.

4. Bruni L, Barrionuevo-Rosas L, Albero G, et al. Human papillomavirus and related diseases in the world. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Summary Report 27 July 2017. Available from: http://www.hpvcentre.net/statistics/reports/XWX.pdf. Accessed February 2, 2018.

5. Zejnullahu, A.Vj. Prevalence of HPV infection and genotypes in women with normal and abnormal cytological results in Kosovo: clinical and diagnostic impact. Medicus 2017; 22(2): 137–147.

6. Kocjan BJ, Bzhalava D, Forslund O, Dillner J, Poljak M. Molecular methods for identification and characterization of novel papillomaviruses. Clinical Microbiology and Infection. 2015;21(9):808-816

7. de Sanjosé S, Brotons M, Pavón MA. The natural history of human papillomavirus infection. Best Practice & Research. Clinical Obstetrics & Gynaecology. 2018;47:2-13

8. Carifi M, Napolitano D, Morandi M, Dall'Olio D. Recurrent respiratory papillomatosis: Current and future perspectives. Therapeutics and Clinical Risk Management 2015;11:731-738

9. Kelly H, Mayaud P, Segondy M, Pant Pai N, Peeling RW. A systematic review and meta-analysis of studies evaluating the performance of point-of-care tests for human papillomavirus screening. Sexually Transmitted Infections. 2017;93(S4):S36-S45

10. Burd EM. Human papillomavirus and cervical cancer. Clinical Microbiology Reviews 2003;16(1):1-17

11. Ginindza TG, Dlamini X, Almonte M, Herrero R, Jolly PE, Tsoka-Gwegweni JM, et al. Prevalence of and associated risk factors for high risk human papillomavirus among sexually active women, Swaziland. PLoS One 2017;12(1):e0170189

12. Lee CH, Peng CY, Li RN, Chen YC, Tsai HT, Hung YH, et al. Risk evaluation for the development of cervical intraepithelial neoplasia: Development and validation of risk-scoring schemes. International Journal of Cancer 2015;136(2):340-349

13. Lin C, Franceschi S, Clifford GM. Human papillomavirus types from infection to cancer in the anus, according to

sex and HIV status: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2018;18(2):198-206

14. Knowles S. & Parker M. Female Genital Cancer in Kosovo; a situational analysis of breast and cervical cancer. UNFPA 2008.

15. Wolniewicz, E. Cervical cancer - knowledge, prevention and exposure to risk factors among students from various countries. Presented at the ISGE World Congress 2016, Volume: 32, Orlando, USA. https://doi.org/10.31 09/09513590.2016.1150635

16. CDC. Human Papillomavirus (HPV) Questions and Answers. 28 December 2015. Archived from the original on 11 August 2016. Retrieved 11 August 2016.

17. CDC ."What is HPV?". 28 December 2015. Archived from the original on 7 August 2016. Retrieved 10 August 2016.

18. Watson RA. Human Papillomavirus: Confronting the Epidemic-A Urologist's Perspective. Reviews in Urology 2005;7 (3): 135–44.

19. WRHA. Human Papilloma Virus (HPV). 18 November 2019. Retrieved 26 March 2019.

20. Charakorn C, Rattanasiri S, Lertkhachonsuk A-A, Thanapprapasr D, Chittithaworn S, Wilailak S. Knowledge of Pap smear, HPV and the HPV vaccine and the acceptability of the HPV vaccine by Thai women. Asia–Pacific Journal of Clinical Oncology 2011; 7(2): 160–167.

21. Juntasopeepun P, Davidson PM, Suwan N, Phianmongkhol Y, Srisomboon, J. Human papillomavirus vaccination intention among young women in Thailand. Asian Pacific Journal of Cancer Prevention 2011; 12(12): 3213-9.

22. Dahlström A L, Sundström K, Young C, Lundholm C, Sparén P, Tran NT. Awareness and knowledge of human papillomavirus in the swedish adult population. Journal of Adolescent Health 2012; 50(2): 204–206. doi:10.1016/j.jadohealth.2011.05.009

23. Di Guiseppe G, Abbate R, Liguori G, Albano L, Angelillo IF. Human papillomavirus and vaccination: knowledge, attitudes, and behavioral intention in adolescents and young women in Italy. British Journal of Cancer 2008: 99(2): 225-229.

24. Nøhr B, Munk C, Tryggvadottir L, Sparén P, Tran NT, Nygård M et al. Awareness of humanpapilloma virus in a cohort of nearly 70,000 women from four Nordic countries. Acta Obstetricia et Gynecologica 2008; 87(10), 1048-1054.

25. Rashwan H, Lubis SH, Ni KA. Knowledge of cervical cancer and acceptance of HPV vaccination among secondary school students in Sarawak, Malaysia. Asian Pacific Journal of Cancer Prevention 2009; 12(7): 1837-1841.

26. Tiro JA, Meissner HI, Kobrin S, Chollette V. What do women in the U.S. know about human papillomavirus and cervical cancer. Cancer Epidemiology Biomarkers & Prevention 2007; 16(2): 288-294.

27. Gerend MA, Shepherd JE. Correlates of HPV knowledge in the era of HPV vaccination: A study of unvaccinated young adult women. Women & Health 2011; 51(1), 25–40.

28. World Health Organization. Human papillomavirus (HPV). 2011. Retrieved 15th February, 2013, from http://www.who.int/nuvi/hpv/en/

PUBLIC HEALTH

ATTITUDE AND PRACTICE ON HUMAN PAPILLOMAVIRUS, HUMAN PAPILLOMAVIRUS VACCINE AND ASSOCIATED FACTORS AMONG POPULATION IN THE REPUBLIC OF KOSOVA

Ernad Kosumi[1], Milaim Kosumi[2], Mome Spasovski[3]

[1] Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Public Health Doctoral Studies, Republic of North Macedonia
[2] "The University of Medicine Tirana, Faculty of Technical Medical Sciences, Tirana, Albania
[3] Faculty of Medicine, Ss. Cyril and Methodius University in Skopje; Institute of Social Medicine, Republic of North Macedonia

Abstract

Approximately 50–80% of sexually active women are exposed to at least one HPV variant during their lifetime. Based on the 2015 annual report of the National Institute of Public Health of Kosova, 68 new cases of cervical cancer were detected. The aim of the study was to investigate the attitude and practice regarding Human Papillomavirus (HPV) infections and the Human Papillomavirus vaccine in the population of the Republic of Kosova. Material and methods: A cross-sectional survey was conducted to assess the knowledge concerning HPV infections among the population aged 18 to 35+ years. The study was conducted during the period of time June 2021 –to August 2021. The sample included 500 participants. The questionnaire was anonymous, and participants were free to end their participation any time, without completing the questionnaire. Results: Regarding the claim „The HPV vaccine is safe", more than half of the respondents, respectively58.6% declare that they do not know, 33.4% of the respondents agree, 6.0% gave an incorrect answer / disagree, and 2.0% did not answer. The percentage difference between the unknown and correct answers is statistically significant for p<0.05. Conclusion: This study found out that the attitude towards the HPV vaccine among people of the Republic of Kosova is low to moderate. HPV vaccines should be included in the national immunization programs, since there is not still established national vaccination program for HPV vaccination.

ЈАВНО ЗДРАВЈЕ

СТАВОВИ И ПРАКТИКИ ЗА ХУМАН ПАПИЛОМАВИРУС, ВАКЦИНА ЗА ХУМАН ПАПИЛОМАВИРУС И ПОВРЗАНИ ФАКТОРИ МЕЃУ НАСЕЛЕНИЕТО ВО РЕПУБЛИКА КОСОВО

Ернад Косуми[1], Милаим Косуми[2], Моме Спасовски[3]

[1] Универзитет Св. Кирил и Методиј во Скопје, Медицински факултет, Докторски студии по јавно здравје, Република Северна Македонија,
[2] „Универзитет за медицина" Тирана, Факултет за технички медицински науки, Тирана, Албанија
[3] Медицински факултет, Универзитет Св. Кирил и Методиј во Скопје; Институт за социјална медицина, Република Северна Македонија

Извадок

Приближно 50-80% од сексуално активните жени се изложени на барем една варијанта на ХПВ во текот на нивниот живот. Врз основа на годишниот извештај на Националниот институт за јавно здравје на Косово за 2015 година, откриени се 68 нови случаи на рак на грлото на матката. Целта на студијата беше да се истражи ставот и практиката во однос на инфекциите со хуман папиломавирус (ХПВ) и вакцината против хуманиот папилома вирус кај населението на Република Косово. Материјал и методи: Спроведена е студија на пресек за да се проце-ни знаењето за ХПВ инфекциите кај популацијата на возраст од 18 до 35+ години. Студијата е спроведена во периодот јуни 2021 година до август 2021 година. Примерокот опфати 500 учесници. Прашалникот беше анонимен, а учесниците можеа слободно да го прекинат своето учество во секое време, без да го пополнат прашалникот. Резултати: Во однос на тврдењето „ХПВ вакцината е безбедна"повеќе од половина од испитаниците, односно 58,6% се изјасниле дека не знаат, 33,4% од испитаниците се согласуваат, 6,0% дале неточен одговор/ не се согласуваат, а 2,0% не одговориле. Процентуалната разлика помеѓу непознатите и точните одговори е статистички значајна за p<0,05. Заклучок: Оваа студија покажа дека односот кон ХПВ вакцината кај жителите на Република Косово е низок до умерен. ХПВ вакцините треба да бидат вклучени во националните програми за имунизација, бидејќи сѐуште не постои воспоставена национална програма за вакцинација против ХПВ.

Introduction

Human papillomavirus (HPV) infection is an increasing public health threat worldwide due to the morbidity, mortality, and expenses associated with cervical, vulvar, anal, penile, head, and neck cancers [1,2]

It is generally accepted that the main risk factors are related to the sexual behavior, for instance, early age of sexual activity at onset, number of lifetime sexual partners, and lack of consistency in condom usage[3-6].

Around 50–80% of sexually active women are exposed to at least one HPV variant during their lifetime[7]. The continuing infections with certain types of HPV is related to advancing HPV-associated infections (non-malignant and malignant)[8].

Cervical cancer is the second most common cancer in women worldwide and the third most common cancer worldwide regardless gender[9-11]. Despite the knowledge on HPV prevention and the causes of HPV, around 274.000 deaths from cervical cancer are recorded each year and above 85% of them are in developing nations[12]. Etiologically, HPV is responsible for90-93% of cases with cancer of the anus, 12-63% of cases with cancer of the oropharynx, 36-40% of cases with cancer of the penis, 40-64% of cases with cancer of the vagina and 40 -51% of cases with cancer of the vulva[13]. About 70% of cervical cancer are caused by HPV variants 16 and 18[14]. Genital warts are benign tumors most frequently triggered by low-risk varieties of HPV [15]. More than 90% of genital warts are associated with low-risk HPV varieties 6 and 11[15].

Data on the cancer cases in Kosovo are not available since a national cancer bureau is in establishing process, and documentation procedures have yet to be formalized. Nevertheless, incidence data offered for 2013 registers 224 breast and 27 cervical cancer cases, although these will undervalue, particularly in the case of cervical cancer. A study guide by Knowles and Packer in 2008 estimated that at least 50% of female genital cancer cases in Kosova are not officially recorded[16]. Based on the 2015 Annual Report of the National Institute of Public Health of Kosova, 68 new cases of cervical cancer were identified in Kosova within the 2015. Another cross-sectional survey in 2016 led by Romejko-Wolniewicz et al. reported a high incidence rate of cervical cancer in Kosova ASR(W)=23.8 and a high mortality rate as well ASR(W)=9.2 [17].

HPV vaccination has provided optimism on decreasing the incidence of infections, morbidity, and mortality from HPV-associated cancer. Recent recommendations include the administration of the vaccine to adolescents and adult females aged 9 to 26 years as well as eligible males of similar age in order to decrease their probability of developing genital warts and avoid cancer[18].Three prophylactic HPV vaccines for high-risk HPV varieties are available in numerousnations worldwide: 2-, 4- and 9-valent vaccines. The vaccines are intended to be administrated, before the first sexual intercourse when possible. The 4-valent vaccine against HPV-6, HPV-11, HPV-16, and HPV-18 was licensed in 2006; the 2-valent vaccine anti HPV-16 and HPV-18 in 2007, and the 9-valent vaccine, anti HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58) in 2014[19]. The first dose is usually recommended at ages 11–12 years old. Immunization

can begin at the age of 9. Only two doses are required if the first dose was delivered before the 15th birthday. Teenagers and young adults who start the vaccination series later, at ages 15 to 26, require three doses of HPV vaccine. Children aged 9 to 14 who have receivedtwo doses of HPV vaccine not more than five months apart need a third dose. Three doses are also suggested for individuals aged 9 to 26 years who have a weakened immune system. Vaccination is not suggested for every person older than age 26 years. Certain adults aged 27 to 45 years who have not been previously vaccinated may opt to get the HPV vaccine after discussing with their doctor their threat from the new HPV diseases and the potential benefits of vaccination for them. HPV vaccination at this age range delivers is less beneficial because more people of this age range have been exposed to HPV[20].

The aim of the study is to examine the attitude and practice regarding Human Papillomavirus (HPV) infections and the Human Papillomavirus vaccine among population in the Republic of Kosova.

Materials and methods

A cross-sectional study assessed the knowledge of HPV infection amongthe residents aged 18 to 35+ years. The investigation was conducted from June 2021 to August 2021.

The sample was obtained based on convenience sampling. From the data of the participants in the population awareness group were collected using a pre-designed questionnaire through an online version: distribution in Google Form via social media.

A study explanation was offered each time it was required. Each part of the questionnaire offers the study's aims and actions for protective privacy. Participation was voluntary, and all data were kept confidential.

Criteria for inclusion was the age of the participants from 18 to 35+ years. This age group was selected because it is the part of the population that are highly exposed to HPV infection and HPV-associated diseases and a critical part of the population that decides to have their children vaccinated against HPV. The latter derives from the fact that the individuals subject to mandatory HPV vaccination are 12-year-old girls who do not decide for themselves and whose vaccination requires parental consent.

Considering the fact that there is no proven questionnaire is in the Republic of Kosova, A series of questions based on Knowledge, Attitude, and Practice (KAP) study in conditions with a related socio-cultural environment in the Republic of Kosova to explore KAP about HPV. A pilot survey was also conducted to test the validity and reliability of the questionnaire. The questionnaire strategy is based on the study and the literature review of the attitude and practice concerning HPV and the HPV vaccine.

The questionnaire was organized into two parts. The first part of the questions presents evidence of the respondent's socio-demographic characteristics, including age, gender, location (urban or rural), level of education, occupation, marital status, and sexual activity. The second part discusses the respondents' attitudes and practices regarding HPV and the HPV vaccine.

The questionnaire was piloted on 50 respondents, and in accordance with the feedback, improvements and modifications were made to the target residents and the study's purposes.

Written approval was not required to protect confidentiality. Participants were set free to terminate their participation without completing the questionnaire.

Results are presented in summary. The data collected were used only for the purpose of this survey.

A password-protected computer was used to store the Excel spreadsheets and to save filled questionnaires.

Results

In the study for attitude and practice on Human papillomavirus and associated factors took part 500 respondents, residents of the Republic of Kosova.

Socio-demographic data of the respondents

The Prevalence of adult groups in the study ranged from 21.0% (age group from 18 to 21 years) to 18.8% (age group from 34+ years). The percentage difference is statistically insignificant for $p<0.05$.

A larger percentage of respondents are female 60.0%, and 40.0% are male.

The respondents surveyed mostly have secondary education n=301 (60.2%), followed by university n=108 (21.6%), with the lowest incidence without education n=24 (4.8%) (Table 1).

N=324 (64.8%) are from the city (urban environment), and n=176 (35.2%) are from the village.

Next, 26.0% of the respondents are married, 40.4% are singles, 31.4% are in a relationship, other 0.4% and 1.8% are divorced. Approximately half of the respondents - 44.8% are employed, 33.4% are students, 14.6% are unemployed, 3.0% are still in school, and 4.2% have some other professional status.

Attitude and Practice on Human Papilloma Virus and Human Papilloma Virus Vaccine

According to the opinion of the respondents to the claim "HPV vaccine is safe", more than half of the respondents 58.6% answer with 'do not know', 33.4% of respondents agree, 6.0% gave an incorrect answer / disagree, and 2.0% of respondents did not answer. The percentage difference between the unknown answer versus the correct answer is statistically significant for $p<0.05$(Difference test. p=0.0000) (Table 1).

Table 1. Presentation of the respondent's answers to the claim „HPV vaccine is safe".

The HPV vaccine is safe.[TRUE]	Count	%
Agree	167	33.4
Disagree	30	6.0
I do not know	293	58.6
Missing answers	10	2.0

A relationship was found between socio-demographic characteristics (age, sex, place of residence, education level, employment status, marital status, condom use) versus attitude to the claim" HPV vaccine is safe" for p<0.05(Pearson Chi-square: 47.6549, df=8, p=.000000; Pearson Chi-square: 9.70680, df=2, p=.007802; Pearson Chi-square: 6.54538, df=2, p=.037904; Pearson Chi-square: 46.9912, df=6, p=.000000; Pearson Chi-square: 36.2230, df=8, p=.000016; Pearson Chi-square: 20.0002, df=8, p=.010335; Pearson Chi-square: 18.9114, df=2, p=.000078).

Based on the respondents opinion-on the statement "I may be infected with HPV in the future",46.6% of the respondents answered "I do not know",41.2% of the respondents gave a correct answer/agree, 9.2% gave an incorrect answer / disagree, and 3.0% of the respondents do not give an answer. The percentage difference between agreeing versus I do not know is statistically insignificant for p>0.05(Difference test. p=0.0853) (table 2).

Table 2. Presentation of respondent's answer to the claim „I may be infected by HPV in the future".

I may be infected by HPV in the future.[TRUE]	Count	%
Agree	206	41.2
Disagree	46	9.2
I do not know	233	46.6
Missing answers	15	3.0

According to the opinion of the respondents' to the claim (to which only females answered) "I may have cervical cancer in the future", more than half of the respondents from females 71.3% answered "I do not know", 7.3% answered that the premise is correct/agreed, 19.0% gave an incorrect answer / disagreed, and 2.4% of respondents do not give an answer. The percentage difference between the unknown answer and the correct answer is statistically significant for p<0.05(Difference test. p=0.0000) (table 3).

Table 3. Presentation of respondent's answer to the claim „I may have cervical cancer in the future".

I may have cervical cancer in the future. [TRUE] Count	Count	%
Agree	22	7.3
Disagree	57	19.0
I do not know	214	71.3
Missing	7	2.3

No relationship was registered between socio-demographic characteristics (age, sex, place of residence, level of education, employment status, marital status, sexual activity, and condom use) versus attitude to the claim "I may have cervical cancer in the future" for p>0.05(Pearson Chi-square: 2.78025, df=8, p=.947382; Pearson Chi-square: .493238, df=2, p=.781438; Pearson Chi-square: .106401, df=2, p=.948190; Pearson Chi-square: 2.53492, df=6, p=.864540; Pearson Chi-square: 6.01943, df=8, p=.645055; Pearson Chi-square: 9.26466, df=8, p=.320464; Pearson Chi-square: 4.60279, df=2, p=.100119; Pearson Chi-square: 3.11747, df=2, p=.210402).

Furthermore only 8.8% of the respondents would give the vaccine to their daughter, 28.0% would not, 62.0% answered "I do not know," and 1.2% did not answer. The percentage difference between not knowing whether to give the vaccine versus the remaining response modules is statistically significant for p<0.05(Difference test. p=0.0000) (table 4).

Table 4. Presentation of respondent's answer to the question „Would you give your daughter the HPV vaccine".

Would you give your daughter the HPV vaccine?	Count	%
Yes	44	8.8
No	140	28
I do not know	310	62
Missing answers	6	1.2

As can be seen on the table below 97% of the respondents did not receive the HPV vaccine, 2.2% did not answer, and 0.8% received the HPV vaccine. The percentage difference between "not receiving the vaccine" versus "yes" is statistically significant for p<0.05(Difference test. p=0.0000) (table 5).

Table 5. Presentation of respondent's answer to the question „Received HPV vaccine".

Received the HPV vaccine?	Count	%
Yes	4	0.8
No	485	97.0
Missing answers	11	2.2

Discussion

Based on our information, the present study is the most widespread and more complete investigation guided to collecting data on the attitude toward Human Papillomavirus (HPV) infections and the HPV vaccine as well as to discover the associated factors among the population in the Republic of Kosova.

Regarding the claim " The HPV vaccine is safe", more than half of the respondents (58.6%) do not have a clue about it, and 6.0% of the respondents gave an incorrect answer / disagree, while only 33.4% of the respondents agreed that the HPV vaccine is safe. Skepticism concerning the safety and efficacyof the vaccine is closely related to the survey conclusions guided by Garbutt JM *et al.* [21].

Currently, the HPV vaccine is confirmed to be safe and effective with no consequences which have been stated for years[22]. HPV vaccination could cause minor consequences fora few recipients, such as muscle pain and headaches[23].Highlighting the safety of HPV vaccination could help decrease the obstacles among the population of the Republic of Kosova toward receiving the vaccines.

When asked about the opinion of the respondents on the statement "I may be infected with HPV in the future", 41.2% of the respondents gave a correct answer/agree, and 46.6% of the respondents answered "I do not know", however only 0.8%of the sampling population received the HPV vaccine.

As a result of the KAP framework, it is well proven that knowledge frequently plays a significant role in healthcare for improving their attitudes and even possible uptake of the HPV vaccine. With a greater level of knowledge, citizens will sense optimistic attitudes, and with additional positive attitudes, respondents will happen to be more inspired to practice prevention associated with HPV-associated diseases. Therefore, more efficient communication policies among physicians and the general public must distribute correct and detailed information[24].

To the opinion of the respondents on the claim (to which only females answered) "I may have cervical cancer in the future", more than half of the respondents, females (71.3%) answered "I do not know". In comparison, only 7.3% answered that the premise is correct/agreed. Community education regarding cervical cancer is low. Traditional rules often stop women from talking or pursuing remedies if they do not have any indications. Women can be examined at local health centers, but it is necessary to be consulted with a regional hospital for curing. Consequently, there is a necessity for a health-education program regarding cervical cancer that includes the media through different channels; such a program might have the major impact.

Ordering the vaccine for youths usually demands parental permission, the highest challenges to HPV vaccine approval are parental knowledge, attitudes, and beliefs[25,26]. Many parents do not recognize the rush to target youths, but this is the most valuable moment to vaccinate them[26,27].As is shown in table 5, only 8.8% of the respondents would give

the vaccine to their daughter, 28.0% would not, and 62.0% answered with "I do not know".

One study has revealed that most of the parents believe that their child is too young and would not start sexual activity for some years to come[26,28,29]. Moreover, some parents believe vaccinating their children against an STI delivers a message that it is acceptable or expect them to become sexually active [28].

Our findings suggest that family doctors should take an active role in distributing health information regarding HPV vaccination in the future. Recent surveys in Italy[30-32] and Argentina[33] showed that people who took information from doctors and medical institutions tended to have improved knowledge and greater perceived demand for additional information regarding the HPV vaccine. Throughout the KAP framework, it is well recognized that knowledge frequently plays a key role in health care for enhancing their attitudes and even possible uptake of HPV vaccination. Once an educational intervention was done, 20% of those who initially did not intend to take the vaccine were ready to have their youngsters vaccinated. Their survey caused us to be conscious of the significance of community education and organizing our patients for this possible intervention.

Regardless of confirmed safety and efficiency, HPV vaccine uptake percentages keep far lower than Healthy People's aim and lower than other vaccines suggested for youths [34,35]. As shown in Table 5, 97% of the respondents did not receive the HPV vaccine, 2.2% did not answer, and 0.8% received the HPV vaccine. Furthermore, while evaluating HPV vaccine uptake, several surveys discovered that a person's gender, race, ethnicity, and socioeconomic condition affected vaccination uptake percentages[36,37]. Reaching the greatest potential HPV vaccination coverage percentage not simply has the possibility to reduce HPV occurrence and related cancer mortality percentages but can also create herd immunity as well. The higher the number of people in a society who are immune to HPV, the less likely the people that are not immune to obtain or get in touch with HPV. Although the coverage is presently inferior for boys than girls, and male HPV vaccination is yet arguable in some circles, the latest survey recommended that an intensified attempt to vaccinate boys is expected to protect more individuals from HPV-related diseases for the same cost[38].

This study has several potential limitations. Firstly, the study questions were investigated by a cross-sectional study design. Such an approach avoids determining essential relations between numerous factors and results. Secondly, the data were collected using a self-reported questionnaire, so there is possibility that several answers could have contained inaccurate information.

Apart from this criticism, the privacy of the survey might have lowered the deviation in the answers.

This investigation, though, presents important strengths: first, it brings evidence from a considerable number of participants, and this permits research of enormously weak associations between variables; second,

the evidence was complete; third, the participation rate was very high, possibly signifying increased interest for this survey.

Conclusions

This study found that the attitude toward the HPV vaccine among people of the Republic of Kosova was low to moderate and that the vaccine uptake was only 0.8%, which was significantly associated with attitude toward HPV, the vaccine, and other factors.

HPV vaccines should be included in national immunization programs, as there is not established national immunization program on HPV vaccination yet.

Consequently, for the effective application of the HPV vaccination in the National Immunization Program of Kosova, health care workers, especially in primary care, are the main component in boosting the vaccination and its acceptability, given that they have an overall impact on the health behavior of their patients.

References

1. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, et al. Global burden of human papillomavirus and related diseases. Vaccine. 2012;30: F12–F23.

2. Giuliano AR, Nyitray AG, Kreimer AR, Pierce Campbell CM, Goodman MT, Sudenga SL, et al. EUROGIN 2014 roadmap: Differences in human papillomavirus infection natural history, transmission and human papilloma-virus-related cancer incidence by gender and anatomic site of infection. Int J Cancer 2015;136: 2752–2760.

3. Veldhuijzen NJ, Snijders PJ, Reiss P, Meijer CJ, van de Wijgert JH. Factors affecting transmission of mucosal human papillomavirus. Lancet Infect Dis 2010;10: 862–874.

4. Oakeshott P, Aghaizu A, Reid F, Howell-Jones R, Hay PE, Sadiq ST, et al. Frequency and risk factors for prevalent, incident, and persistent genital carcinogenic human papillomavirus infection in sexually active women: community based cohort study. BMJ. 2012;344: e4168.

5. Roset Bahmanyar E, Paavonen J, Naud P, Salmerón J, Chow SN, Apter D, et al. Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial. Gynecol Oncol 2012;127: 440–450.

6. Jing L, Zhong X, Zhong Z, Huang W, Liu Y, Yang G, et al. Prevalence of human papillomavirus infection in Guangdong Province, China: a population-based survey of 78,355 women. Sex Transm Dis 2014;41: 732–738.

7. Keam SJ, Harper DM. Human papillomavirus types 16 and 18 vaccine (recombinant, AS04 adjuvanted, adsorbed) [Cervarix] Drugs. 2008; 68:359–72.

8. Moscicki A, Schiffmanb M, Kjaer S, Villa LL. Updating the natural history of HPV and anogenital cancer. Vaccine. 2006;24(Suppl. 3): S42–51.

9. WHO. Report of the consultation on human papillomavirus vaccines. WHO Immunization, Vaccine and Biologicals, Geneva. Available at: www.who.int/vaccines-document/2005. Accessed on: 15th June 2013.

10. Joseph ET, Myriam CD, Lyndsay AR, Maaike D, Eduardo LF. Epidemiology and burden of HPV infection and related disease: Implication for prevention strategies. Preventive Medicine 2011; 53:12-21.

11. McCusker SM, Macqueen I, Lough G, MacDonald AI, Campbell C, Graham SV. Gaps in detailed knowledge of human papillomavirus (HPV) and the HPV vaccine among medical students in Scotland. BMC Public Health 2013; 13: 264.

12. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). (2010).

13. Anil KC. Beyond cervical cancer: Burden of other HPV-related cancers among men and women. Journal of Adolescent Health 2010; 46:20- 26

14. Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006;24(Suppl. 3): S3/26–34..

15. Lacey CJ. Therapy for genital human papillomavirus-related disease. J Clin Virol. 2005;32(Suppl. 1): S82–90.

16. Knowles S. & Parker M. Female Genital Cancer in Kosovo; a situational analysis of breast and cervical cancer. UNFPA 2008.

17. Wolniewicz E. Cervical cancer - knowledge, prevention and exposure to risk factors among students from various countries. Presented at the ISGE World Congress 2016, Volume: 32, Orlando, USA.

18. Centers for Disease Control and Prevention (CDC). Recommendations on the Use of Quadrivalent Human Papillomavirus Vaccine in Males - Advisory Committee on Immunization Practices (ACIP), 2011. MMWR Morb Mortal Wkly Rep 60: 1705–1708.

19. Human papillomavirus vaccines. WHO position paper, May 2017.Wkly Epidemiol Rec 2017;92(19):241–68.

20. HPV vaccination: What Everyone should know [Internet]. Cdc.gov. 2022 [cited 2022 Mar 26]. Available from: https://www.cdc.gov/vaccines/vpd/hpv/public/index.html

21. Garbutt JM, Dodd S, Walling E, Lee AA, Kulka K, Lobb R. Barriers and facilitators to HPV vaccination in primary care practices: a mixed methods study using the Consolidated Framework for Implementation Research. BMC family practice 2018;19(1):53.

22. Scientific Committee on Vaccine Preventable Diseases. Recommendation on the use of human papillomavirus (HPV) vaccine. Hong Kong: Department of Health; 2013 Mar [accessed 2017 Jul 30].

23. Department of Health. Fact sheet on human papillomavirus

(HPV) vaccine for health care professionals. Hong Kong: Department of Health; 2007 Mar 20 [accessed 2017 Jul 30.

24. Venezuela RF, Monetti MS, Kiguen AX, Frutos MC, Mosmann JP, Cuffini CG. Knowledge of the general community in Cordoba, Argentina, on Human Papilloma Virus Infection and its prevention. Asian Pac J Cancer Prev 2016;17(5):2689–94.

25. Juedin P, Liveright E, Carmen M, Perkins R. Race, ethnicity and income as factors for HPV vaccine acceptance and use. Human Vaccines &Immunotherapeutics. 2013; 9(7):1413-1420.

26. Young J, Bernheim R, Korte J, et al. Human papillomavirus vaccination recommendation may be linked to reimbursement: a survey of Virginia family practitioners and gynecologists. Journal of Pediatric & Adolescent Gynecology. 2011; 24:380–5.

27. HPV also known as human papillomavirus. Centers for Disease Control and Prevention Website. https://www.cdc.gov/vaccines/parents/diseases/teen/hpv-indepth-color.pdf. Updated July 2015. Accessed December 29, 2016.

28. Hendry M, Lewis R, Clements A, Damery S, Wilkinson C. HPV? Never heard of it!: a systematic review of girls' and parents' information, views and preferences about human papillomavirus vaccination. Vaccine. 2013; 31(45):5152-5167.

29. Trim K, Nagji N, Elit L, Roy K. Parental knowledge, attitudes, and behaviors toward human papillomaviruses vaccination for their children: a systematic review from 2001 to 2011. Obstetrics and Gynecology International. 2012; 2012:1-12. doi:10.1155/2012/921236.

30. Bianco A, Pileggi C, Iozzo F, Nobile CG, Pavia M. Vaccination against human papilloma virus infection in male adolescents: knowledge, attitudes, and acceptability among parents in Italy. Human Vaccines &Immunotherapeutics. 2014 Sep 2;10(9):2536–42.

31. Napolitano F, Napolitano P, Liguori G, Angelillo IF. Human papillomavirus infection and vaccination: Knowledge and attitudes among young males in Italy. Human Vaccines &Immunotherapeutics. 2016 Jun 2;12(6):1504–10.

32. Di Giuseppe G, Abbate R, Liguori G, Albano L, Angelillo IF. Human papillomavirus and vaccination: Knowledge, attitudes, and behavioural intention in adolescents and young women in Italy. British journal of cancer 2008;99(2):225–9.

33. Venezuela RF, Monetti MS, Kiguen AX, Frutos MC, Mosmann JP, Cuffini CG. Knowledge of the general community in cordoba, argentina, on human papilloma virus infection and its prevention. Asian Pac J Cancer Prev. 2016;17(5):2689–94.

34. Isidean S, Tota J, Gagnon J, Franco E. Human papillomavirus vaccines: key factors in planning cost effective vaccination programs. Expert Review Vaccines. 2014:1-15.

35. National Institutes of Health Website. Recurrent respiratory papillomatosis or laryngeal papillomatosis. http://www.nidcd. nih.gov/health/voice/pages/ laryngeal.aspx. Updated April 1, 2011. Accessed December 29, 2016.

36. Wong K, Do Y. Are there socio-economic disparities in women having discussions on human papillomavirus vaccine with health care providers? BioMed Central Women's Health 2012; 12(33):1-7.

37. Ge S, Gong B, Cai X, Yang X, Gan X, Tong X, et al. Prevent cervical cancer by screening with reliable human papillomavirus detection and genotyping. Cancer Medicine 2012;1(1):59-67.

38. Ryser M, McGoff K, Herzog D, Sivakoff D, Myer E. Impact of coverage dependent marginal costs on optimal HPV vaccination strategies. Epidemics 2015; 11:32-47.

PUBLIC HEALTH

ORGANIZATION OF HEALTH CARE OF ELDERLY PEOPLE IN THE CITY OF SKOPJE - CONDITIONS AND NEEDS

Nadica Totikj[1], Elena Kjosevska[1], Vesna Velikj Stefanovska[2]

[1] Institute of Public Health of the Republic of North Macedonia, Skopje, Republic of North Macedonia
[2] Institute of Epidemiology with Biostatistics and Medical Informatic;, Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Republic of North Macedonia

Abstract

The main goal of the paper was to understand the attitudes of the elderly in the city of Skopje regarding health care, organization and functioning of the health system for ensuring healthy and active aging. Methods: A descriptive-analytical method was used to present the results of the study (cross-sectional study) conducted on the territory of the city of Skopje in the period March-April 2019. A total of 350 respondents aged 65+ were included. A questionnaire containing 53 questions was developed and was designed to suit the age and sex of the respondents. The questionnaire was divided into four parts: first part - general and demographic characteristics; second part - health care; third part - mobility of the elderly; fourth part - conditions and lifestyle. The statistical analysis was performed with Windows 7.0 and SPSS, version 14. A statistical significance was used for two-way tests with a significance level of $p < 0.05$. Results: Of the total of 350 respondents, 133 (38%) were male, and 217 (62%) were female, with a sex ratio of 0.61 : 1. According to the answers given by the respondents in our sample, 50 (37.9%) men and 124 (57.14%) women had chronic diseases. A total of 260 (75.6%) respondents stated that they were satisfied with the health information system „My Appointment" ("MojTermin"). In terms of sex, 101 (78.9%) men and 159 (73.6%) women were satisfied with „My Appointment". A total of 84 (24.4%), 27 (21.1%) men and 57 (26.4%) women, stated that they were dissatisfied with this service. Forty-seven (47.5%) respondents said that discrimination in health care was done by a doctor, 43 (43.4%) said it was done by a nurse and according to 9 (9.1%) respondents, discrimination was done by both the doctor and the nurse. The results obtained showed that more often highly educated people recognized discrimination and abuse by the health personnel than people with lower level of education. Conclusion: The elderly exercise partially their rights to social and health care. The elderly would like to expand the opportunities for social and health benefits. In the self-assessment of the health condition, most of the elderly perceived their health status to be relatively good. The analysis showed a higher percentage of chronic illnesses in women than in men. There is a correlation between active and healthy aging and socioeconomic conditions in which the elderly live.

ЈАВНО ЗДРАВЈЕ

ОРГАНИЗАЦИЈА НА ЗДРАВСТВЕНАТА ЗАШТИТА НА СТАРИТЕ ЛИЦА НА ТЕРИТОРИЈА НА ГРАД СКОПЈЕ – СОСТОЈБИ И ПОТРЕБИ

Надица Тотиќ[1], Елена Ќосевска[1], Весна Велиќ-Стефановска[2]

[1] Институт за јавно здравје на Република Северна Македонија, Скопје, Република Северна Македонија
[2] Институт за епидемиологија со биостатистика и медицинска информатика, Универзитет Св. Кирил и Методиј во Скопје, Медицински факултет, Република Северна Македонија

Извадок

Целта на трудот беше да се согледаат ставовите на старите лица на територија на град Скопје во врска со здравствената заштита и да се добијат одговори за нивните потреби и мислење за организацијата и функционирањето на здравствениот систем. Методи: Беше користен дескриптивно-аналитички метод на работа, со приказ на резултати од истражување (студија на пресек) спроведено на територијата на град Скопје во периодот март-април 2019 година. Беа опфатени вкупно 350 испитаници на возраст од 65+ години. Беше изработен прашалник кој содржеше 53 прашања и беше дизајниран да одговара на возраст и полот на испитаниците. Прашалникот беше поделен на четири дела: прв дел - општи и демографски карактеристики; втор дел - здравствена заштита; трет дел - мобилност на старото лице; четврти дел - услови и начин на живот. Статистичката анализа беше извршена со Windows 7.0 и SPSS, верзија 14. Статистичка значајност беше искористена за двонасочни тестови со ниво на значајност од $p < 0.05$. Резултати: Во студијата беа опфатени 133 (38%) мажи и 217 (62%) жени, со сооднос на полот 0,61 : 1. Според добиените изјави од испитаниците во примерокот, 50 (37.9%) од анкетираните мажи и 124 (57,14%) од анкетираните жени имале хронични заболувања. Од испитаниците, 260 (75,6%) изјавиле дека се задоволни од услугите „Мој термин". Во однос на полот, задоволни од „Мој термин" биле 101 (78,9%) од мажите и 159 (73,6%) од жените во примерокот. Вкупно 84 (24,4%) испитаници изјавиле дека се незадоволни од оваа услуга, и тоа 27 (21,1%) мажи и 57 (26,4%) жени. Кај 47 (47,5%) од испитаниците дискриминација при здравствената заштита направил доктор, кај 43 (43,4%) тоа го направила медицинска сестра, а кај 9 (9,1%) причина биле и докторот и медицинската сестра. Добиените резултати покажале дека најчесто високообразованите лица многу почесто ја препознавале дискриминацијата и злоупотребата од страна на здравствен персонал отколку лицата со пониско образование. Заклучок: Постарите лица делумно ги остваруваат правата од здравствена заштита. Постарите лица би сакале да ги прошират можностите за здравствени придобивки. Во самопроценката на здравствената состојба, најголем дел од постарите лица велат дека нивната здравствена состојба е релативно добра. Постои корелација помеѓу активното и здравото стареење и социоекономските услови во кои живеат постарите лица.

Introduction

People in the world live longer. The 20th century was marked as a revolution in longevity. The average life expectancy has increased by 20 years from 1950 to 66 years and it is expected to continue for another 10 years until 2050. Today, for the first time in history, more people have the opportunity to live 60 years or more. Aging decreases the power of adaptation to the environment which leads to development of a risk of disease or death.[1]

The extension of the life expectancy and increase in the participation of the elderly in the total population is an important fact that significantly increases the interest in research[2]. By 2050 globally, the population over the age of 60 is expected to reach 2 billion, unlike 2015 when it was 900 million.[3] Declining birth rates and extended life expectancy are changing the demographic picture in countries world wide[4] . In terms of age structure, as in the world, the Macedonian population is ageing as it is happening in all parts of the world. In the Republic of North Macedonia, of the total population, 264,964 were elderly people in 2015, while the number of elderly people in 2020 increased to 302,940.[5] In the Republic of North Macedonia, in 2020 the largest number of elderly people over 65+ was from the Skopje region, a total of 98,552, of which 43,066 were men and 55,486 women[6]. The United Nations forecasts that the average life expectancy in the Republic of North Macedonia will continue to increase; the average life expectancy of 74.9 years will reach 79.5 years in 2050. Global policies for the elderly must

scaled up because only sustainability of the pension and health system is not enough. Achieving sustainability should be done by active contribution from the beneficiaries by encouraging positive lifestyles.[8,9] Healthy aging is about creating the environments and opportunities that enable people to be who they are and to do what they have loved and valued throughout life. [10]

The aim of the study was to perceive the attitudes of the elderly in the city of Skopje regarding the health care, and to get answers about their needs and opinions with regards to the organization and functioning of the health system.

Materials and methods

This was a cross-sectional study conducted on the territory of the city of Skopje in the period March-April 2019. The probability sampling technique was used to select the respondents by applying the method of simple random sampling (Simple Random Sampling). A total of 350 respondents aged 65+ were interviewed. The study was conducted with the method of interviews with members of the NGO "Third Age", users of home care services inthe NGO "Humanity", a private institution for social protection of the elderly "Idila Terzieva", as well as users of services in the home for the elderly "Mother Teresa". A questionnaire was used consisting of 53 questions, and it was designed to suit the age and sex of the respondents.The questionnaire was divided into four parts: first part - general and demographic characteristics; second part - health care; third part - mobility of the old person; fourth part-con-

ditions and lifestyle. Statistics for Windows 7.0 and SPSS, version 14, were used for statistical analysis. Two-way tests with a significance level of p <0.05 were used to determine the statistical significance.

Results

According to sex and age, respondents were divided into five age groups as follows: a) 65-69; b) 70-74; c) 75-79; d) 80-84; and e) over 85 years of age. The analysis showed that most of the respondents of both sexes (43.43%) were aged 65-69 years, of which 42.9% were men and 43.8% women.

Respondents in the sample had the opportunity to give a personal assessment of their health by being offered 4 possible answers: a) good; b) relatively good; c) bad and d) very bad (Figure1).

The analysis showed that female respondents compared to males were 2,507 times significantly more likely to have poor and very poor health [OR = 2.5107 (1.11-5.65) 95% CI].

According to the statements received from the respondents, 50 (37.9%) men and 124 (57.14%) women had some chronic disease. A statistically significant association was established between the sex of the respondents and the presence of chronic disease in addition to a significantly higher prevalence of chronic diseases in female respondents (Figure 2).

All 175 (100%) respondents who stated that they had a chronic disease were asked which chronic disease it was, with the possibility of indicating more than one chronic disease.

The prevalence of the most common chronic diseases in the entire sample of 350 respondents indicated that it was hypertension (15.7%), followed by diabetes (8.6%), heart failure (6.6%), rheumatic disease (4.9%), etc.

In terms of sex, 101 (78.9%) men and 159 (73.6%) women were satisfied with the service "My Appointment" ("MojTermin"). A total of 84 (24.4%) were dissatisfied with this service, including 27 (21.1%) men and 57 (26.4%) women (Figure3).

The most common problem in the procurement of medicines pointed out by most of the respondents or 45 (35.7%) was that the medicines they received were not on the positive list. Thirty-seven (29.4%) stated that taking prescribed drugs was problematic, and 27 (21.4%) respondents pointed out to the high drug costs (Figure 4). The need for a special procedure in the procurement of medicines was a problem for 6 (4.8%) respondents, while other problems were stated by 11 (8.7%) respondents.

The respondents were also asked about the number of visits they pay to a family doctor and/or a specialist doctor during a year. This question was answered by 120 (34.29%) respondents of the sample. According to the answers regarding the annual number of visits to a family doctor, it was found that most of the respondents had three visits per year, 34 (28.3%), followed by one visit, 31 (25.8%), and two, 26 (21, 7%). Sixteen (13.3%) respondents paid five or more visits to a family doctor per year, 5 (8.5%) being men and 11 (18%) women.

The respondents were asked how many of them trust the family doctor? A total of 258 (73.7%) stated that they trust their family doctor, 85 (24.3%) lacked confidence, while 7 (2%) of them stated that they did not trust their family doctor.

During the visit to the health institution, i.e., during their contacts with the health staff, a total of 104 (29.7%) respondents of the sample stated that they felt discrimination while receiving health care. Of those who answered positively, 34 (25.6%) were men and 70 (32.3%) were women (Figure 5).

Forty-seven (47.5%) respondents answered that the discrimination in providing health care was done by a doctor, 43 (43.4%) said it was done by a nurse and 9 (9.1%) said discrimination was done by both the doctor and the nurse. The individual analysis of answers according to sex of the respondents indicated that the most common reason for discrimination was the doctor, followed by the nurse (Figure 6).

The respondents were asked about the proposed measures for better health and social protection. Twelve proposed measures were indicated with the option to choose more than one.

Figure 1. Analysis by sex and assessment of health status
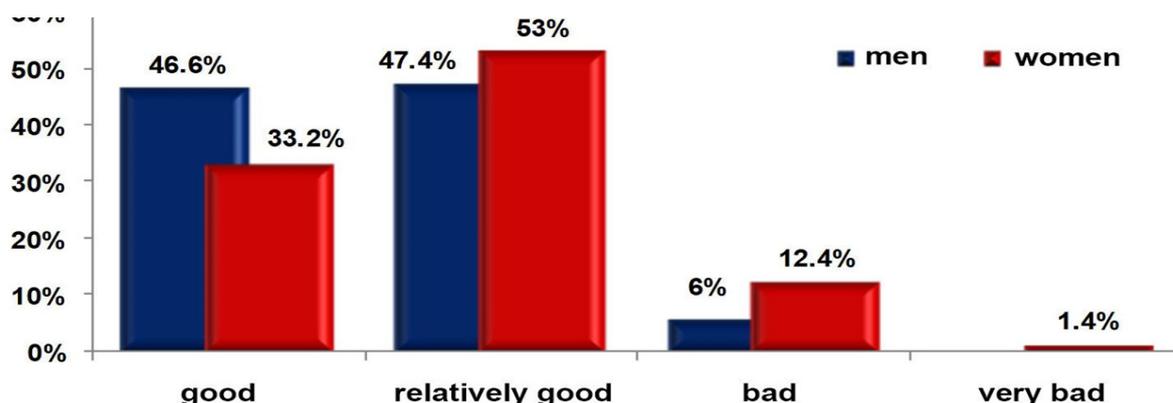
Figure 2. Descriptive analysis of the respondents by sex and presence of chronic disease
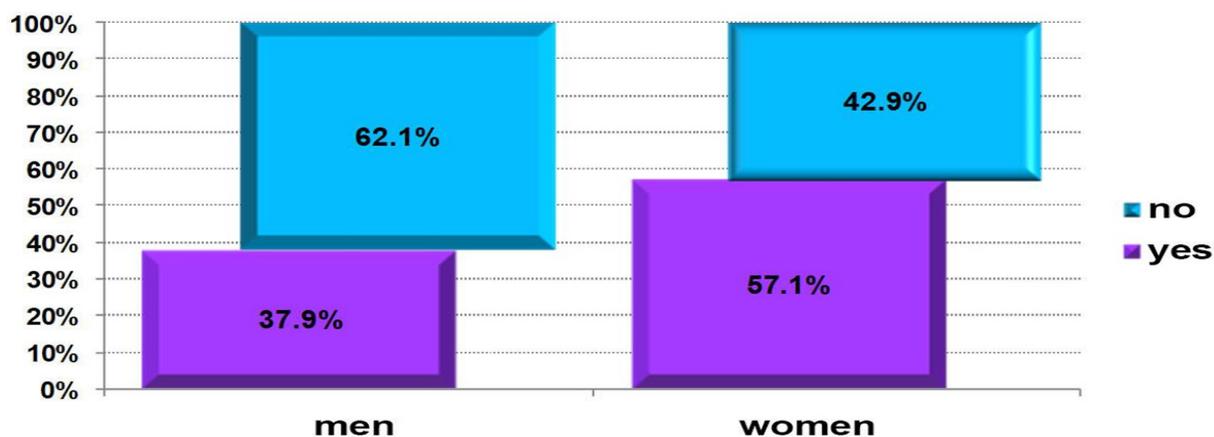
Figure 3 . Analysis by sex and satisfaction with the service „My Appointment"
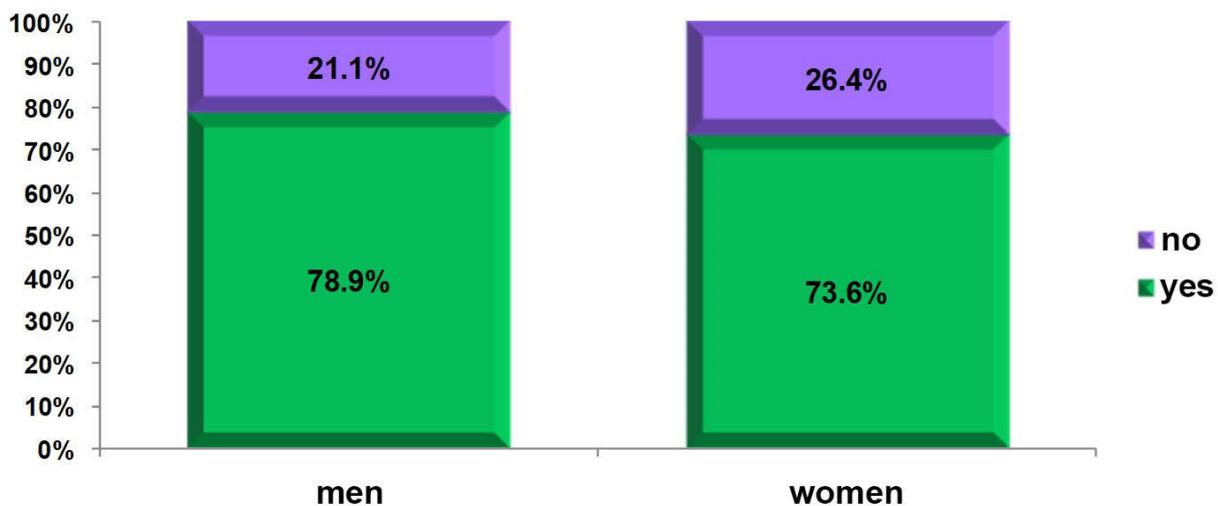
Figure 4 . Distribution by problems in procurement of drugs
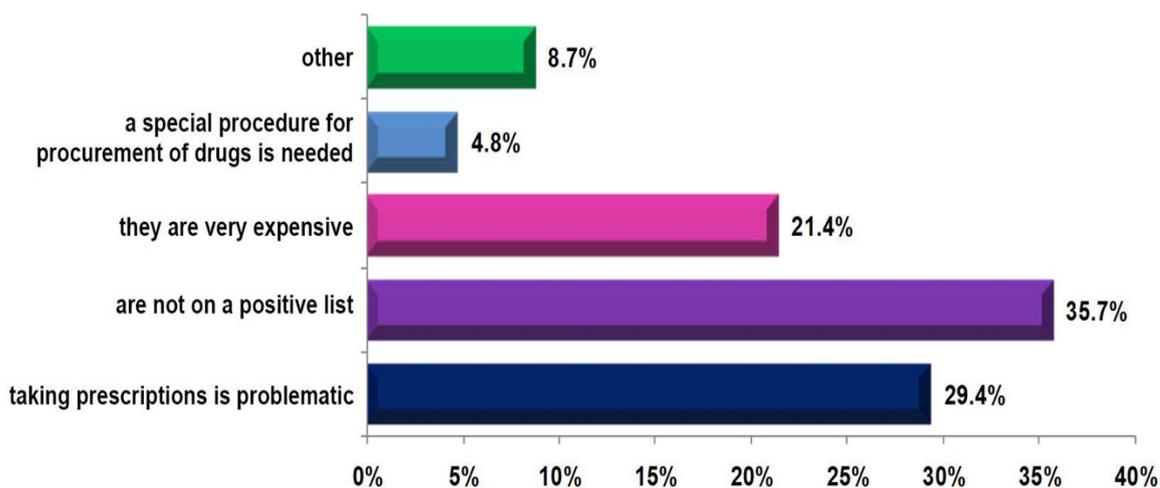
Figure 5 . Descriptive representation of perceived discrimination by sex
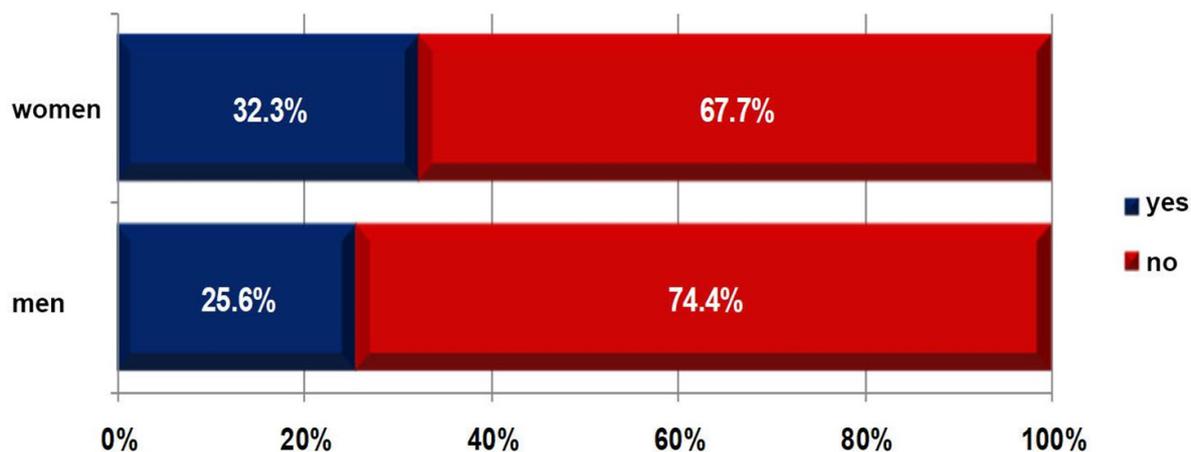
Figure 6 . Distribution by discrimination in health care
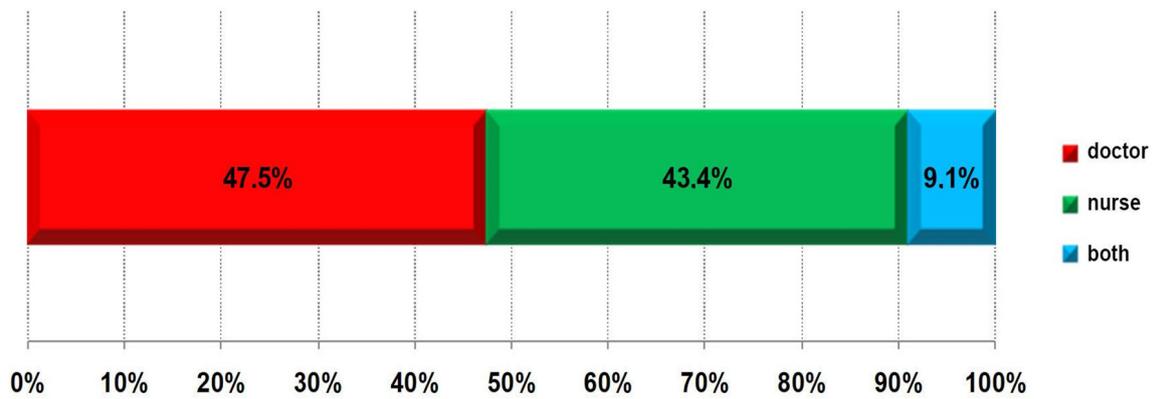
Table 1 . Distribution according to proposed measures for better health and social protection

Proposed measures for better health protection	Respondents (N=350)	
	number	%
Transport to a health facility	86	24.57%
Regular nursing visits for receiving injections	112	32%
Home visit to measure blood pressure and diabetes	148	42.28%
Personal or telephone advice on taking medication	75	21.43%
Home visit to chronically ill people regardingpersonal hygiene	147	42%
Institutional care for chronically ill people	292	83.43%
Day care center for the elderly	228	65.14%
Residential center for the elderly from the village	117	33.43%
Regular delivery of cooked hot meals	93	26.57%
House cleaning and laundry service	59	16.86%
Psychological support	270	77.14%
Other	27	7.71%

According to Table 1, the largest number of respondents, 292 (83.4%), agreed on the need for an institution for care of the chronically ill people. Psychological support was required by 270 (77.1%) respondents, followed by the need for a day care center for the elderly - 228 (65.1%)s. A home visit to measure blood pressure and diabetes was a priority for 148 (42.3%) respondents, followed by a home visit for personal hygiene of the chronically ill people - 147 (42%).117 (33.4%) respondents considered the residential center for the elderly in the countryside to be a necessity. A regular nursing visit for receiving injections was considered to be necessary by 112 (32%) respondents; 93 (26.6%) selected regular delivery of cooked hot meals, while 86 (24.6%) considered transport to a health institution essential.

Discussion

The results of the study have highlighted the health needs of the elderly, but also gave clear directions which actionsare necessary tobe undertaken to achieve positive changes. The governmental institutions /organs of the Republic of North Macedonia have to enable or improve the quality of life of the elderly, that is, to provide them with active and healthy aging.[11] The analysis showed that most of the respondents of both sexes were aged 65-69 years, and of a total of 152 (43.43%) 57 (42.9%) were men and 95 (43.8%) women. The second most common age group of both sexes was 70-74 years old, with a total of 86 (24.6%) persons, i.e., 35 (26.3%) men and 51 (3.5%) women. The smallest number were the respondents in the age

group ≥85 years, who were represented with a total of 11 (3.1%), of which 5 (3.8%) males and 6 (2.8%) females. A similar distribution of respondents according to basic demographic characteristics was found in Albania in three areas, where 475 (52%) men and 438 (48%) women were included in the survey. The sex distribution was more or less similar in all three areas, with 45.8% of participants in Tirana, 51.5% in Shkoder and 48.1% in Vlore.

The adult distribution of respondents in Albania to the elderly was similar in all three areas included in the survey. The share of the very old included in the study, however, was higher in urban areas compared to rural areas (43.4% vs. 28.8%) The mean number of family members was 2.54 ± 2.31, which was a bit higher in rural areas (2.74 ± 2.26) compared to urban areas (2.40 ± 2.28) and in men compared to women (about 3 family members beingmen versus 2 women). There was a greater number of widows, divorced and/or single women (53.9%) versus men (24.4%). Most of the respondents of both sexes in the household lived with the spouse (34.6%), followed by almost a quarter or 27.4% who stated that they lived alone and a fifth or 21.1% who stated that they lived with their children. A total of 3.1% of the respondents stated that they lived with a partner, 3.8% of them being men and 2.8% women.[12]

A survey in Kosovo found that only 5% of older people lived alone, and a similar situation is present in developing countries in general. Figures from the neighbouring Albania and Serbia indicate that the percentage

of elderly people living alone is 20% while in Turkey it is around 30%. The World Economic and Social Survey for 2007 reported that in developed countries more than 35% of individuals aged 60 and over lived alone, while in underdeveloped countries 7% of the elderly lived alone.[13] Surveys show that in countries with traditional family values and views both on the Balkans and beyond, children take care and responsibility for their parents in old age. Besides the tradition, the low economic standard also plays a big role in the multi-generational dwellings. In this study, the respondents were asked about the number of visits to a family doctor and a specialist doctor during a year. This question was answered by 120 (34.29%) respondents in the sample. According to the answers received for the annual number of visits to the family doctor, most of the respondents had three visits per year, 34 (28.3%), followed by one visit, 31 (25.8%), and by two visits, 26 (21, 7%). Sixteen (13.3%) respondents had five or more visits to the family doctor per year, 5 (8.5%) men and 11 (18%) women. The survey conducted in Canada showed that the majority of respondents,> 90%, visited a family doctor regularly and the research showed that with increasing age, the visits to the family doctor also increased (it is more frequent). Excluding physically inactive respondents, more than 80% of respondents perceived their general health to be excellent, very good or good.14 In our study, with ageing, the number of annual visits to the family doctor decreased insignificantly, while in the research in Canada, with increasing age, the number of visits to the family

doctor increased. The Republic of North Macedonia is at the beginning in the field of services for the elderly in home conditions. Assistance and care services in the home have the role of facilitating the daily activities of the elderly. One of those measures is escorting to a health facility.We also see the need for greater involvement of patronage services offered to adults.

According to the statements received from the respondents in our sample, 50 (37.9%) men and 124 (57.14%) women had some chronic disease. A statistically significant association was established between sex of the respondents and the presence of chronic disease in addition to a significantly higher prevalence of chronic diseases in female respondents. Hypertension was pointed out as the most common chronic disease by 55 (31.43%) respondents, followed by diabetes - 30 (17.1%) respondents. In India, the prevalence of hypertension in all respondents was 30.7%, the prevalence in female respondents was 33.9% and in men 25.6%. Out of 407 examinees, 339 or 83.2% had visual impairment; in general, the visual impairment was greater in men and was 90.3% while the visual impairment in women was 98.8%. It was found that 44.7% of respondents suffered from arthritis, and it wasmore prevalent in the rural population than in the urban one. In the survey, 1.71% of the respondents stated that they had a history of malignancy; the prevalence was higher in the urban population (2.46%) compared to the rural one (0.98%).[15]

According to a study conducted in Canada, the most common chronic

diseases in the elderly were: arthritis/rheumatic conditions 24.5%-56%, hypertension 49%, low-back pain 26%, and cardiovascular/cerebrovascular diseases 6%-31%.[14] Our study revealed that women had a higher percentage of chronic diseases than men, and chronic diseases in females lasted longer than in men. This is primarily due to the longer lifespan. There were no significant differences regarding chronic diseases in the studies conducted in Canada, North Macedonia and India, and the most common chronic disease presented in all three studies was hypertension. In all studies, less educated people and people with low economic status hadpoorer results in self-assessment of their health.

A survey by the WHO Regional Office for Europe conducted in 2018/2019 on the elderly and access to health care in North Macedonia indicated similar results to our survey. As it might be expected, the percentage of people who perceived their health as good or very good decreased with age. There can be a drastic reduction in the percentage of people who think their health is good or very good in higher age groups. This percentage decreased from 36.7% among the elderly aged 65 to 74 to 11.1% among the elderly aged 85 and over. In this study, men considered their health to be good or very good unlike women.[16]

To the question How satisfied are you with the "My Appointment" service, the analysis indicated that two thirds of the respondents, 260 (75.6%), stated that they weresatisfied with the "My Appointment" services. In terms of sex, 101 men and 159 women in the sample were satisfied with the "My Appointment" service. A total of 84 respondents were dissatisfied with this service. The "My Appointment" service is one of the most important healthcare services and has been positively accepted by the elderly due to the possibility of completing a specialist examination without waiting for toolong. The waiting time for an appointment appears to bethe main reason for dissatisfaction with this service. A survey conducted in England revealed that 36.8% of adult respondents over the age of 65 had experienced age discrimination. Descriptive analysis indicates that all socio-demographic factors, with the exception of marital status, are related to perceived age discrimination. Multivariate analyses have shown that with increasing age, discrimination also increases, the peak is 70-79 years.[17] Inboth England and in our country, surveys have shown that age discrimination certainly exists.

A characteristic feature of our studyis the form of discrimination and how many people recognized it. The results obtained showed that most often highly educated people recognized discrimination and abuse by health personnel compared topeople with lower education. Several respondents also showed insincerity in the answers to this topic. There is still stigma among these generations and a small number of older people admit age discrimination. [18]

The elderly in R.N. Macedonia have equal access to rights and services, but the respondents pointed out that it is necessary for the health institutions to be closer to them. The

need for institutional care comes first, small capacities cannot meet the expectations for care of these people. The change in the structure of families and the influence of the environment in which the elderly live contributes to the special needs and demands, loneliness and psychological support.[19,20] The elderly due to loneliness and the need for socializing, go to the doctor more than necessary to have some day activity and for socialization[21].

Conclusion

This study enabled us to perceive the real picture of the needs and way of life of the elderly in the city of Skopje. The elderly are not sufficiently informed about their rights and services that they can use. The elderly would like to expand the opportunities for social and health benefits. The most important obstacles in terms of access to services are the lack of information on existing services, the lack of information of citizens about their rights, the lack of sufficient health and propaganda materials in the languages of the communities.

It is necessary to bring closer the already developed forms and services for social and health protection of the elderly in the environment in which they live (day care centers, help centers at home) and to have easily accessible resources. In the self-assessment of their health status, most of the elderly perceived their health condition to berelatively good; the analysis indicated a higher percentage of chronic diseases in women than in men. This is primarily due to the longer life expectancy of women as opposed to men and because of easier expression, openness or recognition that women suffer from a certain disease unlike men presented to the interviewer. The elderly have been discriminated against by health professionals. Abuse of older adults happens too often, but it remains a largely hidden problem. Adult Prejudice or "Ageism" includes the broader meaning of gerontophobia, unwarranted fear, and hatred of the elderly. Negative stereotypes and discriminatory attitudes towards adults must change. The low standard in the country does not bypass the elderly who are part of the marginalized groups in society. Low income after retirement plays a key role.

This study found that older people do not want to be constantly at home; they indicate financial difficulties, and not a reduced interest in social interaction. It is necessary for the society to be an environment that supports and maintains the internal capacities and functional abilities of the elderly that is certainly the key to healthy aging.

References

1. Word Health Organization. Ageing and health- Key facts. 2021 Accessed: 18.03.2022.

2. Galic S, Tomasovic Mrcela N et al. Practitioner for gerontology, geriatrics and psychology of old age- psychology of aging. Medical School Osijek, 2013

3. World Health Organization. Age-friendly environments in Europe: Indicators, monitoring and assessments. Available at: http://www.

euro.who.int/en/health-topics/
Life-stages/healthy-ageing/publi-
cations/2018/age-friendly-environ-
ments-in-europe-indicators,-mon-
itoring-and-assessments-2018.
Accessed: November 30, 2019

4. Eurostat Statistics Explained. Popu-
lation structure and ageing. Avail-
able at:https://ec.europa.eu/eu-
rostat/statisticsexplained/index.
php?title=Population_structure_
and_ageing.Accessed: March 19,2022

5. State Statistical Office. North
Macedonia in numbers. 2021.
Availableat:https://www.stat.
gov.mk/PrikaziPublikacija_1.
aspx?rbr=833.Accessed: 25.02.2022.

6. State Statistical Office. Deaths
by age, by municipality, 2005-
2020 Available at: http://mak-
stat.stat.gov.mk/PXWeb/pxweb/
mk/MakStat/MakStat__Nasele-
nie__Vitalna/225_VitStat_Op_
UmrVoz_mk.px/?rxid=a92d871e-
9d22-40c1-bfb2-de922334917d.
Accessed: February 25, 2022

7. Data World Bank. Live expectancy
at birth. Total (years)-North Mace-
donia. Availableat:data.world-
bank.org/ indicator/SP.DYN.LE00.
IN?locations=MKAccessed: March
10,2022

8. World Atlas. Countries with the
largest aging population in the
world. Available at:https://www.
worldatlas.com/articles/coun-
tries-with-the-largest-aging-pop-
ulation-in-the-world.html. Ac-
cessed: 19.05.2019

9. ActiveAgeingIndex2014 Summa-
ry. Available at:https://statswiki.
unece.org/display/AAI/VI.+Doc
uments+and+publications?pre
view=/76287849/117178632/ex-
tract_for_translation3%20up-

dated--.pdf. Accessed: June, 2015

10. National institute of ageing.
Global ageing, Available at:
https://www.nia.nih.gov/re-
search/dbsr/global-aging. Ac-
cessed: November 21,2019

11. Vlahovic V, et al. Guide to imple-
menting the National Strategy
for Equality and Non-Discrimi-
nation on the basis of ethnicity,
age, mental and physical disabil-
ity, and on a sex basis. Skopje:
British Council in Macedonia,
2014, pp.33-34.

12. Ylli, A. Health and social condi-
tions of older people in Albania:
Baseline data from a national
survey. Public Health Rev 2010;
3:, 549–560.

13. Jerliu N, Toçi E, Burazer, G. et al.
Socioeconomic conditions of el-
derly people in Kosovo: a cross-
sectional study. BMC Public
Health 2012;12: 512.

14. Frontiers in Public Health. Pre-
dicting Self-Rated Health in Dia-
betes and Chronic Heart Failure
– A Multiple Mediation Model
Front. Available at: https://www.
frontiersin.org/articles/10.3389/
fpubh.2015.00266/fullPublic
Health Accessed: 21.02.2022

15. World Health Organization. Re-
gional Office for Europe (WHO).
European report on preventing
elder maltreatment.Copenha-
gen;2011. Available at: https://
www.euro.who.int/__data/
assets/pdf_file/0010/144676/
e95110.pdf

16. World Health Organization. Old-
er people and access to health
care in North Macedonia. 2021.
Available at:https://www.euro.
who.int/en/countries/north-

macedonia/publications/older-people-and-access-to-health-care-in-north-macedonia-2021 Accessed:15 March,2022.

17. Rippon I, KnealeD, Demakakos P, Steptoe A. Perceived age discrimination in older adults. Age and Ageing 2014; 43(3):379-386

18. Jordanova Peshevska D, Markovik M, Sethi D, Serafimovska E, Jordanova T. Prevalence of Elder Abuse and Neglect: Findings from First Macedonian Study. OA Maced J Med Sci 2014; 2(2):353-359.

19. Liotta G, Canhao H, Cenko F, Cutini R, Vellone E, Illario M, et al. Active ageing in Europe: Adding healthy life to years. Front Med 2018; 5:123.

20. Kjosevska E, Totikj N. Report for health status and health care of elderly people. Institute of Public Health of Republic of Macedonia. Skopje, 2021.

CLINICAL SCIENCE

THE ROLE OF SOME INFLAMMATORY MARKERS, CYTOKINS AND TUMOR MARKERS IN DIAGNOSIS OF ENDOMETRIOSIS

Jadranka Georgievska[1], Gligor Tofoski[1], Goran Dimitrov[1], Ana Daneva-Markova[1], Viktorija Jovanovska[1], Dragi Dabeski[1], Sashe Jovcevski[1], Elena Dzikova[1], Aleksandra Atanasova[1]

[1] *University Clinic for Gynecology and Obstetrics; Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia*

Abstract

Endometriosis is a multifactorial disease which etiopathogenesis has not been elucidated. One of the theories of etiopathogenesis is the inflammatory theory. Aims of the study: To develop a practical non-invasive test for the diagnosis of endometriosis by examining some inflammatory markers and cytokines; to compare the highly sensitive C-reactive protein (hsCRP), cytokines (interleukin-6-IL-6 and tumor necrotizing factor alpha) and the tumor marker cancer antigen 125 (CA-125) among healthy patients and patients with endometriosis; to determine the sensitivity and specificity of each biomarker separately in the diagnosis of endometriosis and to determine their role in the diagnosis of endometriosis. Materials and methods: In a prospective study conducted at the University Clinic for Gynecology and Obstetrics, Ss. Cyril and Methodius University in Skopje, North Macedonia 138 patients were included of a reproductive age between 18-50 years (83 with diagnosis endometriosis operated laparoscopically or with laparotomy) and a control group of 55 healthy women, in a period between 01.09.2018 to 01.05.2021. Serum levels of IL-6, TNF-α, hs-CRP and tumor marker CA-125 were evaluated in both groups. Results: Serum levels of CA-125, IL-6 and TNF-α and hs-CRP were significantly higher in patients with endometriosis compared to the control group. The surface under the ROC curve (AUC) for IL-6, CA-125, hs-CRP, and TNF-α has shown that as individual markers they all have a discriminatory capacity to diagnose patients with endometriosis. Conclusions: Results obtained in our study showed statistically significantly higher serum concentrations of CA-125, IL-6 and TNF-α and hs-CRP in patients with endometriosis compared to the control group of patients. However, none of these biomarkers showed a high sensitivity for diagnosis of endometriosis. It is necessary to find a panel combination of biomarkers with a high sensitivity of about 100% that will enable early diagnosis of endometriosis.

КЛИНИЧКИ ИСТРАЖУВАЊА

УЛОГАТА НА НЕКОИ ИНФЛАМАТОРНИ МАРКЕРИ, ЦИТОКИНИ И ТУМОР МАРКЕРИ ВО ДИЈАГНОЗА НА ЕНДОМЕТРИОЗАТА

Јадранка Георгиевска[1], Глигор Тофоски[1], Горан Димитров[1], Ана Данева-Маркова[1], Викторија Јовановска[1], Драги Дабески[1], Саше Јовчевски[1], Елена Џикова[1], Александра Атанасова[1]

[1] *Универзитетска клиника за гинекологија и акушерство; Медицински факултет, Универзитет „Св. Кирил и Методиј" во Скопје, Република Северна Македонија*

Извадок

Ендометриозата е мултифакторно заболување, чија етиопатогенеза не е разјаснета. Една од теориите за етиопатогенезата е инфламаторната теорија. Цели на истражувањето: Да се развие практичен неинвазивен тест за дијагноза на ендометриозата со иследување нанекои инфламаторни маркери и цитокини; да се направи споредба на високосензитивниот Ц-реактивен протеин (hsCRP), цитокините (интерлеукин 6 –IL-6 и тумор-некротизирачки фактор алфа - TNF-α) и туморскиот маркер cancer antigen 125 (CA-125) кај здрави пациентки и пациентки со ендометриоза; да се утврди сензитивноста и специфичноста на секој биомаркер посебно во дијагнозата на ендометриозата и да се утврди нивната улога во дијагноза на ендометриозата. Материјал и методи: Во проспективна студија спроведена на Универзитетската клиника за гинекологија и акушерство, Универзитет „Св. Кирил и Методиј" во Скопје, Северна Македонија беа вклучени 138 испитанички на репродуктивен возраст помеѓу 18-50 години (83 со дијагноза ендометриоза, оперирани со лапароскопија или лапаротомија) и контролна група од 55 здрави жени, во период од 01.09.2018 година до 01.05.2021. Серумските вредности на интерлеукин 6 (IL-6), тумор-некротизирачки фактор алфа (TNF-α), високоспецифичен Ц-реактивен протеин (hsCRP) и туморскиот маркер CA-125 беа евалуирани во двете групи. Резултати: Серумските вредности на CA-125, IL-6 и TNF-α и hsCRP беа сигнификантно повисоки кај пациентките со ендометриоза во споредба со оние во контролната група. Површината под ROC кривата (AUC) за IL-6, CA-125, hs-CRP и TNF-α покажа дека како поединечни маркери сите имаат дискриминаторен капацитет за дијагноза на пациентки со ендометриоза. Заклучоци: Иследувањата во нашата студија покажаа статистички сигнификантно повисоки концентрации на CA-125, IL-6 и TNF-α и hsCRP кај пациентките со ендометриоза во однос на контролната група пациентки. Меѓутоа, ниту еден од овие биомаркери не покажа висока сензитивност за дијагноза на ендометриозата. Потребно е да се најде панел комбинација на биомаркери со висока сензитивност од околу 100% кои ќе овозможат рана дијагноза на ендометриозата.

Introduction

Endometriosis is a disease characterized by the presence of endometrial glands and endometrial stroma outside the uterine cavity. This tissue responds to reproductive hormones and this results in the formation of endometrial cysts in the ovaries and endometrial foci along the peritoneum, intestines, and other sites in the abdominal cavity.

The clinical picture of these patients includes: chronic pelvic pain, dysmenorrhea, dyspareunia, menstrual disorders, infertility, but the disease can be asymptomatic and can be detected as a random finding by laparoscopy or laparotomy. It is found in 6-10% of women in the reproductive period and in 30-50% of patients with infertility[1]. Ultrasound and magnetic resonance imaging[2] can help diagnose this disease. Today the gold standard for the diagnosis of endometriosis is laparoscopy, which allows visualization of endometrial foci as well as their biopsy for histopathological confirmation of the diagnosis[3].

At the same time, laparoscopy as well as laparotomy make it possible to determine the stage of the disease according to the revised classification of endometriosis by the American Society for Reproductive Medicine - rASRM [4]. Because laparoscopy as well as laparotomy are risky and expensive methods, several blood markers are being investigated for non-invasive diagnosis of endometriosis that would allow early diagnosis of this disease.

Despite a long history of clinical experience and experimental research, the pathogenesis of endometriosis has not yet been accurately established.

There are several theories, such as: implantation theory as a consequence of retrograde menstruation, theory of complete metaplasia, genetic theory, immune theory, inflammation theory and others[5, 6].

According to inflammatory theory, the peritoneal environment in patients with endometriosis may be involved in the pathogenesis of the disease[7]. Peritoneal fluid in patients with endometriosis is thought to be filled with activated macrophages that secrete a range of local products such as growth factors and cytokines, and therefore endometriosis is considered a chronic inflammatory disease. Chronic inflammation is accompanied by fibrous tissue formation and local peritoneal adhesions, angiogenesis, and proliferation. The inflammatory process in endometriosis causes pelvic pain and infertility [8].

There are many studies suggesting elevated levels of activated macrophages and several cytokines in the peritoneal fluid in patients with endometriosis, such as: interleukin-6 (IL-6), interleukin 1ß, interleukin-8 (IL-8), tumor necrotizing factor α (TNF-α), vascular endothelial growth factor (VEGF), macrophage migration inhibitory factor (MIF), and others [9, 10, 11].

Elevated values of several inflammatory biomarkers have also been found in the serum of patients with endometriosis: C-reactive protein (CRP), interleukins (interleukin-4, interleukin-6, interleukin-8, TNF-α) and others [12].

IL-6 is thought to play a major role

in the growth and survival of ectopic endometrial tissue. Interleukin-6 regulates inflammation and the immune response by modulating the secretion of other cytokines, promoting T-cell activation, B-cell differentiation, and inhibiting the growth of other cell lines.Interleukin-6 (IL-6) is a cytokine that is a mediator of the immune system and has a number of biological actions. It is also known as B-cell stimulating factor.

TNF-α is secreted by activated macrophages and has inflammatory, cytotoxic and angiogenic effects. TNF-α stimulates the expression of matrix metalloproteinase from endometrial tissue involved in endometrial invasion and remodeling.

Serum cancer antigen (CA-125) is a surface cellular antigen with elevated serum values in most patients with endometriosis and therefore this tumor marker is recommended in the screening of this disease [13,14]. Several studies have combined studies of serum concentrations of CA-125, CA 19-9, and CA 15-3, but the diagnostic value of these tumor markers in endometriosis is still inconsistent [15].

C-reactive protein (CRP) is a protein of the acute inflammatory phase and is widely used in clinical practice as a marker of inflammation. Some studies have found higher CRP and hs-CRP values in patients with endometriosis, especially in stages 3 and 4 of the disease, but without a significant difference between patients with endometriosis and patients without endometriosis[16]. On the other hand, in a study by Lermann J. *et al.* hs-CRP values were significantly lower than CRP values

in women without endometriosis and therefore hs-CRP could serve as a marker for the absence of endometriosis [17].

Objectives of the study were to determine the role of serum markers of inflammation (hs-CRP), cytokines (IL-6, TNF-α) and tumor marker CA-125 in the diagnosis of endometriosis, to determine the sensitivity and specificity of each biomarker separately in the diagnosis of endometriosis. Based on the results obtained, to determine which biomarkers can be used in the early diagnosis of endometriosis.

Materials and methods

In a prospective study conducted at the University Clinic for Gynecology and Obstetrics, Ss. Cyril and Methodius University in Skopje, North Macedonia 148 women were included of a reproductive age between 18-50 years. Of these, 93 were diagnosed with endometriosis and were hospitalized at the Clinic for surgical treatment (laparoscopy or laparotomy) and 55 patients were in the control group. In 83 patients the diagnosis of endometriosis was confirmed histopathologically postoperatively and they were included in the examination.

Ten patients whose diagnosis of endometriosis was not confirmed postoperatively were excluded from the study. The intraoperative stage of endometriosis was determined according to a revised classification ofthe American Society for Reproductive Medicine (rASRM). The control group consisted of 55 healthy women in the reproductive period in whom the presence of endometri-

osis or some other form of inflammation was excluded.

The study was performed in the period from 01.09.2018 to 01.05.2021 and was approved by the Human Research Ethics Committee of the Faculty of Medicine in Skopje.

Exclusion criteria were: malignancy, menopause, pelvic inflammatory disease, previous anti-inflammatory therapy for a period shorter than 6 months before the start of the study.

After receiving an informed consent forparticipation in the study, a detailed history was taken from each woman who voluntarily participated in the study. A preoperative echosonographic evaluation was then performed. After appropriate preoperative preparation, patients were operated on by laparoscopy or laparotomy in the proliferative phase of the menstrual cycle. Intraoperative stage of the disease was determined according to the rASRM classification. Venous blood (5 ml) was taken from each patient preoperatively in the proliferative phase of the menstrual cycle. Blood samples were left at room temperature for 60 minutes to coagulate. The sample was then centrifuged for 10 min at 3000 rpm. Serum concentrations of interleukin-6 (IL-6), tumor necrotizing factor alpha (TNF-α), highly specific C-reactive protein (hs-CRP) and tumor marker CA-125were determined in the Clinical Biochemical Laboratory of the University Clinic for Gynecology and Obstetrics in Skopje.

Method for determination of interleukin-6 (IL-6) concentration

Serum IL-6 concentration was quantified by the immunometric assay (Immulite 2000 HP, Diagnostic Products Corp). Analytical sensitivity of the test is 2 pg / ml with a measuring range up to 1000 pg / ml. Reference values for IL-6 <5.9 pg / ml.

Method for determination of tumor necrotizing factor α (TNF-α) concentration

TNF-α concentrations were quantified by the immunometric method (Immulite 1000, Diagnostic Products Corp.). TNF-α is a solid phase, chemiluminescence immunometric essay. The test shows an analytical sensitivity of 1.7 pg / ml with a measuring range up to 1000 pg / ml. Reference value for TNF-α <8.1 pg / ml.

Method for determination the concentration of highly sensitive CRP (hs-CRP)

The serum concentration of hs-CRP in patients was determined by the immunoturbidimetric method at 522 nm, on a biochemical analyzer Cobas Integra 400 plus, Roshe Diagnostic, Germany. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically 552 nm with a measurement range of 0.1-20mg / L (0.952-190 nmol / L). The lowest level of detection is 0.1mg / L (0.952 nmol / L). Reference values for hs-CRP <5mg / ml.

Method for determination of CA-125 concentration

Serum CA-125 tumor marker concentration was quantified by the immunometric assay (Immulite 2000 HP, Diagnostic Products Corp). It is

a sequential, two-sided, solid-phase, enzymatic chemiluminescence essay. Analytical sensitivity of the test is 2 pg / ml with a measuring range up to 1000 pg / ml. Reference value for CA-125 <35mIU / ml.

Statistical analysis

The statistical analysis of the data was performed with the statistical program SPSS 23.0. The Kolmogorov-Smirnov test was used to test the normality of data distribution. The statistical characteristics of the categorical variables are presented by absolute and relative numbers, while the quantitative variables are presented by average, standard deviation, minimum and maximum values, median value and interquartile range.

The statistical significance of the intergroup differences was tested with the Chi-square test and the Mann-Whitney test.

Logistic regression analysis was used to determine the independent predictors of endometriosis.

Sensitivity, specificity, positive and negative predictive value were calculated for each marker. Because the study was designed as a case-control study, predictive values were calculated by Bayesian approximation, assuming an endometriosis prevalence of 10%.

ROC analysis by constructing a ROC curve was used to determine the discriminant ability of IL-6, hs-CRP, TNF-alfa, and CA-125 as tests for the diagnosis of endometriosis.

A ROC curve was constructed for all of these tests, as a graphical representation of the sensitivity and specificity of each possible test result.

Statistical significance was defined at the level of p <0.05.

Results

A total of 138 patients of a reproductive age from the University Clinic for Gynecology and Obstetrics were included in the study, of which 83 patients with endometriosis (IG) and 55 patients without endometriosis (control group - CG). Both groups were homogeneous in terms of age of patients (p = 0.56); the mean age was 33.4 ± 6.4 and 32.8± 5.5 years, respectively in the study and control groups(Table 1).

Table 1. Patients from both age groups

Variable	Variable calculated	groups		p-level
		CG	IG	
Age	mean ±SD	32.8 ±5.5	33.4± 6.4	t=0.58 p=0.56 ns
	min - max	22 – 45	22 – 54	

IG-investigated group with endometriosis
 t - (Student's t-test)
CG - control group

Table 2. Comparison of investigated biomarkers (IL-6, CA-125, hs-CRP and TNF-α) between CG and IG

Calculated parameter	groups		p-level
	CG	**IG**	
IL - 6			
min – max	2.5 – 18.3	2 – 367	Z=2.84
median (IQR)	4.98(3.99 – 5.9)	6.97(3.99 – 13.2)	**p=0.0045 sig
IL - 6			
min – max	1.2 – 29.5	2.53 – 384.4	Z=8.12
median (IQR)	7.4(5.2 – 10.7)	27.6(16.3 – 64.5)	***p=0.000000 sig
IL - 6			
min – max	0.2 – 23.5	0.2 – 243	Z=3.41
median (IQR)	1.1(0.5 – 3.3)	2.7(1.0 – 9.2)	***p=0.00064 sig
IL - 6			
min – max	4 – 25.7	4 – 238	Z=3.89
median (IQR)	5.3(4.6 – 6.25)	6.34(5.2 – 9.64)	**p=0.0001 sig

IG- investigated group   **Z** (Mann-Whitney test)
CG- control group   *p<0.05 **p<0.01 ***p<0.0001
The results presented in Table 2 show that patients with endometriosis had higher values for all 4 analyzed biomarkers compared to patients from CG.

Significantly higher median serum IL-6 values were confirmed for p = 0.0045 in the endometriosis group compared to CG (6.97 vs. 4.98) (Figure 1).

Median serum concentrations of the tumor marker CA-125 were significantly higher in the endometriosis group compared toCG (27.6 *vs.* 7.4, p <0.0001). These results are shown in Figure 2.

The biomarker of inflammation, hs-CRP, showed significantly higher serum concentrations in patients with endometriosis versus the control group (2.7 *vs.* 1.1, p = 0.00064) (Figure 3). Significantly higher serum concentrations of TNF-α were measured for p = 0.0001 in the group of patients with endometriosis compared to CG patients (6.34 *vs.* 5.3) (Figure 4).
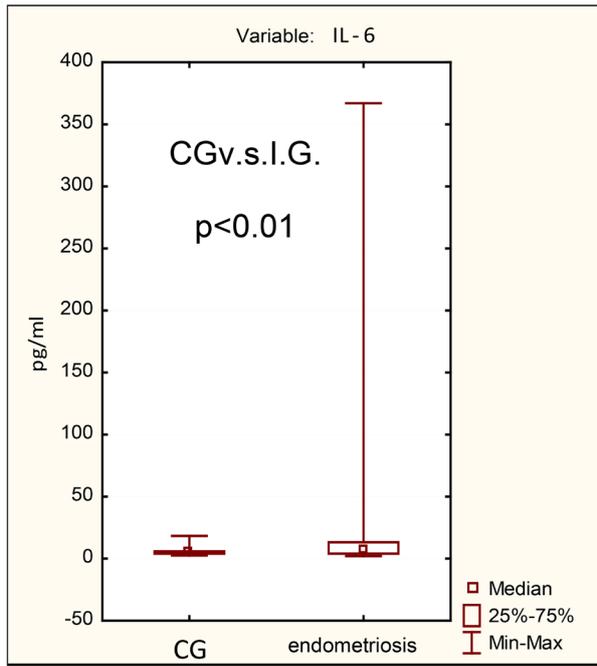
Fig 1. Distribution of IL-6 (CG vs.IG),
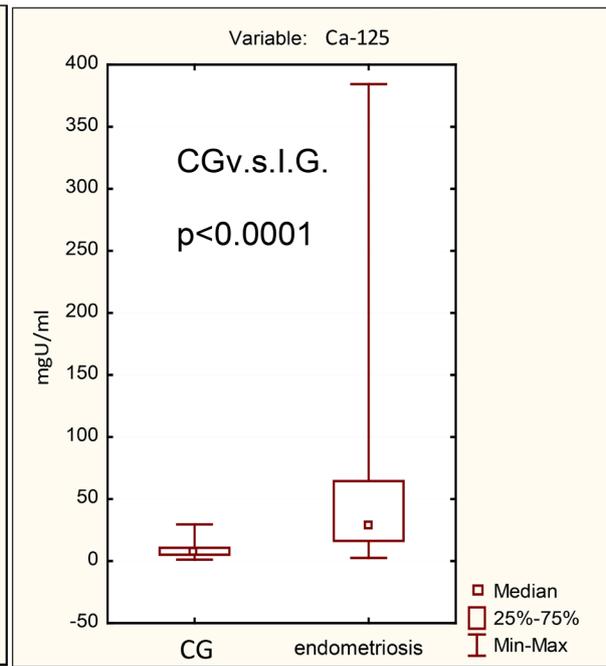
Fig. 2 Distribution of CA-125 (CG vs. IG)
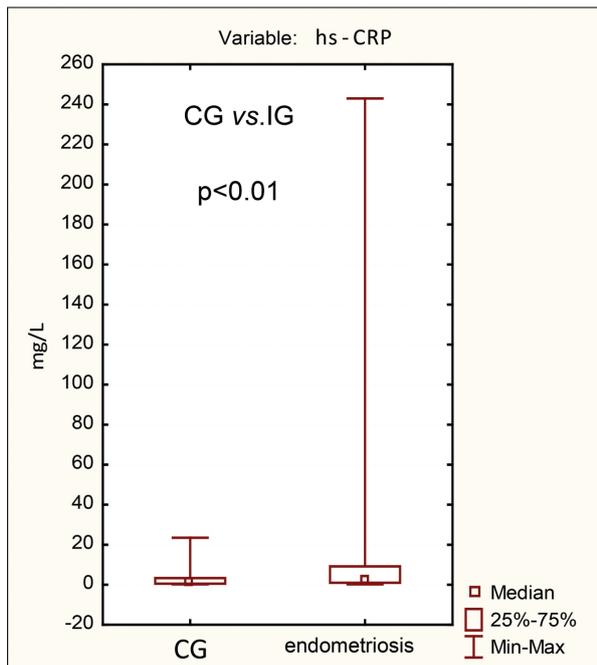
Fig 3. Distribution of hs-CRP (CG vs. IG),
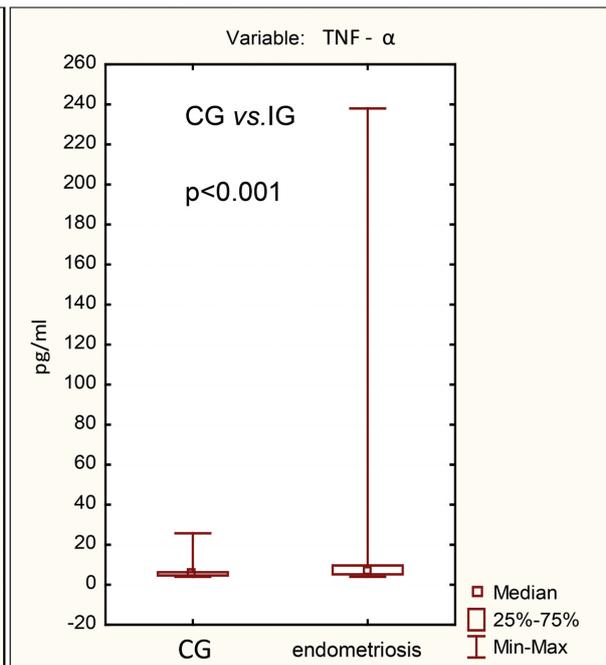
Fig. 4 Distribution of TNF-α (CG vs. IG)

Table 3 presents the results of the univariate and multivariate logistic regression analysis showing the variables associated with endometriosis. The variables that proved to be significant by univariate analysis (IL-6, CA-125, hs-CRP and TNF-α) were included in a multivariate analysis model, which determined serum CA-125 as a marker significantly associated with endometriosis. Increasing serum concentrations of CA-125 by 1mIU / ml increased the chance of endometriosis by 19.7% (OR = 1.197; 95% CI 1.108-1.294).

Table 3. Univariate and multivariate analysis of the examined biomarkers (IL-6, CA-125, hs-CRP and TNF-α)

	Univariate analysis				Multivariate analysis			
			95% CI for Exp (B)				95% CI for Exp (B)	
	p	Exp (B)	Lower	Upper	p	Exp (B)	Lower	Upper
age	0.557	1.017	0.961	1.077				
IL6	0.011	1.117	1.025	1.218	0.714	1.031	0.875	1.215
CA 125	0.000	1.220	1.132	1.314	0.000	1.197	1.108	1.294
CRP	0.019	1.093	1.015	1.177	0.617	1.030	0.917	1.157
TNF alfa	0.023	1.161	1.021	1.320	0.119	1.105	0.975	1.252

Elevated serum IL-6 levels were significantly more frequently reported in patients with endometriosis compared to CG (56.6% vs. 23.6%, p = 0.00013). Table 4 shows the diagnostic performance of IL-6 as a predictor of endometriosis. IL-6 had a sensitivity of 56.63% and a specificity of 76.36% in discrimination between patients with endometriosis and the control group at cut-off values of 5.9 pg / ml.

Table 4. Contingency table of the diagnostic performance of IL-6 as a predictor of endometriosis

IL-6	IG-with endometriosis	CG	Total
>5.9	47 (56.63%)	13 (23.64%)	60
<5.9	36 (43.37%)	42 (76.36%)	78
total	83	55	138

Chi-square = 14.65 ***p=0.00013

sensitivity	56.63%
specificity	76.36%
positive predictive value	21.02%
negative predictive value	94.06%

***p<0.0001

There were no patients in the CG with elevated serum CA-125 concentrations, while in the endometriosis group 37.35% of patients had elevated CA-125. The intergroup comparison in the frequency of elevated serum CA-125 was statistically significant (p <0.0001).

Table 5 shows the diagnostic performance of CA-125 as a predictor of endometriosis at a cut-off value of 35 mIU / ml.

Table 5. Diagnostic performance of CA-125 as a predictor of endometriosis

CA – 125	IG	CG	Total
>35	31(37.35%)	0	31
<35	52(62.65%)	55(100%)	107
total	83	55	138
Chi-square = 26.65 ***p<0.0001			
sensitivity	37.35%		
specificity	100%		
positive predictive value	100%		
negative predictive value	93.49%		

Table 5 shows that CA-125 had a sensitivity of 37.35% and a specificity of 100% in the diagnosis of endometriosis.

For p = 0.011, a statistically significant difference in the frequency of increased hs-CRP in the serum between the two groups was confirmed. Values were increased in 38.55% and 18.2% of patients, respectively in the endometriosis and control groups.

Table 6 illustrates the diagnostic performance of hs-CRP as a predictor of endometriosis. Hs-CRP had a sensitivity of 38.55% and a specificity of 81.82% in the diagnosis of endometriosis, at a cut-off value of 5mg / ml.

Table 6. Diagnostic performance of CA-125 as a predictor of endometriosis

hs-CRP	IG	CG	Total
>5	32(38.55%)	10(18.18%)	42
<5	51(61.45%)	45(81.82%)	96
total	83	55	138
Chi-square = 6.48 *p=0.011 sig			
Chi-square = 6.48 *p=0.011 sig	37.35%		
sensitivity	38.55%		
specificity	81.82%		
positive predictive value	19.07%		
negative predictive value	92.30%		

*p<0.05

The TNF-α biomarker was elevated in the serum of 30.1% of patients with endometriosis, and in 9.1% of CG patients, with a significant difference of p = 0.0034.

Table 7 shows the diagnostic performance of TNF-α as a predictor of endometriosis. TNF-α had a sensitivity of 30.12% and a specificity of 90.91% in the diagnosis of endometriosis at a cut-off value of 8.1pg / ml.

Table 7. Contingency table of the diagnostic performance of IL-6 as a predictor of endometriosis

TNF-α	IG	CG	Total
>8.1	25(30.12%)	5(9.09%)	30
<8.1	58(69.88%)	50(90.91%)	108
total	83	55	138
Chi-square = 8.59 **p=0.0034			
sensitivity	30.12%		
specificity	90.91%		
positive predictive value	26.91%		
negative predictive value	92.13%		

**p<0.01

ROC analysis of the area under the curves was performed to determine the discriminant ability of IL-6, Ca-125, hs-CRP, and TNF-alfa as tests for the diagnosis of endometriosis. A ROC curve was constructed for all of these tests.

The results showed that based on the size of the area under the ROC curve (AUC), IL-6, CA-125, hs-CRP and TNF-alpha were tests with a satisfactory ability to distinguish patients with endometriosis from the control group: IL-6 (AUC = 0.665), CA-125 (AUC = 0.687), hs-CRP (AUC = 0.602), TNF-alpha (AUC = 0.605).

Discussion

In patients with severe endometriosis, the clinical picture is dominated by pelvic pain, dysmenorrhea, infertility, and ultrasound evaluation indicating the presence of endometriotic cysts in the ovaries. In these patients, gynecologists will advise laparoscopic extirpation of the endometriomas and endometriotic lesions, so that the diagnosis of endometriosis will be made quickly. However, a large percentage of patients with mild endometriosis can be asymptomatic. Early diagnosis is important for them, which will allow them to start therapy for endometriosis immediately, before severe irreversible damage to the

female genitals occurs. The use of non-invasive biomarkers would allow early suspicion of endometriosis and early therapy and invasive procedures such as laparoscopy would be avoided.

In our study we found elevated values of markers of inflammation (hs-CRP), cytokines (IL-6, TNF-α) and tumor marker CA-125 in the investigated group of patients with endometriosis compared to the control group of healthy patients, which corresponded to the results obtained in the studies of Irungu S. et al., and May KE et al. [18,19]. None of the patients operated for endometriosis in our study had an infection of the operative wound postoperatively, and the blood for analysis was taken preoperatively, so that the surgical treatment had no effect on the results of the study.

In the group of patients with endometriosis, we found elevated values of IL-6 compared to the levels in the control group of patients (6.97 vs. 4.98, p = 0.0045). Similar results were presented by Kashanian M. et al., who in their study found higher CA-125 and IL-6 values in patients with endometriosis than in the control group of patients, but AUC (CA-125) and AUC (IL -6) did not show significant difference and diagnostic value ofthese tests in the diagnosis of endometriosis[20].

Recent studies have shown that there is a correlation between CA-125 values and the stage of endometriosis. In the study of Tian Z. et al., the sensitivity of this biomarker was found to be 63.1% in stage 3 and 4 endometriosisversus 24.8% for the first and second stage endometriosis[21]. The CA-125 tumor marker

values had diagnostic value in diagnosing advanced stages of endometriosis as well as in monitoring the treatment of these patients in thestudy of Wu MH et al.[22] and inthe study of Maiorana A. et al.[23].

In the study of Sütcü H. K et al. significantly elevated values of the tumor marker CA-125 were found in patients in all stages of endometriosis in comparisonto the control group of patients, as well as in patients with stages 3 and 4 of the disease compared to the control group of patients (p<0.05). CA-125 had a sensitivity of 70% and a specificity of 79% in discrimination between patients with stage 3 and 4 endometriosis in comparison to the control group of patients, at a cut-off value of 21.7U / ml [24].

In our study, in CG there were no patients with elevated serum CA-125 concentrations. In the group of patients with endometriosis, 37.35% of patients had elevated CA-125 values. Based on the area under the CA-125 curve (AUC) in the diagnosis of endometriosis, at a cut-off value of 35 mIU / ml, the sensitivity was 37.35% and the specificity 100%. Similar results were obtained in a study by Bilibioet al., who found higher sensitivity and specificity of CA-125 in the diagnosis of advanced (third and fourth stage) endometriosis [25].

In our study patients with endometriosis were not divided into groups according to the stage of the disease, so the sensitivity and specificity of CA-125 in the diagnosis of endometriosis were lower.

IL-6 is a pleotropic cytokine produced by a range of cell types such as monocytes, lymphocytes, fibroblasts, endothelial cells, and kerati-

nocytes. IL-6 is also produced in the eutopic and ectopic endometrium. IL-6 is a mediator in the active phase of inflammation and angiogenesis.

Bedaiwy et *al.* in their study found that serum IL-6 levels could be used to discriminate patients with and without endometriosis. They found a sensitivity of 90% and a specificity of 67% for IL-6 in the diagnosis of endometriosis, at a cut-off value of 2 pg / ml 26. In our study we found significantly elevated IL-6 values in patients with endometriosis compared to the levels in the control group (56.6% vs. 23.6%, p = 0.00013).

For IL-6 we found a sensitivity of 56.63% and a specificity of 76.36% in the prediction of endometriosis, at a cut-off value of 5.9 pg / ml. According to these results, IL-6 showed the highest sensitivity and specificity of all other inflammatory markers and cytokines in the diagnosis of endometriosis.

Hs-CRP is a marker of inflammatory response and can be used as a non-invasive biomarker of endometriosis. In our study, the hs-CRP biomarker presented significantly different serum concentrations in patients of both groups. Hs-CRP presented elevated serum values in 38.55% of patients with endometriosis versus 18.2% of patients without endometriosis. At cut-off values of 5 mg / ml, the sensitivity of hs-CRP in the diagnosis of endometriosis was 38.55%, and the specificity 81.82%

In their study, Abrao et *al.* reported elevated hs-CRP levels in patients with severe endometriosis (stage 3 and 4) [27].

Endometriosis patients and CG patients in our study differed significantly in their serum TNF-α values, with significantly higher median serum TNF-α concentrations in the endometriosis group compared to CG patients (6.34 vs. 5.3, p =0.001).

The TNF-α biomarker was elevated in the serum of 30.12% of patients with endometriosis and in 9.09% of patients with CG, with a significant difference between IG versus CG (p = 0.0034). TNF-α as a single marker is useful in distinguishing patients with endometriosis from healthy patients (AUC = 0.605), with a sensitivity of 30.12% and a specificity of 90.91%, at a cut-off value of 8.1 pg / ml.

In the study of Furucu FN. et *al.* from 2020, the authors observed the expression of TNF-α and IL-6 in normal and endometrial tissue using the immunohistochemical method. They found increased immunoreactivity of TNF-α and IL-6 in the endometrial tissue of the examined patients compared to healthy patients and stated that these two biomarkers play an important role in the pathogenesis of endometriosis [28].

Conclusions

In this study, the use of several biomarkers in the diagnosis of endometriosis (CA-125, IL-6, hs-CRP and TNF-α) was evaluated. The results showed statistically significantly higher concentrations of CA-125, IL-6 and TNF-α and hs-CRP in patients with endometriosis compared to the concentrations in the control group of patients. The results of our study showed that none of these biomarkers individually has high sensitivity and specificity for the diagnosis of endometriosis.

The limitation of our study is the

smaller number of patients examined compared to other studies. It is necessary to find a panel combination of biomarkers with a high sensitivity of about 100% that will enable early diagnosis of endometriosis. Also, this combination of biomarkers will allow patients with infertility and pelvic pain to be singled out as patients at high risk for endometriosis who will undergo laparoscopy with excision of the endometrial foci.

References

1. Missmer SA, Cramer DW. The epidemiology of endometriosis. Obste Gynecol Clin North Am 2003; 30: 1-19.

2. Novak E, Berek JS. Berek-Novak's gynecology; Lippincott Williams-Wilkins; 2007.

3. Duffy JM, Arambage K, Correa FJ et *al.* Laparoscopic surgery for endometriosis. Cochrane Database Syst Rev (4) (2014) CD011031

4. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 1997; 67: 817-821.

5. Sourial S, Tempest N, Hapangama DK. Theories on the pathogenesis of endometriosis. Int J Reprod Med 2014; 2014: 179515

6. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril 2012; 98(3): 511-519.

7. Lousse JC, Van Langendonckt A, Defrere S, et *al.* Peritoneal endometriosis is an inflammatory disease. Front Biosci (Elite Ed). 2012; 4:23-40.

8. Malutan M A, Drugan T, Costin N, Ciortea R, Bucuri C, Rada P.M et Mihu D. Pro-inflammatory cytokines for evaluation of inflammatory status in endometriosis. Cent Eur J Immunol 2015, 40 (1): 96-100.

9. Sütcü K.H, Celik T.G, Akpak K.Y, Akar E.M, Taskin Ö, Özdem S, Uzun G. The value of CA-125, CA 19-9, Interleukin-6, interleukin-8 and HSCRP in the diagnosis of endometriosis. Acta Med Mediterr 2015, 31:793-799.

10. Ilie I, Ilie R. Cytokines and endometriosis-the role of immunological alterations. Biotechnology, molecular biology and nanomedicine 2013, 2: 8-19.

11. Wu MY, Ho HN. The role of cytokines in endometriosis. Am J Reprod Immunol 2003; 49: 285-296.

12. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS et *al.* Blood biomarkers for non-invasive diagnosis of endometriosis. Cochrane database syst rev 2016; (5): CD012179.

13. Amaral VF, Ferriani RA, Sa MF, et *al.* Positive correlation between serum and peritoneal fluid CA-125 levels in women with pelvic endometriosis. Sao Paolo Med J 2006; 124: 223-227.

14. Trojnar K.Ž, Pilszyk A, Niebrzydowska M, et *al.* The potential of non-invasive biomarkers for early diagnosis of asymptomatic

patients with endometriosis. J Clin Med 2021;10(13):2762.

15. Airong Shen, Shengnan Xu, Yange Ma, et *al.* Diagnostic value of serum CA125, Ca19-9 and CA 15-3 in endometriosis: A meta-analysis. J International Med Research 2015; 43(5): 599-609.

16. Xavier P, Belo L, Rebelo I, et *al.* Serum levels of VEGF and TNF-alfa and their association with C-reactive protein in patients with endometriosis. Arch Gynecol Obstet 2006; 273:227-231.

17. Lermann J, Mueller A, Körber F et *al.* Evaluation of high-sensitivity C-reactive protein in comparison with C-reactive protein as biochemical serum markers in women with endometriosis. Fertil Steril 2010; 93(7):2125-2129.

18. Irungu S, Mavrelos D, Worthington J, Blyuss O, et *al.* Discovery of non-invasive biomarkers for the diagnosis of endometriosis. Clin Proteomics 2019; 16:14 .

19. May K.E, Conduit-Hulbert S.A, Villar J, et *al.* Peripheral biomarkers of endometriosis: A systematic review. Hum Reprod Update 2010; 16: 651-674.

20. Kashanian M, Sariri E, Vahdat M, et *al.* A comparison between serum levels of interleukin-6 and CA 125 in patients with endometriosis and normal women. Med J Islam Repub Iran 2015; 29: 280.

21. Tian Z, Chang X.H., Zhao Y., et *al.* Current biomarkers for the detection of endometriosis. Chin Med J 2020; 133:2346-2352.

22. Wu M-H, Hsiao K-Y and Tsai Sh-J. Endometriosis and possible inflammation markers. Gynecol Minim Invasive Ther 2015; 4: 61-67.

23. Maiorana A, Cicerone C, Niceta M, et *al.* Evaluation of serum CA 125 levels in patients with pelvic pain related to endometriosis. Int J Biol Markers 2007; 22: 200-202.

24. Sütcü HK, Çelik GIT, Akrak YK, et *al.* The value of CA-125, CA 19-9, interleukin-8 and HSCRP in the diagnosis of endometriosis. Acta Med Mediterr 2015; 31: 793.

25. Bilibio JP, Souza CA, Rodini GP, et *al.* Serum prolactin and CA 125 levels as biomarkers of peritoneal endometriosis. Gynecol Obstet Invest 2014; 78:45-52.

26. Bedaiwy MA, Falcone T, Sharma RK, et *al.* Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. Hum Reprod 2002; 17 (2): 426-431

27. Abrao MS, Podgaec S, Filho BM, et *al.* The use of biochemical markers in the diagnosis of pelvic endometriosis. Hum Reprod 1997; 12: 2523-7.

28. Furucu F.N, Sencar L, Çetin T.M, et *al.* Expression of interleukin 6 and tumor necrosis factor alpha in endometriotic tissue: an immunohistochemical study. Cukurova Med J 2020; 45(3):1115-1125.

CLINICAL SCIENCE

SECONDARY SJOGREN'S SYNDROME IN PATIENTS WITH RHEUMATOID ARTHRITIS

Filip Gucev[1], Ljubinka Damjanovska[1], Georgi Bozhinovski[1], Snezhana Perchinkova-Mishevska[1], Natali Jordanovska-Guceva[2]

[1] University Clinic for Rheumatology; Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Republic of North Macedonia
[2] University Clinic for Traumatology, Orthopedics, Anesthesiology, Reanimation and Intensive Care; Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Republic of North Macedonia

Abstract

Secondary Sjogren's syndrome (sSS) is a connective tissue disease characterized by xeropthalmia and xerostomia, associated with another autoimmune disease. The prevalence of sSS in patients with rheumatoid arthritis (RA) is different in different countries, but is assumed at 10% of all RA patients and 20% of these have sub-clinical manifestations. This is a large subpopulation of patients with RA, especially taking into account that the clinical implications of their coexistence are not well explored. Aims: To analyze the effects of sSS on RA, the association between this syndrome and disease activity and disease evolution, presence of serological and immunological markers, disease duration and quality of life in patients with RA. Material and methods: We examined 42 patients, at the age of 18 to 70 years, diagnosed according to the criteria for classification and diagnosis by EULAR (2010). Twenty patients were diagnosed with RA and sSS, and 22 patients with RA without sSS. The groups were comparable regarding age, sex and disease duration. We analyzed the incidence of sSS, association with age, sex, demographic data, disease duration, extraarticular manifestations, and serologic tests (positive RF, anti-CCP) were also made. Disease activity was assessed by disease activity score (DAS28) and quality of life by the health assessment questionnaire-disability index (HAQ-DI). The number of tender and swollen joints was assessed, as well as pain level by using the visual analogue scale (VAS), sedimentation rate (ESR), CRP, and immunological tests (SSA, SSB, antidsDNA, ANA, antiU1snRNP) were also made. Results: In the analyzed patients there was no statistically significant difference in ESR, CRP, DAS28, HAQ-DI, seropositivity of RF and anti-CCP and the presence of antidsDNA, ANA or antiU1snRNP and disease duration. Patients in the RA group had more tender, swollen joints and VAS. There was a statistically significant difference in SSA and SSB levels. There was no significant difference in the treatment of patients from both groups. Conclusion: There was no statistically significant difference in the level of disease activity and quality of life in patients with RA compared to sSS group.

КЛИНИЧКИ ИСТРАЖУВАЊА

СЕКУНДАРЕН СЈЕГРЕНОВ СИНДРОМ КАЈ ПАЦИЕНТИ СО РЕВМАТОИДЕН АРТРИТИС

Филип Гучев[1], Љубинка Дамјановска[1], Георги Божиновски[1], Снежана Перчинкова-Мишевска[1], Натали Јордановска-Гучева[2]

[1] Универзитетска клиника за ревматологија; Универзитет „Св.Кирил и Методиј" во Скопје, Медицински факултет, Република Северна Македонија.
[2] Универзитетска клиника за трауматологија, ортопедија, анестезиологија, реанимација и интензивно лекување; Универзитет Св. Кирил и Методиј во Скопје, Медицински факултет, Република Северна Македонија.

Извадок

Секундарниот Сјегренов синдром (сСС) претставува заболување на сврзното ткиво кое се карактеризира главно со појава на ксерофталмија и ксеростомија, во склоп на други автоимуни заболувања. Преваленцијата на сСС кај пациенти со ревматоиден артритис (РА) е различна во различни држави, но се претпоставува дека изнесува околу 10% од сите пациенти со РА, при што околу 20% од овие имаат супклинички манифестации. Ова претставува голема суппопулација на пациенти со РА, при што клиничките импликации на коегзистирањето на двете болести сѐуште се недоволно испитани. Цели: Да се анализираат ефектите на појавата на сСС врз болеста, односно поврзаноста на овој синдром со активноста и еволутивноста на болеста, присуството на серолошки и имунолошки маркери, времетраењето на болеста и квалитетот на животот кај пациенти со РА. Материјал и методи: Беа испитани 42 пациенти на возраст од 18 до 70 години, дијагностицирани според критериумите на EULAR (2010). Дваесет пациенти беа со РА и сСС и 22 пациенти со РА без придружен сСС. Групите беа со слични карактеристики во однос на пол, возраст и времетраење на болеста. Беа анализирани: појавата на сСС и нејзината зависност од возраста и полот на пациентите, демографските податоци, траењето на болеста, присуството на вонзглобни манифестации, а беа направени и серолошки тестови (позитивниот RF, anti-CCP). Активноста на болеста беше анализирана преку индексот на активност на болест - DAS28 (Disease Activity Score 28), а квалитетот на живот на пациентите преку HAQ-DI (Health Assessment Questionnaire – Disability Index). Беше анализиран бројот на болни и отечени зглобови, VAS (визуелна аналогна скала на болка), седиментација на еритроцити (ESR), CRP, а беа направени и имунолошки тестови (SSA, SSB antidsDNA, ANA, antiU1snRNP) и по потреба други имунолошки и серолошки тестови). Резултати: Кај испитаните пациенти немаше статистички сигнификантна разлика во ESR, CRP, DAS28, HAQ-DI, серопозитивност кон RF или anti-CCP, како и присуството на antidsDNA, ANA или antiU1snRNP, како и траење на болеста. Пациентите во РА групата беа во поголем број, со повеќе отечени зглобови и повисока VAS. Постоеше статистички сигнификантна разлика во позитивноста на SSA и SSB антителата во двете групи. Немаше разлика во лекувањето на пациентите во двете групи. Заклучок: Не постои статистички сигнификантна разлика во нивото на активност на болеста и квалитетот на живот на пациентите со РА во споредба со оние со сСС.

Introduction

Sjogren's syndrome (SS) is a chronic autoimmune disease which afflicts mainly the exocrine glands and is characterized by keratoconjunctivitis sicca and xerostomia[1]. The beginning of the disease is generally slow and patients at this time usually complain of generalized myalgia, arthralgia and fatigue.

Sjogren's syndrome is considered secondary when it is present in association with another autoimmune disease, even when SS was present before the "primary" disease. Lasarus et al. followed 114 patients with primary SS in a period of 10 years and showed that 13 of them had developed another autoimmune disease, which pointed out to a more generalized dysfunction of the immune system[2].

Secondary Sjogren's syndrome (sSS) is considered an extraarticular manifestation of rheumatoid arthritis (RA)[1]. The exact prevalence of sSS in patients with RA varies significantly, depending on the definition of sSS, duration of RA and the geographic region. The cumulative prevalence of sSS in patients with RA is 17% and 25% for duration of RA of 10 and 30 years correspondingly[3]. Another study from the UK confirms the association of sSS and RA according to disease duration[4], but a study from Norway doesn't show this[5]. This Norwegian study shows that the minimal prevalence of sSS in patients with RA is 7% and that there is no association between sSS and disease activity in RA. The same authors point out that there is a significant association between lower salivary production and disease activity in RA patients[5]. A study from Austria shows sSS prevalence of 22% in RA patients[6].

The relevance of sSS in patients with RA is clearly illustrated in the doubling of the risk of non-Hodgkin's lymphoma compared to patients with RA without sSS [7]. Some reports show increased mortality in patients with sSS[8,9].

The presence of sSS may point to a shorter life span in patients with RA and possibly higher disease activity. According to this, sicca symptoms should not be considered as irrelevant. It is important to explore whether sSS influences disease activity and the approach to treatment in patients with RA in everyday clinical practice.

The aims of our study were to examine the association of RA and SS in regard to disease activity, functional ability and quality of life, extraarticular manifestations, as well as possible correlation with serologic markers such as RF and anti-CCP and autoantibodies such as SSA, SSB, ANA, antidsDNA, antiU1snRNP. This should help us recommend a more optimal model for treatment and follow-up of patients with RA and sSS.

Materials and methods

This study was conducted at the University Clinic for Rheumatology in Skopje, North Macedonia. All patients were recruited and followed at this Clinic. We examined 42 patients, aged 18 to 70 years, who were previously diagnosed with RA and sSS, coming to the Clinic for a follow-up visit. All patients were informed about the goals of the study, and they could ask relevant questions.

After signing the informed consent, all the necessary procedures in the study were started.

Patients were divided into two groups, patients with RA and patients with RA and sSS. All patients were diagnosed according to the EULAR criteria for classification and diagnosis of RA and SS. The groups were comparable according to size, age and sex distribution.

Patients with other autoimmune diseases such as but not limited to systemic lupus, sarcoidosis, ankylosing spondylitis, Lyme disease etc. were excluded from the study. Similarly, patients with severe infections, history of irradiation of the neck or head, history of lymphoma or other malignant disease, or regular use of anticholinergics were excluded.

The analysis of RF and CRP were done on a biochemical analysis machine (BioSystems A15). Levels of anti-CCP and antiU1snRNP were determined by ELISA (Elisis Duo Human), while antidsDNA, SSA and SSB were done on Mindray MR-96A ELISA. The antinuclear antibodies (ANA) were analyzed by using immunofluorescence, on an Olympus CX31 microscope, by the same doctor certified for the use of this method.

After collecting data, disease activity was calculated by the use of DAS28-ESR scale. Quality of life was assessed with the HAQ-DI questionnaire. The average pain level was assessed by the patient marking the VAS scale incorporated in the HAQ-DI questionnaire. Disease activity and quality of life was compared between both groups.

All data was collected in digital tables and SPSS v19.1 was used for statistical analysis. Comparation between groups was done by Student's t-test and Chi-square tests. Correlation analysis was done by Pearson's analysis. P values <0.05 were considered statistically significant.

Results

We examined 42 patients, aged 18 to 70 years, with an average age of 61.22 years. Twenty-eight (66.67%) were women and 14 (13.33%) were men. The average disease duration of RA was 10.34 years and 34 patients (80.95%) had positive RF.

As shown in Table 1, there was no statistically significant difference in demographic, clinical and serologic characteristics between the groups. There was a statistically significant difference in levels of SSA and SSB, and ANA Hep2 positivity (which correlates to SSA and SSB levels), which was expected. These antibodies are specific for patients diagnosed with Sjogren's syndrome. There was no significant difference in the levels of antidsDNA and antiU1snRNP.

Table 1. Demographic, clinical, serologic and immunologic data

	Patients with RA and sSS (SD) n=20	Patients with RA (SD) (n=22)	P value
Duration of RA	10.45 (9.98)	10.23 (10.11)	NS
Age	62.12 (12.43)	60.33 (13.19)	NS
ESR (mm/1 hour)	14.82 (8.18)	20.21 (14.48)	NS
CRP (mg/l)	1.42 (5.99)	1.74 (7.89)	NS
Number of tender joints	0.89 (3.11)	0.32 (0.97)	NS
Number of swollen joints	2.33 (3.45)	1.31 (2.75)	NS
VAS (pain level inmm)	38.98 (27.34)	28.79 (22.91)	NS
DAS28-ESR	2.69 (0.97)	3.12 (1.19)	NS
Anti-CCP (U/ml)	127.33 (160.1)	138.21 (141.13)	NS
RF IgG (U/ml)	53.90 (44.2)	133.74 (220.12)	NS
Anti-dsDNA (U/ml)	56.18 (58.55)	72.34 (102.45)	NS
SSA (U/ml)	102.44 (29.19)	28.15 (34.1)	<0.05
SSB (U/ml)	88.23 (20.73)	26.67 (12.29)	<0.05
antiU1snRNP (U/ml)	26.12 (75.76)	28.47 (56.22)	NS
ANA Hep2 positivity	13	3	<0.05
HAQ-DI	0.89 (1.23)	0.94 (1.46)	NS

The value of RF IgG was higher in patients but without statistical significance. The number of tender and swollen joints, pain according to the VAS scale were higher in the sSS group, but this was also statistically insignificant. All patients were treated with a disease modifying anti-rheumatic drug (DMARD).

The comparison of disease activity of RA according to DAS28-ESR and the quality of life according to the HAQ-DI questionnaire showed no statistically significant difference.

Discussion

Secondary Sjogren's syndrome is a common extraarticular occurrence in patients with rheumatoid arthritis. This has beenshown in several studies, such as the one from Norway where 60.7% of the examined patients with RA complained of at least one symptom of dryness (mouth, eyes, trachea, etc.) [5]. One study from Turkey showed significantly less sicca symptoms (11.4%) in patients with RA than data from the UK, USA, but more present than information gathered in east Asia or Africa[10]. Haga et al. showed that 28% of patients with RA complained of at least one symptom of dryness, though sSS prevalence wasminimal, 3.6%[11]. The prevalence of sSS varies greatly, form 43% in Greece to 17% in patients with RA in Great Britain[12].

One study of patients with early arthritis[4] discovered an association between RA disease duration and the prevalence of sSS. As in the Norwegian study, our data did not support this notion.

Although sicca symptoms are considered to be difficult for patients with RA, serious complications are rare[13]. We still have to remain aware of data showing the association of sSS and the doubling of risk for non-Hodgkin's lymphoma, compared to patients with RA without sSS[7], as well as increased mortality[8,9]. There is a suggestion that sSS is associated with higher disease activity in patients with RA 4 and there is a correlation between lower saliva production and higher RA disease activity[5]. We, like Haga et al. did not find an association between DAS28-ESR and sSS. There were more tender and swollen joints in patients with sSS, but it was not statistically significant.

The value of RF was higher in the RA group, but this was statistically insignificant. All patients were treated with DMARDs, but no patients in the sSS group were treated with a biologic drug.

The number of patients with sSS in this study was small(n=20), and hence, there was a high standard deviation and it wa smore difficult to show statistically significant differences. We assume that the increased number of tender and swollen joints in patients with sSS to be a result of the stated statistical weakness, and not showing more severe disease activity (DAS28-ESR had no statistically significant difference between the groups).

We believe that there may be an increased disease activity in the RA group of patients. This is based on the higher levels of the inflammatory markers, HAQ-DI score, as well as presence of patients treated with biologics which is a sign of intran-

sigent and severe disease. With the increase of the number of examined patients, these differences may be better elaborated and the statistical significance may increase.

In conclusion, we found no statistically significant difference in disease activity or quality of life in patients with RA in comparison with those with sSS.

References

1. Jonsson R, Haga H-J, Gordon T. Sjogren's syndrome. In Koopeman WJ (ed.) Arthritis and Allied Conditions. A Textbook of Rheumalology, 14th edn, 2001; pp 1736–59. Lippincott & Williams & Wilkins, Philadelphia, PA.

2. Lazarus MN, Isenberg DA. Development of additional autoimmune diseases in a population of patients with primary Sjogren's syndrome. Ann Rheum Dis 2005; 64(7): 1062–1064.

3. Carmona L, Gonzales-Alvaro I, Balsa A, et al. Rheumatoid arthritis in Spain: occurrence of extra-articular manifestations ad estimates of disease severity. Ann Rheum Dis 2003; 62(9): 897–900.

4. Young A, Koduri G. Extra-articular manifestations and complications of rheumatoid arthritis. Best Pract Res Clin Rheumatol 2007; 21(5): 909–927.

5. Uhlig T, Kvien TK, Jensen JL, et al. Sicca symptoms, saliva and tear productions, and disease variables in 636 patients with rheumatoid arthritis. Ann Rheum Dis 1999; 58(7): 415–422.

6. Skoumal M, Wottawa A. Long term observation study of Austrian patients with rheumatoid arthritis. Acta Med Austriaca 2002; 29(2): 52–56.

7. Kauppi M, Pukkala E, Isoma¨ki H. Elevated incidence of hematologic malignancies in patients with Sjogren's syndrome compared with rheumatoid arthritis (Finland). Cancer Causes Control 1997; 8(2): 201–204.

8. Martens PB, Pillemer S, Jacobsson LTH, et al. Survivorship in a population based cohort of patients with Sjogren's syndrome 1976–1992. J Rheumatol 1999; 26(6): 1296–1300.

9. Turesson C, O'Fallon WM, Crowson C, et al. Occurrence of extra-articular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis. J Rheumatol 2002; 29(1): 62–67.

10. Calguneri M, Ureten K, Akif Ozturk M, et al. Extraarticular manifestations of rheumatoid arthritis: results of a university hospital of 526 patients in Turkey. Clin Exp Rheumatol 2006; 24(3): 305–308.

11. Haga HJ, naderi Y, Moreno AM, Peen E. A study of the prevalence of sicca symptoms and secondary Sjogren's syndrome in patients with rheumatoid arthritis, and its association to disease activity and treatment profile. Int J Rheum Dis. 2012;15(3):284-288.

12. Drosos AA, Lanchbury JS, Panayi GS. Rheumatoid arthritis in Greek and British patients. A comparative clinical, radiology and serology study. Arthritis Rheum 1992;35(7):745-8.

13. Asmussen KH, Bowman SJ. Outcome measures in Sjogren's syndrome. Rheumatology (Oxford), 2001; 40(10): 1085–1088.

CLINICAL SCIENCE

DETECTION OF BIOFILM PRODUCTION AND ANTIMICROBIAL SUSCEPTIBILITY IN CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII* AND *PSEUDOMONAS AERUGINOSA*

Radomir Jovchevski[1], Kakja Popovska[2], Aneta Todosovska Ristovska[1], Maja Lameski[1], Ardian Preshova[3], Mumin Selmani[1], Sara Nedelkoska[1], Hristijan Veljanovski[4], Marija Gjoshevska[1]

[1] Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[2] Institute of Microbiology and Parasitology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
[3] Institute of Public Health of the Republic of North Macedonia, Skopje, Republic of North Macedonia
[4] University Clinic for Neurosurgery; Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Acinetobacter baumannii and *Pseudomonas aeruginosa* are commensal which commonly colonize humans. As a result of their ubiquitous nature, reservoirs in hospital environment and resistance to many antimicrobial agents they are responsible for hospital – acquired infections. Additionally treatment of these infections is difficult because of the ability for biofilm formation. Aim of the paper was to determine the association between biofilm formation on medical devices and antibiotic resistance profile, compared to respiratory samples in clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Material and methods: The study comprised 50 clinical samples (36 from medical devices and 14 as a control group from respiratory secretions). *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were identified by routine microbiological methods. Modification of the microtiter plate assay described by Stepanovic et al. was used to investigate the formation of biofilm. The antimicrobial susceptibility testing was performed according to EUCAST guidelines. Results: Of the 50 analyzed strains, 16 (32%) were non-biofilm producers, and 34 (68%) were producing biofilms. Out of these, 29 (58%) were from medical devices, and 5 (10%) from the control group. Acinetobacter baumannii showed biofilm formation in 19 (67.9%), of which 17 (60.7%) from medical devices, and 2 (7.1%) from control group. *Pseudomonas aeruginosa* produced biofilm in 15 (68.1%), of which 12 (54.5%) from medical devices, and 3 (13.6%) from the control group. Multidrug resistance was detected in 40 (80%). All strains of *Acinetobacter baumannii* were multidrug resistant (MDR). For *Pseudomonas aeruginosa*, 11 (73.3%) biofilm forming isolates were MDR, and 1 (14.2%) non-biofilm forming isolate was MDR. Conclusion: Biofilm production was higher in strains from medical devices. Eighty percent of isolates were MDR. This is a serious challenge for treatment of these hospital-acquired infections.

КЛИНИЧКИ ИСТРАЖУВАЊА

ДЕТЕКЦИЈА НА СПОСОБНОСТА ЗА ФОРМИРАЊЕ БИОФИЛМ И АНТИМИКРОБНАТА ОСЕТЛИВОСТ НА КЛИНИЧКИТЕ ИЗОЛАТИ НА *ACINETOBACTER BAUMANNII* И *PSEUDOMONAS AERUGINOSA*

Радомир Јовчевски[1], Каќа Поповска[2], Анета Тодосовска Ристовска[1], Маја Ламески[1], Ардиан Прешова[3], Мумин Селмани[1], Сара Неделкоска[1], Христијан Велјановски[4], Марија Ѓошевска[1]

[1] Медицински факултет, Универзитет „Св. Кирил и Методиј" во Скопје, Република Северна Македонија
[2] Институт за микробиологија и паразитологија, Медицински факултет, Универзитет „Св. Кирил и Методиј" во Скопје, Република Северна Македонија
[3] Институт за јавно здравје на Република Северна Македонија, Скопје, Република Северна Македонија
[4] Универзитетска клиника за неврохирургија; Медицински факултет, Универзитет „Св. Кирил и Методиј" во Скопје, Република Северна Македонија

Извадок

Acinetobacter baumannii и *Pseudomonas aeruginosa* се комензали кои нормално ги колонизираат луѓето. Нивната убиквитарна природа, присуството во хоспиталната средина како и резистенцијата кон голем број антимикробни средства ги прави одговорни за инфекциите кои се стекнуваат во болничка средина. Дополнително, третманот на овие инфекции е отежнат поради способноста за продукција на биофилм. Целта на оваа студија беше да се одреди способноста на *Acinetobacter baumannii* и *Pseudomonas aeruginosa* да продуцираат биофилм на медицински помагала и нивната резистенција кон антимикробни средства споредена со истите изолати изолирани од примероци од респираторен тракт. Материјал и методи: Примероци од 50 пациенти (36 од медицински помагала, 14 како контролна група од респираторни секрети). *Acinetobacter baumannii* и *Pseudomonas aeruginosa* беа идентификувани со рутински микробиолошки методи. Модификација на микротитарскиот метод опишан од Stepanovic et al. бешеупотребен за детекција на продукција на биофилм. Тестирањето за антимикробна осетливост беше спроведено според препораките на EUCAST (Европски комитет за тестирање на антимикробна осетливост). Резултати: Од 50 анализирани соеви, 16 (32%), не создадоа биофилм, додека, пак, 34 (68%) продуцираа биофилм. Од овие, 29 (58 %) беа од медицински помагала, а 5 (10%) од контролната група. Деветнаесет соеви на *Acinetobacter baumannii* (69.7%) имаа способност за продукција на биофилм. Од нив, 17 (60.7%) соеви потекнуваа од медицински помагала, а 2 (7.1%) од контролната група. Петнаесет соеви на *Pseudomonasaeruginosa* (68.1%) имаа способност за продукција на биофилм. Од нив, 12 (54.5%) соеви потекнуваа од медицински помагала, а 3 (13.6%) од контролната група. Мултирезистентност беше детектирана во вкупно 40 (80%) соеви. Сите соеви на *Acinetobacter baumannii*беа мултирезистентни. Кај *Pseudomonas aeruginosa*,11 (73.3%) соеви што продуцираа биофилм беа мултирезистентни, додека, пак, 1 (14.2%) сој без способност за продукција на биофилм, беше мултирезистентен. Заклучок: Создавањето на биофилм е повисоко кај соевите од медицинските помагала. Осумдесет и еден процент од изолатите беа мултирезистентни. Тоа е сериозен предизвик за третман на овие болнички стекнати инфекции.

Introduction

Acinetobacter and *Pseudomonas* are biochemically inert, opportunistic bacteria that are commensals which commonly colonize humans and animals. These Gram-negative bacteria have the ability to persist and multiply on abiotic surfaces.[1] Although they are ubiquitous in nature, *Acinetobacter* and *Pseudomonas* have a key role and are responsible for hospital-acquired infections, particularly in patients with compromised immune system.[2,3] The highest frequency of infections is found in the intensive care units (ICUs), in which they are most common causative agents of ventilator-associated pneumonia, catheter-associated-bacteremia, urinary tract infections or surgical site infections and are associated with high morbidity and mortality. The increased use of indwelling medical devices, such as central venous catheterization, mechanical ventilation and antimicrobial therapy are considered as crucial factors for upraise of these infections.[4,5]

Both bacteria are intrinsically resistant to many antibacterial agents, but also can acquire resistance to almost any antibiotic. Resistance mechanisms include production of beta lactamase, efflux pumps, or modification of specific target sites. Multidrug resistance (MDR) can occur as a result of single mechanism, or action of different mechanisms in a single isolate.[6] Extensively drug resistant (XDR) and pandrug resistant (PDR) strains are cumulating in various countries.[7] Additionally, treatment of these infections is difficult, because *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have ability to form biofilms.[8]

Biofilms are defined as structured aggregate of bacterial cells, surrounded by extracellular polymeric substance (EPS), which they self - produce and are embedded in, attached to a biotic or abiotic surface. Bacteria in these biofilms are more protected from host immune response, antibiotics and adverse environmental conditions than the free-living planktonic cells. In this communities, bacteria are producing chemical signaling QR (quorum sensing) molecules for cell-to-cell communication during changes in the environment such as temperature, oxygen level, pH etc. Moreover, antibacterial agents when administrated below the minimum inhibitory concentration (MIC) act like stressors and induce biofilm formation.[8, 9]

Extracellular polymeric substance (EPS) is crucial element which provides attachment to surface, adhesion of cells and aggregation. It functions as a three-dimensional structure that enables unity, stability and protection from antimicrobial agents and immune system of the host.[10]

Bacteria in biofilms have a higher resistance against antibiotics than their planktonic cells. This is thought to be caused by different factors such as: the extracellular matrix which acts like a barrier and prevents penetration of the drug, different compounds in the matrix inactivating the drug, metabolic alterations in bacteria within the biofilm, a large number of bacteria compared to available antibiotic and close proximity of bacteria enables exchange of genetic material.[11]

Foreign body implants in vivo are coated with blood components such as collagen, fibrin, fibronectin, etc. which represents a higher risk factor for biofilm formation. Consequently, this results in severe hospital acquired-infections. These biofilms on medical devices with high resistance to antibacterial drugs and constant reinfections pose a huge danger for chronic infections, tissue damage and therapeutic failure.[11, 12]

According to the published data *Acinetobacter* and *Pseudomonas* are the most common nosocomial Gram-negative pathogens, which is contributed to their high resistance to the known antibiotics and persistence in hospital environment. This notorious persistence is attributed to formation of biofilm as one of the virulence factors of these bacteria and consequently causing device-related infections.[8, 13] In this study, we had aim to determine the rates of biofilm production and antibiotic resistance profile among the clinical strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in association with the origin of the sample.

Materials and methods

Our study comprised a total of 50 samples taken from hospitalized patients. Thirty-six of them were from medical devices: tip from central venous catheter, swabs from endotracheal and tracheostomy tubes, swabs from abdominal drain tubes, cerebrospinal liquor from ventricular shunt and hemoculture from catheter. Fourteen samples as a control group were from respiratory secretions (sputum, tracheal aspirate

and bronchoalveolar lavage). All of the samples were derived from the University Clinical Center in Skopje and were submitted for routine laboratory testing at the Institute of Microbiology and parasitology, Faculty of Medicine.

Acinetobacter baumannii and *Pseudomonas aeruginosa* were initially identified using standard laboratory methods including growth on blood agar at 37°C, Gram stain and biochemical tests. Final identification was done with Vitek 2 system (Biomerieux, France).

After the identification, few colonies with identical morphology were stored in trypticase soy broth supplemented with 20 % glycerol at -80°C until further analysis.

The formation of biofilm was assessed with the method previously described by Stepanovic *et al.* with certain modifications.[14]

Bacterial cultures were refreshed from trypticase soy broth (after defreezing, one whole loop was inoculated on blood agar and incubated aerobically for 18 – 24 hours at 37°C). After verifying purity of the strain, few colonies were suspended in saline to acquire McFarland 0.5 suspension containing (~10 8 CFU/ml). This was performed using a photometric device. This blend was homogenized by vortexing for 1 minute.

Tissue culture plates (Laboglob, Germany) with 96 polystyrene, flat bottomed wells were filled with 180 µl trypticase soya broth (TSB) supplemented with 1% dextrose (medium for biofilm cultivation). From the previously prepared bacterial suspension 20 µl were added to each

well. Three wells were used for negative control and contained 200 μl TSB with 1% dextrose per well. Because phenotypic expression of biofilm formation is highly susceptible to different in vitro conditions, to minimize errors, each strain was tested in three wells (triplicate) and each test was carried out two times. The inoculated plates were covered with a lid and incubated aerobically 24 hours at 35 – 37°C under static conditions.

After these 24 hours, the supernatant containing the unattached bacterial cells was gently removed with a pipette and discarded and the plates were washed three times with 300 μl PBS (pH = 7.2) and then drained in an inverted position. Long fixation was done for 20 minutes with 150 μl methanol, thereafter the plates were emptied and left to air dry at room temperature.

To visualize the biofilm formed on each well, at the bottom and at the walls 150 μl crystal violet was used for 15 minutes. This is a cationic dye that stains negatively charged biofilm constituents based on ionic interactions. The stain was removed gently with micropipette and discarded, excess stain was removed with running tap water and the plates were air dried at room temperature. 150 μl 95% ethanol was added for 30 minutes at room temperature to resolubilize the dye from attached cells. Addition of alcohol enables indirect measurement of bacteria attached to the wells.

The absorbance of each well with ELISA microplate reader at 570 nm was measured. Average (mean) OD values were calculated for all tested strains and negative controls, be-cause all tests were performed two times and in triplicate. The cut–off value (ODc) is defined as three standard deviations (SD) above the average OD of the negative controls. For each microtiter plate the cut–off value was determined.

The strains were divided in four categories for easier interpretation of the results, based upon previously calculated mean OD values: OD ≤ ODc = no biofilm formation; ODc < OD ≤ 2*ODc = weak biofilm formation; 2*ODc < OD ≤ 4* ODc = moderate biofilm formation; 4*ODC < OD = strong biofilm formation.

Standard disc diffusion method guidelines[15] by EUCAST (The European Committee on Antimicrobial Susceptibility Testing) were used to test susceptibility to frequently prescribed antibiotics which are active against these bacteria. Antibacterial categories included in the test were: beta–lactams, aminoglycosides, quinolones, sulfonamides and polymyxins. Commercial antibiotic discs (Oxoid, England) that were used for both bacteria were: piperacillin – tazobactam (PTZ, 36 μg), imipenem (IMI, 10 μl), meropenem (MER, 10 μg), ceftriaxone (CAZ, 10 μg), cefepime (FEP, 30 μg), amikacin (AM, 30 μg), ciprofloxacin (CIP, 5 μg). For *Pseudomonas aeruginosa* additionally were used: tobramycin (TB, 10 μg) and levofloxacin (LEV, 5 μg) and for *Acinetobacter baumannii*: ampicillin (AMP, 10 μg), amoxicillin – clavulanate (AMC, 30 μg), ertapenem (ETP, 10μg), cefuroxime (CXM, 30μg), ceftriaxone (CRO, 30μg), gentamicin (GM, 10 μg) and co-trimoxazole (SXT, 25 μg).

Muller Hinton agar (Oxoid) with antibiotic discs was incubated for 24

hours at 37°C and examined for inhibition zones.

Susceptibility for colistin was done with broth microdilution test (Merlin) to determine the MIC according to EUCAST.[16]

Statistical analysis

Biofilm detection by tissue culture plate method was graded as weak/none, moderate and strong. High and moderate production was considered positive and weak/none biofilm production was considered negative. Association of two or more set of variables was analyzed using the Chi – square test. A p value < 0.05 was considered as statistically significant. IBM SPSS version 28 was used for data analysis.

Results

A total number of 50 isolates were obtained. Bacteriological profile included *Acinetobacter baumannii* (n=28) and *Pseudomonas aeruginosa* (n=22). Thirty six were isolated from indwelling medical devices, *Acinetobacter baumannii* (n=21), *Pseudomonas aeruginosa* (n=15) and fourteen respiratory samples served as a control group, *Acinetobacter baumannii* (n=7) and *Pseudomonas aeruginosa* (n=7). In this study, strains were evaluated for their ability to pro-
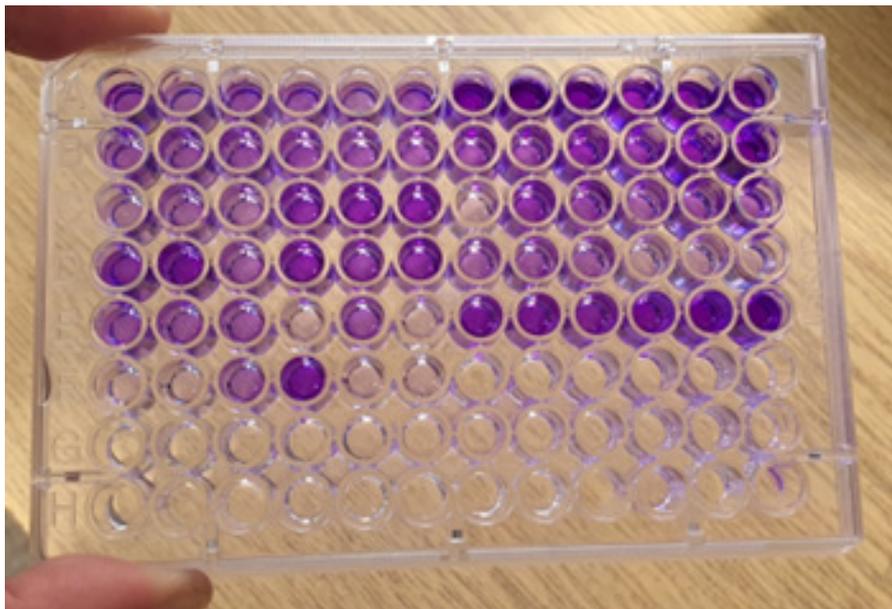

Fig 1. Biofilm formation by the TCP method

duce biofilms by the tissue culture plate method (TCP) Figure 1.

Of the 50 bacterial strains tested, 16 (32%) were non-biofilm producers while 34 (68%) produced biofilms. From those which were biofilm producers, 29 (58%) were from medical devices and 5 (10 %) from the control group.

From 28 isolates of *Acinetobacter baumannii*, biofilm production was detected in 19 (67.9%). In 15 (68.1%) isolates of *Pseudomonas aeruginosa* formation of biofilm was detected.

Out of these, 14 (28%) isolates showed a strong biofilm production, 20 (40%) moderate and 16 (32%) showed weak/ none production.

Acinetobacter baumannii isolated from medical devices showed biofilm formation in 17 (60.7%) strains and only 2 (7.1%) strains were producing biofilm from the control group.

Pseudomonas aeruginosa isolated from medical devices showed biofilm formation in 12 (54.5%) strains and in 3 (13.6%) strains from the control group. The rate of biofilm production between strains from medical devices and control group was found to be statistically significant. The Pearson's chi – square showed a strong statistical significance ($\chi2$ = 9.314; df = 1, p = 0.002), with p < 0.05 (Table 1).

Table 1. Detection of biofilm formation by Tissue culture plate method

Organism	Total number of isolates (N)	Number of BF* isolates by TCP† from medical devices N (%)	Number of BF* isolates by TCP† from control group N (%)
Acinetobacter baummanii	28	17 (60.7%)	2 (7.2%)
Pseudomonas aeruginosa	22	12 (54.5%)	3 (13.7%)
Total number of isolates	50		

*biofilm forming; †tissue culture plate method

Quantification of biofilm formation is shown in Table 2. Seven (25%) strains of Acinetobacter baumannii from indwelling medical devices were strong biofilm producers, 10 (35.7%) were moderate producers and 4 (14.2%) were weak/none biofilm producers. From the control group, there were no strong biofilm producers, 2 (7.1%) were moderate producers and 5 (17.8%) were weak/none biofilm producers.

Five (22.7%) strains of Pseudomonas aeruginosa isolated from medical devices were strong biofilm producers, 7 (31.8%) were moderate and 3 (13.6%) strains were weak/none biofilm producers. From the control group, 2 (9.09%) strains were strong biofilm producers, 1 (4.5%) was moderate and 4 (18.1%) strains were weak/none biofilm producers.

Table 2. Quantification of biofilm formation

	Isolates from medical devices		Control group	
Biofilm formation	Acinetobacter baumannii N (%)	Pseudomonas aeruginosa N (%)	Acinetobacter baumannii N (%)	Pseudomonas aeruginosa N (%)
Strong	7 (33.3%)	5 (33.3%)	0	2 (28.5%)
Moderate	10 (47.6%)	7 (46.6%)	2 (28.5%)	1 (14.2%)
Weak/None	4 (19.04%)	3 (20%)	5 (71.4%)	4 (57.1%)
Total	21 (100%)	15 (100%)	7 (100%)	7 (100%)

All isolates of *Acinetobacter baumannii* (n=28,100%) were resistant to ampicillin, amoxicillin – clavulanate, piperacillin – tazobactam, ertapenem, imipenem, meropenem, cefuroxime, ceftazidime, ceftriaxone, cefepime. Seventeen (89.4%) strains isolated from medical devices showed resistance to gentamicin, 15 (78.9%) isolates showed resistance to trimethoprim – sulfamethoxazole and 1 (5.2%) isolate was resistant to colistin. Out of non–biofilm forming isolates, 8 (88.8%) were resistant to gentamicin and trimethoprim– sulfamethoxazole (Table 3).

Table 3. Antibiotic resistance pattern of biofilm forming and non–biofilm forming Acinetobacter baumannii isolates

Antibiotics	Resistance in BF* isolates Total N=19 (100%)	Resistance in NBF† isolates Total N=9 (100%)
Ampicillin	19 (100%)	9 (100%)
Amoxicillin– clavulanate	19 (100%)	9 (100%)
Piperacillin– tazobactam	19 (100%)	9 (100%)
Ertapenem	19 (100%)	9 (100%)
Imipenem	19 (100%)	9 (100%)
Meropenem	19 (100%)	9 (100%)
Cefuroxime	19 (100%)	9 (100%)
Ceftazidime	19 (100%)	9 (100%)
Ceftriaxone	19 (100%)	9 (100%)
Cefepime	19 (100%)	9 (100%)
Gentamicin	17 (89.4%)	8 (88.8%)
Amikacin	19 (100%)	9 (100%)
Ciprofloxacin	19 (100%)	9 (100%)
Trimethoprim– sulfamethoxazole	15 (78.9%)	8 (88.8%)
Colistin	1 (5.2%)	0

*biofilm forming; †non-biofilm forming

The antimicrobial resistance pattern of *Pseudomonas aeruginosa* isolates which were biofilm producing and non–biofilm producing, is shown in Table 4. A high resistance in biofilm forming isolates was detected against piperacillin–tazobactam 14 (93.3%), followed by cefepime 12 (80%), ceftazidime 11 (73.3%), meropenem, tobramycin, ciprofloxacin, levofloxacin 10 (66.6 %), amikacin 9 (60%), imipenem 6 (40%) and colistin 1 (6.6%). Non-biofilm producing isolates showed resistance to ciprofloxacin, levofloxacin, cefepime 4 (57.1%), piperacillin – tazobactam, imipenem, meropenem, cefepime 3 (42.8%), tobramycin 1 (14.2%).

Table 4. Antibiotic resistance pattern of biofilm forming and non–biofilm forming Pseudomonas aeruginosa isolates

Antibiotics	Resistance in BF* isolates Total N=15 (100%)	Resistance in NBF† isolates Total N=7 (100%)
Piperacillin tazobactam	14(93.3%)	3(42.8%)
Imipenem	6(40%)	3(42.8%)
Meropenem	10(66.6%)	3(42.8%)
Cefepime	12(80%)	3(42.8%)
Ceftazidime	11(73.3%)	4(57.1%)
Tobramycin	10(66.6%)	1(14.2%)
Amikacin	9(60%)	0
Ciprofloxacin	10(66.6%)	4(57.1%)
Levofloxacin	10(66.6%)	4(57.1%)
Colistin	1(6.6%)	0

*biofilm forming; †non-biofilm forming

Multidrug resistance (MDR) was seen in 40 (80%) isolates, of which 30 (88.2%) biofilm forming isolates and 10 (62.5%) non–biofilm producing isolates were MDR. All isolates of *Acinetobacter baumannii*, 19 (100%) of biofilm forming and 9 (100%) of non–biofilm forming, were MDR. Eleven (73.3%) of biofilm forming Pseudomonas aeruginosa isolates were MDR and 1 (14.2%) non–biofilm forming isolate was MDR. In P*seudomonas aeruginosa* isolates, there was a statistically significant difference in MDR in relation to biofilm production ($\chi 2= 6.712$; df = 1; p = 0.001), with p< 0.05 (Table 5).

Table 5. Comparison of multidrug resistance among biofilm forming and non–biofilm forming isolates

Organism	Number of BF* isolates	BF* MDR†	Number of NBF‡ isolates	NBF‡ MDR† N (%)	p value
Acinetobacter baumannii	19	19 (100%)	9	9 (100%)	/
Pseudomonas aeruginosa	15	11 (73.3%)	7	1 (14.2%)	0.001
Total	34	30 (88.2%)	16	10 (62.5%)	

*biofilm forming; †multu–drug resistant; ‡non-biofilm forming

Discussion

In our study out of the 50 isolates, 29 (58%) from medical devices and 5 (10%) from the control group, had the ability to form biofilm. Our results showed that there was a statistically significant difference in biofilm formation in association with origin of the sample (p = 0.002). Analysis of the antibiotic susceptibility of all isolated strains allowed classifying the bacteria in MDR and non–MDR strains. Our data detected 80% and 20% of MDR and non–MDR strains, respectively. Thirty MDR strains (88.2%) were biofilm forming and ten MDR strains (62.5%) were non–biofilm forming. All isolates (n=28) of *Acinetobacter baummannii* were MDR with no difference between biofilm forming and non – biofilm forming, but there was a statistically significant difference in *Pseudomonas aeruginosa* strains which were MDR in association with biofilm formation (p = 0.001).

In the present study, biofilm production in isolates of *Acinetobacter baumannii* from medical devices was observed in 17/21 (60.7%), out of which 25% were strong and 35.7% were moderate producers. In 2/7 (28.5%) isolates from the control group moderate biofilm formation was detected. This result shows that strains from other microbiological samples have significantly lower ability for biofilm production compared to strains from medical devices. This was supported by the results obtained by Revdiwala *et al.*[17] who noted 17/23 (73.9%) biofilm forming isolates from inserted devices, 69.5% were moderate and 4.3% strong producers, while only in 1 isolate from non-ventilator associated respiratory tract infection biofilm formation was detected by Rodriguez et *al.*[18] On the other hand, Lee HW et *al.*[19] in their study demonstrated that all 23 clinical isolates of *Acinetobacter baumanni* had the ability to form biofilm.

In our study, *Pseudomonas aeruginosa* isolates from medical devices had the ability for biofilm formation in 12/15 (54.5%), 5 (33.3%) strong and 7 (46.6%) moderate producers. In 3/7 (13.7%) isolates from the control group, 2 (28.5%) were strong producers from patients with cystic fibrosis and 1 (14.2%) isolate was a moderate producer. These results were in agreement of those presented by Danin PE et *al.*[20] who demonstrated

that from 22 biofilm forming isolates, the most frequent organism found in endotracheal tubes was Pseudomonas aeruginosa (58.3%). Diez–Aguilar M et al.[21] in their study demonstrated that from 53 respiratory samples from patients with cystic fibrosis, 32 (60.4%) were biofilm producers.

All *Acinetobacter baumannii* strains in our study were resistant to commonly prescribed antibiotics (100%), except in biofilm forming isolates where two were sensitive to gentamicin and four to trimethoprim sulfamethoxazole. One (5.2%) isolate was resistant to all antibacterial categories including colistin. In contrast to this, the study by Eze EC et *al.*[22] noted resistance of 17% to all antibiotics in all categories. In non–biofilm forming isolates, one isolate was sensitive to gentamicin and one to trimethoprim sulfamethoxazole. This susceptibility pattern was supported by Konca K et al.[23] who analyzed the antimicrobial susceptibility in MDR *Acinetobacter baumannii* strains. Resistance to colistin was 2.2% and to trimethoprim – sulfamethoxazole 73.9%, so these antibiotics are therapy choice.

For biofilm forming isolates of *Pseudomonas aeruginosa*, high resistance was detected for beta-lactams, 93.3%, 80% and 73.3% for piperacillin–tazobactam, cefepime and ceftazidime respectively, followed by fluoroquinolones 66.6%. This is in contrast with the results obtained by Folliero V et al.[24] who noted, based on the sensitivity level of biofilm forming isolates, that fluoroquinolones were a potential treatment for these infections. Low resistance was noted for amikacin (60 %). In our study, one strain (6.6%) was resistant to colistin. Non–biofilm forming isolates showed

resistance to fluoroquinolones with 57.1%, followed by beta-lactams with 42.8%. Low resistance of 14.2% for tobramycin and all strains sensitive to amikacin shows that these antibiotics are therapy choice in patients without cystic fibrosis which has been supported by Tanriverdi E et al.[25] They found that treatment with inhalation of tobramycin decreased hospitalization rates and improved the symptoms.

In the current study, all isolates (n=28) of *Acinetobacter baummannii* were MDR with no difference between biofilm forming and non–biofilm forming. As for Pseudomonas aeruginosa, 11/15 (73.3%) biofilm forming isolates were MDR and only 1/7 (14.2%) non–biofilm forming strain was MDR. This coincided with the results of Abidi SH et al.[26] who showed that production of biofilm was higher among MDR *Pseudomonas aeruginosa* strains than in non-MDR strains.

We documented a high rate of biofilm production in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from medical devices. These bacteria as hospital pathogens are responsible for chronic and multidrug resistant infections. This represents a serious challenge to clinicians in the treatment and care of these patients. Antibiotics belonging to the class of polymyxin E (colistin) and in small percentages aminoglycosides and trimethoprim – sulfamethoxazole, are effective for biofilm producing strains.

Conclusion

This study was based only on phenotypic method for biofilm detection. This is a simple, reliable, accurate

method and can be utilized for biofilm screening. Future studies with molecular methods should identify genes responsible for biofilm production and resistance to antimicrobial agents.

References

1. Lupo A, Haenni M, Madec JY. Antimicrobial resistance in *Acinetobacter spp.* and P*seudomonas spp.* Microbiol Spectr 2018;6(3).

2. Weber BS, Harding CM, Feldman MF. Pathogenic Acinetobacter: from the cell surface to infinity and beyond. J Bacteriol 2015;198(6):880-887.

3. Moradali MF, Ghods S, Rehm BH. *Pseudomonas aeruginosa* Lifestyle: A paradigm for adaptation, survival and persistence. Front Cell Infect Microbiol 2017;7:39.

4. Jurado-Martin I, Sainz-Mejias M, McClean S. *Pseudomonas aeruginosa*: An audacious pathogen with an adaptable arsenale of virulence factors. Int J Mol Sci 2021;22(6):3128.

5. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and pathophysiological overview of *Acinetobacter infections*: A century of challenges. Clin Microbiol Rev 2017; 30(1):409-447.

6. Djordjevic ZM, Folic MM, Jankovic SM. Previous antibiotic exposure and antimicrobial resistance patterns of *Acinetobacter spp.* and *Pseudomonas aeruginosa* isolated from patients with nosocomial infections. Balkan Med J 2017;34(6):527-533.

7. Moubareck CA, Halat DH. Insights into *Acinetobacter baumannii*: a review of microbiological, virulence and resistance traits in a threatening nosocomial pathogen. Antibiotics (Basel). 2020;9(3):119

8. Thi MTT, Wibowo D, Rehm BH. *Pseudomonasaeruginosa*biofilms. Int J Mol Sci 2020;21(22):8671.

9. Eze EC, Chenia HY, El Zowalaty ME. *Acinetobacter baumannii biofilms*: effects on physisochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. Infect Drug Resist 2018;11:2277-2299.

10. Jiang Y, Geng M, Bai L. Targeting biofilms therapy: Current research strategies and development hurdles. Microorganisms 2020; 8(8):1222.

11. Schulze A, Mitterer F, Pombo JP, Schild S. Biofilms by bacterial human pathogens: Clinical relevance – development, composition and regulation – therapeutical strategies. Microb Cell 2021;8(2):28-56.

12. Magana M, Sereti C, Ioannidis A et al. Options and limitantions in clinical investigation of bacterial biofilms. Clin Microbiol Rev 2018;31(3):e00084-16.

13. Brisse S, Milatovic D, Fluit AC et al. Molecular surveillance of European quinolone-resistant clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter spp.* using automated ribotyping. J Clin Microbiol 2000; 38(10):3636-45.

14. Stepanović S, Vuković D, Hola V et al. Quantification of bio-

film in microtiter plates: over-view of testing conditions and practical recommendations for assessment of biofilm produc-tion by staphylococci. APMIS. 2007;115(8):891-9.

15. EUCAST. Antimicrobial suscep-tibility testing EUCAST disk dif-fusion method. Version 9.0. 2021.

16. EUCAST. Reading guide for broth microdilution. Version 3.0. 2021.

17. Revdiwala S, Rajdev BM, Mulla S. Characterization of bacterial etiologic agents of biofilm forma-tion in medical devices in critical care setup. Crit Care Res Pract 2012:945805.

18. Rodríguez-Baño J, Martí S, Soto S et al. Biofilm formation in A*cinetobacter baumannii*: asso-ciated features and clinical im-plications. Clin Microbiol Infect 2008;14(3):276-8.

19. Lee HW, Koh YM, Lee JC et al. Capacity of multidrug – resis-tant clinical isolates of *Acineto-bacter baumannii* to form bio-film and adhere to epithelial cell surfaces. Clin Microbiol Infect 2008;14(1):49-54.

20. Danin PE, Girou E, Legrand P et al. Description and microbiol-ogy of endotracheal tube biofilm in mechanically ventilated sub-jects. Respir Care 2015;60(1):21-9.

21. Diez – Aguilar M, Ekkelenkamp M, Morosini MI *et al*. Anti – biofilm activity of murepavadin against cystic fibrosis P*seudomonas aeru-ginosa isolates*. J Antimicrob Che-mother 2021;76(10):2578-2585.

22. Eze EC, El Zowalaty ME, Pillay M. Antibiotic resistance and biofilm formation of *Acinetobacter bau-mannii* isolated from high – risk effluent water in tertiary hospi-tals in South Africa. J Glob Anti-microb Resist 2021;27:82-90.

23. Konca C, Tekin M, Geyik M. Sus-ceptibility patterns of multi-drug – resistant *Acinetobacter baumannii*. Indian J Pediatr 2021;88(2):120-126.

24. Folliero V, Franci G, Dell'Annunziata F et al. Evalua-tion of antibiotic resistance and biofilm production among clini-cal strain isolated from medi-cal devices. Int J Microbiol 2021; 2021:9033278.

25. Tanriverdi E, Yildirim BZ, Gul S et al. Results of tobramycin in-halation therapy in patients with nocystic fibrosis bronchiectasis with P*seudomonas aeruginosa colonization*: real life manage-ment. J Aerosol Med Pulm Drug Deliv 2021;34(5):274-279.

26. Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, Kazmi SU. Drug re-sistance profile and biofilm form-ing potential of *Pseudomonas ae-ruginosa* isolated from contact lenses in Karachi – Pakistan. BMC Ophthalmol 2013;13:57.

CLINICAL SCIENCE

TREATMENT OF VENOUS MALFORMATIONS IN PEDIATRIC POPULATION – THREE- YEAR EXPERIENCE

Roza Sokolova[1], Shaban Memeti1, Toni Risteski[1], Biljana Andonovska[1], Njomza Lumani-Bakiji[1], Aleksandar Stepanovski[1]; Borche Kocevski[2]

[1] University Clinic of Pediatric Surgery; Ss. Cyril and Methodius University in Skopje, Faculty, of Medicine, Republic of North Macedonia.
[2] Clinical Hospital Tetovo, Surgical department, Republic of North Macedonia

Abstract

Venous malformations (VMs) are a type of vascular malformations that result in abnormal development of veins that become extensible over time due to an error in vascular morphogenesis. They usually appear in newborns or in early adulthood as a bluish, soft, swollen and eventually painful skin formation. Treatment includes conservative therapy, sclerotherapy, and surgical excision. Aim of the paper is to evaluate the therapeutic effect of sclerotherapy in pediatric patients with venous malformations. Material and methods: In a three-year period, from 2019 to 2021, venous malformations were found in 33 patients aged 4 to 14 years (average age: 8 years). Pain as a symptom occurred in 8 patients. Two patients had lesions measuring up to 5 cm and 5 cm, respectively, while in the remaining subjects the lesion was over 5 cm. Ultrasound was performed routinely in all subjects, and MRI in two patients. Conservative treatment was instituted in 13 patients with venous malformations of the extremities; surgical excision with local reconstruction was performed in 11 patients, and sclerotherapy with bleomycin under general anesthesia was performed in 8 patients. Combined treatment was used in one patient that presented with venous malformation of the upper arm that underwent partial sclerotherapy with subsequent operative excision due to a phlebolith. Follow-up examinations revealed regression of the change not only from functional but from aesthetic aspect as well. Conclusion: Sclerotherapy is the established golden standard, first-line treatment for venous malformations. Excellent results were achieved as the reduction of the lesions was below 50% of the initial size. However, the modality of treatment should be individualized to each patient as it can sometimes require a combination of more than one treatment option. Venous malformations are best treated early, but they usually recur over time. Treatment helps relieve symptoms and control the growth of vascular malformations.

КЛИНИЧКИ ИСТРАЖУВАЊА

ТРЕТМАН НА ВЕНСКИ МАЛФОРМАЦИИ КАЈ ПЕДИЈАТРИСКА ПОПУЛАЦИЈА – ТРИ-ГОДИШНО ИСКУСТВО

Роза Соколова[1], Шабан Мемети[1], Тони Ристески[1], Биљана Андоновска[1], Њомза Љумани-Бакији[1], Александар Степановски[1], Борче Коцевски[2]

[1] Универзитетска клиника за детска хирургија; Универзитет „Св. Кирил и Методиј“ во Скопје, Медицински факултет, Република Северна Македонија
[2] Клиничка болница – Тетово, Хируршки оддел, Република Северна Македонија

Извадок

Венските малформации се тип на васкуларни малформации што резултираат со патолошки развој на вените, кои стануваат проширени со тек на време како резултат на нарушување во морфогенезата. Обично се манифестираат во новороденечка или рана адултна возраст како сини, меки, отечени и болни на палпација кожни формации. Третманот вклучува: конзервативна терапија, склеротерапија и хируршка ексцизија. Цел на трудот е да се евалуираат терапевтските ефекти од склеротерапијата кај педијатриски пациенти со венски малформации. Материјали и методи: Во 3-годишен период (2019-2021), венските малформации беа дијагностицирани кај 33 пациенти на возраст од 4 до 14 години (средна возраст: 8 години). Болка како симптом се јави кај 8 пациенти. Кај двајца пациенти лезијата беше поголема од 5 цм и еднаква на 5 цм соодветно, додека кај останатите пациенти лезиите беа под 5 цм. Ултрасонографија беше ординирана рутински кај сите пациенти и МРИ кај двајца пациенти. Конзервативниот третман беше ординиран кај 13 пациенти со венски малформации на екстремитетите, хируршка ексцизија со локална реконструкција кај 11 пациенти, додека кај 8 пациенти беше изведена склеротерапија со блеомицин во услови на општа анестезија. Комбинирана терапија се употреби кај еден пациент со клиничка слика на венска малформација на надлактица, кај кого третманот вклучуваше парцијална склеротерапија со последователна оперативна ексцизија заради флеболит. Контролните прегледи покажаа регресија на промената, не само од функционален туку и од естетски аспект. Заклучок: Склеротерапијата е воспоставен „златен стандард“ и прва линија на третман на венските малформации. Беа постигнати одлични резултати, со оглед на редукција на лезијата под 50% од иницијалната големина. Сепак овој тип на третман потребно е да се индивидуализира за секој пациент, со оглед на тоа што побарува комбинација на повеќе од еден тераписки модалитет. Венските малформации најоптимално се лекуваат рано заради тенденцијата за рекурентност. Со помош на третманот се подобруваат симптомите и се контролира растот на венските малформации.

Introduction

Vascular anomalies account for 5% of pathological conditions in the pediatric population. The presence of a vascular anomaly means abnormal growth and development of capillaries, veins, arteries, and/or lymphatic vessels.

Venous malformations (VMs) are a type of vascular malformations that result in abnormal development of veins that become extensible over time due to an error in vascular morphogenesis. They belong to the group of slow-flow or low-flow malformations with lesions composed of capillaries, veins and/or lymphatic blood vessels. Venous malformations usually appear in newborns or in early adulthood as a bluish formation of the skin that is soft on palpation, swollen and eventually painful[1-3]. They are characterized by irregular blood flow and an increased risk of intralesional and/or systemic thrombosis. The presence of thrombosis is manifested by episodes of pain and small palpable superficial thrombi. The very presence of thrombi leads to a loss of elasticity of the vessel wall. The walls of the veins that make up the venous malformation lack smooth muscle compared to normal veins. The cause of this type of malformation is unknown, but research shows the involvement of certain genetic mutations[4].

VMs can be quite sensitive and accompanied by pain. These types of malformations appear as bluish skin discoloration and can express as a simple or multiple lesions. In terms of depth, the lesions can be deep or superficial. VMs can cover one or more areas of the body with focal, multifocal or diffuse distribution. The most common occurrence is on the extremities (40%), followed by the neck, mouth, part of the face, scalp etc. Superficial VMs range from small dots to more extensive changes[5]. The grading is based on the findings of MR with VMs less than 5 cm, equal to 5 cm and those larger than 5 cm.

The symptoms of venous malformations depend on their location. By growing larger they can exert compressive effect on the surrounding tissues and organs.

The International Society for the Study of Vascular Anomalies (ISSVA) is used as a standard nomenclature for diagnosis and treatment of vascular anomalies[6]. According to this classification vascular anomalies are divided into vascular tumors and vascular malformations. It was approved in 2014 and further updated in 2018 with the incorporation of new anomalies and causal genes.

The diagnosis is made by clinical examination, ultrasonography, magnetic resonance imaging or magnetic resonance imaging with venography. Ultrasonography can help if the VM is superficial. It is a useful non-invasive technique. Color Doppler is recommended for a more accurate diagnosis. MRI or MR venography as a method for further evaluation shows the relationship of surrounding tissues with the pathological change, depth of lesion, blood flow, presence of phleboliths etc.

Positive clinical signs include bluish discoloration of skin, soft and compressible formation, pain, swelling and the involvement of superficial or deeper structures. Negative clini-

cal signs include pulsation, thrill and hyperemia.

Asymptomatic venous malformations do not require treatment.

The basic non-surgical treatment consists of appropriate elastic compression with socks or gloves, depending on whether the VM is located on the leg or arm, so as to alleviate the symptoms of swelling and pain. Minimal doses of aspirin are administered to prevent phleboliths. Other modalities of treatment include sclerotherapy, laser therapy, embolization and surgical intervention.

Sclerotheapy is a minimally invasive method that is used as monotherapy or in combination with surgical treatment. Ethanol and bleomycin are commonly used sclerosing agents. Direct percutaneous puncture (DPP) is a method of injecting a fluid under fluoroscopic control. The volume of ethanol injected is 0.15-1mL/kg of body weight every 10 minutes. General anesthesia is required due to the pain and the treatment is repeated as needed after 3 to 4 weeks. Bleomycin as a sclerosing agent is administered at 1 mL/kg of body weight with no more than 15 mL per sclerosing intervention[7]. It is performed under ultrasonographic control and general anesthesia. The treatment can be repeated after 3 to 4 months. Sclerotherapy with sodium decyl sulfate. Several treatments are usually needed for complete withdrawal. Extensive lesions are usually treated with 95% ethanol, whereas smaller lesions could be treated with sodium tetradecyl sulfate. Sclerotherapy is usually performed by an experienced pediatric surgeon.

Aim of the paper is to evaluate the therapeutic effect of scleraotherapy in pediatric patients with venous malformations.

Material and methods

During a 3-year period, from 2019 to 2021, at the Clinic for Pediatric surgery - Skopje, 33 patients with venous malformation were treated. The patients were initially presented to the outpatient department with a diagnosis of hemangioma. A history carefully taken revealed that the lesion had been present since birth in all patients. It increased with crying and exertion with no signs of regression over time. The diagnosis was reached by ultrasonography examination, and MRI was performed in two children. The latter imaging modality was used to rule out the presence of arteriovenous malformation. Our series included 17 males and 16 female patients, in the age range from 4 to 14 years, with mean age of 8.6 years. The venous malformation was located on the extremities in 24 patients (12 patients with lesions on the upper and lower extremity respectively), on the back in 3 patients and the neck region was affected in 2 patients. In the remaining 4 patients the lesion was noticed in other areas (face, inguinal region etc.). Predominant symptom was pain and swelling in the affected area reported by almost all subjects. In terms of size, two patients had lesions measuring up to 5 cm and 5 cm, while in the remaining subjects the lesion was over 5 cm. Conservative treatment was instituted in 13 patients.

Results

In 8 patients sclerotherapy with bleomycin was performed due to the presentation with pain (Fig.1,2). Side effects included local skin rash and swelling that were alleviated by topically applied dressings with boric acid solution and oral analgesics. Sclerotherapy was also performed in a child with venous malformation on the neck due to an intermittent pain that limited mobility of the neck (Fig.3). The procedure with bleomycin as sclerosing agent was performed in the operative theater under general anesthesia. Follow-up examinations revealed regression of the change not only from functional but from aesthetic aspect as well (Fig.4).

Fig 1. VM on the arm before sclerotherapy

Fig 2. Outcome after one session of sclerot herapy

Fig 3. VM on the neck before sclerosing intervention

Fig 4. VM after two sessions of sclerotherapy

Surgical treatment was indicated in 11 patients as a result of palpable phleboliths and painful local lesion. Surgical excision with local reconstruction was performed (Fig.5,6). In two of the surgically treated children the lesion was located on the back and limb respectively. Intraopera-tive phlebolithswere found in all children. No postoperative complications were reported with normal findings at the follow-up ultrasonographic studies. The histopathologicfindings described: "benign formation of widened venous vessels with no signs of malignancy".

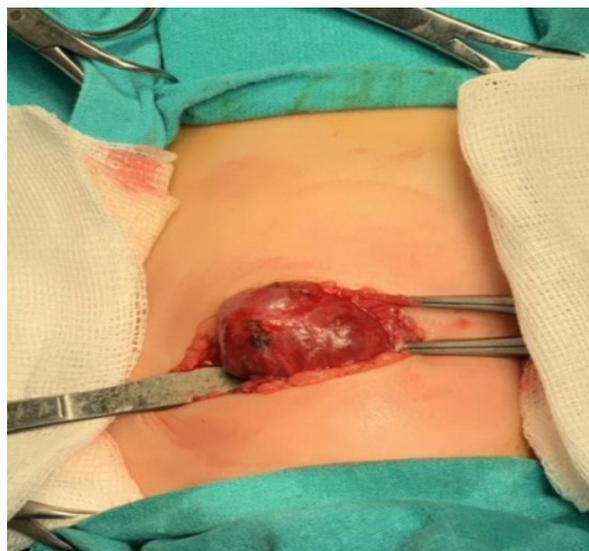**Fig 5.** VM of left axillar region, preoperative

Fig 6. VM of left axillar region, intraoperative finding

Combined treatment was used in one patient that presented with venous malformation of the upper arm that underwent partial sclerotherapy with subsequent operative excision due to a phlebolith.

Klippel-Trenaunay syndrome was diagnosed in 3 patients that were presented to us.

Table 1. Types of treatment in all patients

Treatment	Conservative	Sclerotherapy	Operative	Combined
Number of patients	13	8	11	1

In children with venous malformations of the extremities, a conservative treatment was initiated by applying an elastic bandage (sock or glove) in 6 patients, heparin-sodium ointment topical-ly in 6 patients and application of minimal doses of anti-coagulant therapy in 1 patient. The conservative treatment was institut-ed in 13 patients. They all achieved good results at the 2-month follow-up examination (Table 1).

Eight patients were treated with sclerosing agent bleomycin. In VMs less than 5 cm in size, sclerotherapy in one session in 3 patients yielded excellent results at the 2 month fol-low-up. The remaining 5 patients

had VMs more than 5 cm. In 2 of them sclerotherapy was performed in one session and in 3 patients in more than one session. They achieved good and excellent results respectively at the 2-month follow-up. Small lesions required only one sclerotherapy treatment, while widespread bulky lesions required second treatment after 3 months (Table 2).

Surgical treatment was used in 11 patients. In 7 of them the lesion was less than 5 cm and in the remaining 4 patients the lesion was more than 5 cm. Both groups showed good results in the reduction of the venous malformations and the combined treatment was used in one patient that presented with venous malformation of the upper arm that underwent partial sclerotherapy with subsequent operative excision due to a phlebolith.

Table 2. Patients treated with sclerotherapy

Category	Treatment	Patients	Results
Group I Venous malformations (less than 5 cm in size)	Sclerotherapy as monotherapy	3	Excellent
Group II Venous malformations (more than 5 cm in size)	Sclerotherapy as monotherapy	2	Good
Group III	Sclerotherapy as monotherapy – repetitive after 3 to 4 months	3	Good

Excellent results can be considered having in regard that the reduction of the lesion is below 50% of the initial size. Good results can be considered having in regard that the reduction of lesion is up to 50%, with unsatisfactory result meaning less than 20% of the initial size. In terms of long-term outcomes, further research and follow-up is required to investigate the durability of the amelioration of pain and the rates of recurrence following treatment after 2-month follow up.

Discussion

Venous malformations occur with an incidence of 1-5 in 10.000 births and prevalence of 1% and constitute two thirds of all congenital vascular malformations. They arise at different body regions including the head and neck (40%), extremities (40%) and trunk (20%), and their size varies from superficial asymptomatic to extensive and disfiguring lesion8. VMs are soft-tissue formations, characterized by slow blood circulation, which can cause blood clots to form calcifications. These are the so-called phleboliths or venous stones, they tend to grow without spontaneous regression. One of the features of VM is that it becomes larger during effort when the child cries. The symptoms of venous malformations depend on their location. By growing larger, they can exert compressive effect on the surrounding tissues and organs. Extremely large venous malformations can cause inflammation with activa-

95

tion of endothelial cells, intravascular coagulation with many blood clots of proteins resulting in the so-called local intravascular coagulation characterized by disturbance of plasma fibrinogen, factor V, factor VII and factor VIII levels of coagulation and elevation of D-dimers. They present mostly as sporadic cases, although familial inheritance is possible. Complex combined vascular malformations include several syndromes such as Klippel-Trenaunay syndrome, Parkes Weber Syndrome, extensive diffuse low-flow venous malformations, Bannayan-Riley syndrome, Maffuci's syndrome, cutis marmorata teleangiectatica congenita, CLOVES syndrome, Proteus sydrome etc[9,10].

The ISSVA classification system first distinguishes CVMs between vascular tumors and vascular malformations. This is especially important as vascular malformations are frequently missdiagnosed as hemangiomas. The Hamburg Classification classifies vascular malformations into five main categories: arterial, venous, arteriovenous, lymphatic, and combined vascular malformations. Furthermore, it divides these categories into two embryological based subcategories, extratruncular or truncular lesions. Extratruncular malformations occur in earlier embryonic stages, while truncular anomalies form during the later stages of embryonic development[11].

According to their vascular hemodynamics, vascular malformations are divided into high-flow malformations witch include arteriovenous malformations, and low-flow malformations that include lymphatic malformations, capillary-venulous malformations, venous malforma-

tions, glomovenous malformations, and non-shunting mixed lesions[12]. The most common type of low-flow vascular lesions are VMs, subdivided into sporadic VMs (94%), dominantly inherited cutaneomucosal VMs (1%) and, dominantly inherited and non-inherited glomuvenous malformations (5%) first described by Vikkula et al[13].

Accurate diagnosis has been a limiting factor in vascular malformation management. Non-invasive imaging methods are preferred. Doppler ultrasound and magnetic resonance imaging are key imaging methods used todiagnose vascular malformations. Contrast-enhanced MRI and MRA are the preferred imaging modalities for pre-procedure diagnosis and interventional planning as well as post-procedure evaluation of the vascular malformation. Conventional MRI has 100% sensitivity and 24% to 33% specificity in differentiating VMs from non-VMs 14. Dynamic contrast MR angiography increases specificity to 95%. Direct percutaneous puncture with contrast injection or phlebography (DPP) is the fine-needle puncture of the VM with subsequent contrast injection under fluoroscopy. It is the gold standard diagnostic tool for specificity when confirmation of a VM is required after alternative imaging approaches have not yielded definitive results, in cases when treatment planning is required or when a neoplasm must be ruled out.

Prior to treatment, the surgeon should consider lesion's pathophysiology, etiology, and consequences of the procedure. There may be instances when it is prudent to delay intervention in favor of observation, or to avoid intervention if there are

no significant symptoms and risks. Lesions with severe symptoms and/ or potential complications should be treated. Extratruncular subtype of venous malformation is more likely to require treatment, as they display worse symptoms and higher recurrence rate than truncular forms.

Treatment includes conservative therapy, sclerotherapy and surgical excision. Surgical intervention was traditionally considered to be the initial form of treatment if the lesion could be completely resected and had minimal anatomic and functional consequences. However, the emergence of sclerotherapy as a viable yet cost-effective and minimally invasive technique has spurred its use as mono-therapy or in conjunction with surgery[15]. As excision of complex lesions remains difficult due to secondary intraoperative bleeding, the favored approach is now sclerotherapy. Therefore, sclerotherapy is the established golden standard, first-line treatment for VMs[16]. Bleomycin is derived from Streptomyces verticillus as antibiotic with cytotoxic and antineoplastic features and induces both single- and double-stranded DNA break down in endothelial sclerosants. It has also been found that it elicits the least inflammatory response[17]. It shows similar efficacy as alcohol in superficial venous malformations but with fewer side effects. The overall response of bleomycin treatment is reported in range from 70-100% with a complication rate of 6%. Mucositis, alopecia, pulmonary toxicity and hyperpigmentation have been mentioned as adverse effects 8. The reported effectiveness of bleomycin is between 43–82.7% [18-20]. However, the modality of treatment should be individualized to each patient as it can

sometimes require a combination of more than one treatment option and the application of appropriate sessions. Ahmad et al. in his case series of seven patients with venous malformations in the orbital and paraorbital region treated with percutaneous sclerotherapy with bleomycin reported higher efficacy of bleomycin than other sclerosing agents, no major systemic adverse effects and emphasised the safeness of this procedure. In the reported 2 female children of 5 and 7 years of age, he noticed improvement in the outcome at 9 and 12 months of follow-up respectively[17]. Shigematsu et al. in his retrospective review of 18 patients with VM of the eyelid reported more than 80% reduction and 50-80% reduction in seven and eight patients respectively by using bleomycin sclerotherapy. Recurrence was reported in one patients, with no complication related to the procedure. He concluded that the use of bleomycin appeared to be a simple, safe and effective treatment for VM affecting the eyelid and that it avoided the more complex procedures of surgery and laser interventions[21]. Mohan et al. came to the similar conclusion in their retrospective study of the outcome of 32 children with proven VM treated with intralesional bleomycin injection[22]. Although Zhang et al. in their large randomised study involving 138 children demonstrated superior curative effects of absolute ethanol in comparison to bleomycin, ethanol therapy showed greater incidence of adverse effects compared to bleomycin, in first place skin necrosis, localized swelling, muscle fibrosis and even brain embolism[23]. Some studies demonstrated more successful results of a combined sclerotherapy with laser or surgical therapy in

the cervicofacial venous malformations[24,25].

Post therapy follow up assessment is vital in a clinical setting. A significant percentage of patients will suffer from their VMs again after treatment. In particular female patients who undergo pregnancy or younger patients undergoing puberty as hormonal changes trigger recurrence or proliferation. MRI is most suited to evaluate clinical outcomes after successful sclerotherapy. There is not enough evidence to the superior effect of one treatment over the other in terms of obtaining favorable results.

Conclusion

Our series showed that there are different treatment approaches in the management of venous malformations in children. The modality of treatment depends on the location of lesion, accompanying symptoms, size of formation, presence of phlebolith etc. The study determines the prevalence of this pathology by sex, age and associated syndromes if present. It emphasizes the importance of monitoring these children for a certain period of time for possible additional treatment. A multidisciplinary approach is important for the management of pediatric vascular abnormalities especially for venous malformations. For proper treatment it is recommended to include a team of pediatric hematologist, interventional radiologist, surgeon and anesthetist. US and MRI are the two central imaging techniques in the work-up of VMs. DPP is a golden standard diagnostic tool utilized when other imaging is equivocal. It also plays a central role in aiding decisions during sclerotherapy.

In our series almost all of the patients in the sclerotherapy group achieved excellent results i.e. reduction for more than 50% of the initial size either by monotherapy or repetitive therapy in comparison to the conservative and surgical therapy group. Sclerotherapy is the gold standard for treating these malformations and is the first line of treatment. The usage of preoperative sclerosing therapy with N-butyl cyanoacrylate (n-BCA) has been described in literature 24 to 48 hours before the surgical excision. Combined therapy is sometimes necessary in order to achieve satisfactory results.

Venous malformations are best treated early; however, they usually recur over time. It is important to note that treatment helps relieve symptoms and control the growth of VMs.

References

1. Redondo P, Aguado L, Martínez-Cuesta A. Diagnosis and management of extensive vascular malformations of the lower limb: part II. Systemic repercussions, diagnosis, and treatment. Journal of the American Academy of Dermatology 2011; 65: 909-923.

2. Lobo-Mueller E, Amaral JG, Babyn PS, Wang Q, John P. Complex combined vascular malformations and vascular malformation syndromes affecting the extremities in children. Semin Musculoskelet Radiol 2009; 13(3):255-276.

3. Lee MS, Liang MG, & Mulliken JB. Diffuse capillary malformation with overgrowth: a clinical subtype of vascular anomalies with hypertrophy. Journal of the

American Academy of Dermatology 2013; 69: 589-594.

4. Mazoyer E, Enjolras O, Laurian C, Houdart E, & Drouet L. Coagulation abnormalities associated with extensive venous malformations of the limbs: differentiation from Kasabach–Merritt syndrome. Clinical & Laboratory Haematology 2002; 24: 243-251.

5. Enjolras O, Ciabrini D, Mazoyer E, Laurian C, Herbreteau D. Extensive pure venous malformations in the upper or lower limb: a review of 27 cases. Journal of the American Academy of Dermatology 1997; 36: 219-225.

6. ISSVA classification for vascular anomalies. (n.d.). Retrieved March 10, 2022, from https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf

7. Ernemann U, Kramer U, Miller S, Bisdas S, Rebmann H, Breuninger H, et al. Current concepts in the classification, diagnosis and treatment of vascular anomalies. European journal of radiology 2010; 75: 2-11.

8. Hage A N, Chick J, Srinivasa R N, Bundy J J, Chauhan N R, Acord M, Gemmete JJ. Treatment of venous malformations: The data, where we are, and how it is done. Techniques in vascular and interventional radiology 2018; 21(2): 45–54.

9. Glovkzki P, & Driscoll D (2007). Klippel–Trenaunay syndrome: current management. Phlebology 22: 291-298.

10. Noel AA, Gloviczki P, Cherry Jr KJ, Rooke TW, Stanson AW, & Driscoll DJ (2000). Surgical treatment of venous malformations in Klippel-Trenaunay syndrome. Journal of vascular surgery 32: 840-847.

11. Lee BB, Baumgartner I, Berlien P, et al. Diagnosis and Treatment of Venous Malformations. Consensus Document of the International Union of Phlebology (IUP): updated 2013. Int Angiol 2015

12. Richter GT, Friedman AB. Hemangiomas and vascular malformations: current theory and management. Int J Pediatr 2012; 2012:645678.

13. Vikkula M, Boon LM, Mulliken JB. Molecular genetics of vascular malformations. Matrix Biol 2001;20:327-335.

14. Van Rijswijk CS, van der Linden E, van der Woude HJ, et al. Value of dynamic contrast-enhanced MR imaging in diagnosing and classifying peripheral vascular malformations. AJR Am J Roentgenol 2002; 178(5):1181-1187.

15. Legiehn GM, Heran MK. Venous malformations: classification, development, diagnosis, and interventional radiologic management. Radiol Clin North Am 2008;46 (3):545-597.

16. Horbach SE, Lokhorst MM, Saeed P, et al. Sclerotherapy for low-flow vascular malformations of the head and neck: A systematic review of sclerosing agents. J Plast Reconstr Aesthet Surg 2016; 69(3):295-304

17. Ahmad S, Akhtar FK. Percutaneous sclerotherapy of para-orbital and orbital venous malformation: A single center, case series. Phlebology 2019; 34(5), 355–361.

18. Bai Y, Jia J, Huang XX, et al. Sclerotherapy of Microcystic Lymphatic Malformations in Oral and Facial Regions. J Oral Maxillofac Surg 2009; 67(2):251-256.

19. Hassan Y, Osman AK, Altyeb A. Noninvasive management of hemangioma and vascular malformation using intralesional bleomycin injection. Ann Plast Surg 2013; 70(1):70-73.

20. Yang Y, Sun M, Ma Q, et al. Bleomycin A5 sclerotherapy for cervicofacial lymphatic malformations. J Vasc Surg 2011; 53(1):150-155.

21. Shigematsu T, Sorscher M, Dier E C and Berenstein A. Bleomycin sclerotherapy for eyelid venous malformations as an alternative to surgery or laser therapy. Journal of neurointerventional surgery 2019; 11(1), 57–61.

22. Mohan A T, Adams S, Adams K, Hudson D A. Intralesional bleomycin injection in management of low flow vascular malformations in children. Journal of plastic surgery and hand surgery 2015; 49(2), 116 –120.

23. Zhang J, Li HB, Zhou SY, Chen KS, Niu CQ, Tan XY, Jiang YZ, Lin QQ. Comparison between absolute ethanol and bleomycin for the treatment of venous malformation in children. Exp Ther Med 2013;6(2):305-309.

24. Gregory S, Burrows P E, Ellinas H, Stadler M, Chun R H. Combined Nd:YAG laser and bleomycin sclerotherapy under the same anesthesia for cervicofacial venous malformations: A safe and effective treatment option. International journal of pediatric otorhinolaryngology 2018; 108: 30–34.

25. MacArthur CJ, Nesbit G. Simultaneous intra-operative sclerotherapy and surgical resection of cervicofacial venous malformations. International journal of pediatric otorhinolaryngology 2019; 118: 143 –146.

CASE REPORT

FACIAL PALSY IN A NEWBORN: A CASE REPORT

Snezana Jancevska[1], Sanja Ristovska[1], Igor Isjanovski[2], Nikolina Zdraveska[3]

[1] University Clinic for Gynecology and Obstetrics; Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Republic of North Macedonia

[2] University Clinic for Eye Diseases; Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Republic of North Macedonia

[3] University Clinic for Children's Diseases; Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Republic of North Macedonia

Abstract

Unilateral congenital facial palsy in the newborn occurs more often as isolated than in the context of syndromes or developmental defects. Recognition of the etiological factor, the severity of the clinical presentation, the dynamics of recovery are a guide for a multidisciplinary approach, a range of investigations and treatment. In a non-therapeutic approach, unsupported by sufficiently reliable data from the literature, there is a dilemma whether and how long one has to wait and observe the improvement of clinical results, before determining the range of examinations in the newborn. In facial paresis of prenatal or perinatal origin, identical to non-congenital Bell's palsy, infectious and anatomical-structural causes should initially be excluded. This paper presents a case of a symmetrical hypotrophic premature infant with a manifestation of acute Bell's palsy at birth, with negative biomarkers for infectious etiology and a normal brain ultrasound. The newborn underwent a special neonatal care with targeted local treatment. Towards the end of the first postnatal week, there was an evident withdrawal of clinical symptomatology.

ПРИКАЗ НА СЛУЧАЈ

ФАЦИЈАЛНА ПАРЕЗА КАЈ НОВОРОДЕНЧЕ: ПРИКАЗ НА СЛУЧАЈ

Снежана Јанчевска[1], Сања Ристовска[1], Игор Исјановски[2], Николина Здравеска[3]

[1] Универзитетска клиника за гинекологија и акушерство; Универзитет Св. Кирил и Методиј во Скопје, Медицински факултет, Република Северна Македонија

[2] Универзитетска клиника за очни болести; Универзитет Св. Кирил и Методиј во Скопје, Медицинскифакултет, РепубликаСеверна Македонија

[3] Универзитетска клиника за детски болести; Универзитет Св. Кирил и Методиј во Скопје, Медицински факултет, Република Северна Македонија

Извадок

Еднострана вродена парализа на лицето кај новороденчето почесто се јавува изолирано, одошто во склоп на синдроми или од дефект во развојот. Препознавањето на етиолошкиот фактор, тежината на клиничката презентација, динамиката на опоравувањето ќе бидат смерница за мултидисциплинарен пристап, палета на иследувања и третман. При вонтерапевтски приод, непоткрепен со доволно сигурни податоци од литературата, постои дилема дали и колку да се чека и обсервира подобрувањето на клиничките резултати, пред да се одреди палетата на иследувања кај новороденото дете. Кај фацијалната пареза од пренатално или перинатално потекло, идентично како кај неконгениталната парализа на Бел, првично треба да се исклучат инфективни и анатомско-структурни причини. Во овој труд се презентира случај на симетрично хипотрофично прематурно новороденче со манифестација на акутна парализа на Бел на раѓање, со негативни биомаркери за инфективна етиологија и уреден ултрасонографски наод на мозочните структури. Новороденото дете беше на специјална неонатална нега со целно спроведуван локален третман. Кон крајот на првата постнатална седмица покажа евидентно повлекување на клиничката симптоматологија.

Introduction

The presentation of congenital facial palsy in neonatal period is represented by asymmetric facial movements on the affected side, incomplete eye closure, and possible feeding problems. In the future development of the child, the untreated facial palsy may affect the speech, the facial emotional expression or chewing and may also lead to cosmetic discomfort and social isolation. The congenital facial palsy can be divided into traumatic or non-traumatic (developmental), unilateral or bilateral, total (palsy) or incomplete (paresis); it can occur as an isolated change or as part of other syndromes. The incidence of facial palsy in neonatal period is 0.8-2.1 per 1,000 live births. Bell's palsy is an idiopathic mononeuritis. It is a unilateral facial palsy and occurs both in adults and in children[1]. There is insufficient data in the literature on the incidence and frequency of this condition in premature newborns.

The seventh cranial nerve (nervus facialis) is a mixed nerve that contains motor, parasympathetic and sensory fibers. This nerve provides innervations to the facial muscles and is responsible for the perception of taste in the first two thirds of the tongue. The movements of the lateral side of the mouth and of the nasolabial fold are enabled by a group of four muscles, the most important of which is the musculus depressor angularis oris. All of these muscles are innervated by a branch of the seventh cranial nerve, and, in case of injury, there is a dysfunction of these muscles, which results in asymmetric facial expression.

From etiological point of view, the congenital facial palsy can be considered a traumatic or developmental disorder of brain structures or of facial nerves[2] The traumatic cause for the occurrence of facial palsy is much more common, and the risk factors include: termination of vaginal birth by using typical forceps with extended second delivery time; a primipara mother, giving birth to a macrosomic fetus (> 3,500 g), cranio-pelvic disproportion[3]. Facial nerve palsy as a secondary trauma when using forceps at birth was first studied by Landouzy as a research subject of his doctoral thesis in 1839[4]. Intrauterine trauma occurs due to increased pressure on the face of the fetus with the sacral bone at birth[5]. Recent studies show that traumatic facial palsy may be secondary to the use of continuous positive air pressure (CPAP) nasal application, due to the continuous and severe pressure on the newborn's face[6]. It is considered that the underlying patho-physiological process is ischemic tissue damage, mediated by free radicals[7]. Given that this is about a very young age group and that there is nolinear correlation with the type and duration of injury, the full knowledge of the patho-physiological mechanism remains unknown[8].

From the point of view of developmental disorders of brain structures and of facial nerves, the congenital facial palsy can be presented as part of a series of other syndromes, such as Möbius syndrome, hemifacial microsomia, velocardiofacial syndrome (DiGeorge Syndrome), CHARGE syndrome, facio-scapulo-humeral muscular dystrophy, Goldenhar syndrome. The intrauterine

hypoplasia of the nucleus of the seventh cranial nerve is often associated with congenital malformations and permanent facial palsy[9].

Teratogenic risk factors during pregnancy, such as thalidomide and misoprostol, are responsible for the occurrence of facial palsy in newborns.

A Case Report

The presented case is a third child from a third planned, desired and controlled pregnancy of a 33-year-old mother. In this pregnancy, the mother was exposed to multiple risk factors: pre-existent arterial hypertension, treated with methyldopa, as well as with gestosis of hypertension. In the prenatal period, the fetus was diagnosed with intrauterine growth retardation (IUGR), decreased amniotic fluid (Olygohydramnion). Due to the condition of fetal distress, the birth was terminated via abdominal surgical route, with emergency caesarean section; the fetus was extracted in capital presentation; a male newborn was born with birth weight of 2,100 g, Apgar score 7/8, with gestational maturity suitable for 36 gestational weeks or a late preterm newborn. The newborn was in good general condition. According to the birth weight and gestational age illustrated on the percentile curve, the newborn was hypotrophic. The head was properly configured, with craniofacial symmetry. Decreased movements on the left side of the face were noticeable. When crying, the nasolabial fold was lost; the newborn was unable to move the lower muscle of his lip, he had the so-called „limp"lip and pulled the lip to the side of the mount that was not affected. The eye on the left side was partially closed (Figure 1 and Figure 2).
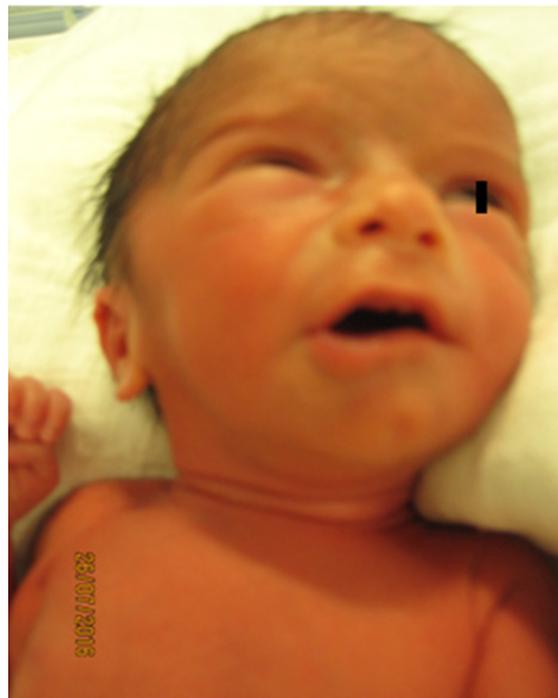

Figure 1 and Figure 2   Newborn with left-sided facial palsy, presented when crying and when not crying

In the first hours after birth, peripheral venous blood was collected for examination of biomarkers to rule out congenital infection (complete blood count with leukocyte formula, C-reactive protein, procalcitonin, blood culture). The results of the examination were negative. Ultrasonographic examination of the central nervous system showed an orderly finding of brain structures.

The newborn infant was treated with special care, which included gentle minimal manipulation, general measures for protection from cold and drafts and local treatment of the eyes with a solution for prevention of eye irritation, bathing the face with warm water.

Discussion

There is a logical link between intrauterine risk factors and the clinical expression of facial palsy, as well as the absence of facial asymmetry in the newborn. The reduced volume of amniotic fluid prevents free movement of the fetus and leads to anchoring and complete immobility, followed by pressure on the supine side in the pelvic bone structures (sacral part). All these unfavorable conditions for the fetus indicate a possible etiology of facial palsy in the newborn.

The compromised fetal mobility and dull pressure affect blood circulation, causing mild to moderate tissue ischemia. Timely completion of the delivery prevents reflection on soft and bony structures in terms of facial developmental asymmetry of the supine side relative to the free side.

Differentially diagnosed, facial palsy should be distinguished from congenital hypoplasia of musculus depressor angularis oris, which is a benign change and causes facial asymmetry when the newborn is crying[9].

There are grading systems and scales for assessing facial palsy. The most commonly used are the House-Brackmann Scale[10] (Table 1) and the Terzis-Noah Scale[11].

Table 1. House-Brackmann Scale

Grade	Characteristics
I. Normal	Normal facial function in all areas
II. Mild dysfunction	• Slight weakness noticeable on close inspection; • May have very slight synkinesis; • At rest: normal symmetry and tone. • Motion: Forehead - moderate to good function; Eye - complete closure with minimum effort; Mouth - slight asymmetry.
III. Moderate dysfunction	• Obvious weakness and asymmetry; • At rest: normal symmetry and tone. • Motion: Forehead - none; Eye - incomplete closure; • Mouth - asymmetric with maximum effort.
IV. Severe dysfunction	• Only barely perceptible motion. • At rest: asymmetry. • Motion: Forehead - none; Eye - incomplete closure; Mouth - slight movement.
V. Totalpalsy	• No movement.

The case presented belongs to the third grade according to the House-Brackmann Scale, although there is no strict mathematical limit in ranking patients from one grade to another.

The management and treatment approach of neonatal facial palsy is careful. The artificial tears are the first protection against dry eye. The use of corticosteroids in neonatal facial palsy is controversial. The use of corticosteroids in adult facial palsy population gives satisfactory results. However, there are still not enough randomized and controlled trials for this condition, which would justify the use of corticosteroids in the neonatal period[12, 13].

The surgical treatment of traumatic facial palsy depends on the clinical and electro-physiological tests. Total facial palsy (Grade V. on the House-Brackmann scale), temporal bone injury proven by computed tomography of the brain, and lack of improvement after 5 weeks of birth are possible indications for surgical treatment. The surgical treatment carries a risk of iatrogenic injuries.

Physiotherapy is a possible supportive therapy in newborns with facial palsy, through stimulating exercises, opening/closing of the eyelid and provoking a smile.

There is a complete withdrawal of facial palsy in most newborns. This process may take 3-6 months and does not require surgery[14, 15].

Negative prognostic factors for traumatic facial palsy are unilateral total palsy present at birth, temporal bone dislocation fracture, absence of spontaneous and evoked motor unit responses in all muscles innervated by the facial nerve for 3 to 5 days of life, and impaired function of the facial nerve by the fifth week after delivery[16]. The available literature on the prognosis of isolated congenital unilateral facial palsy is scarce and inconsistent, probably due to the more frequent encounter of patients with traumatic etiology.

Developmental monitoring and regular check-ups by a neonatologist and pediatric neurologist are important in newborns with congenital unilateral facial palsy to detect additional deficits such as abnormal ocular mobility, hypoglossal or trigeminal nerve involvement, which are not visible in the neonatal period[17].

Conclusion

Recovery time and degree significantly differ in patients with facial nerve paresis compared to patients with total palsy. Since the term "palsy" includes both entities, the term "palsy" should be used only to describe the total loss of nerve function. Patients, including newborns with incomplete acute Bell's palsy (paresis), should show early improvement in facial function (1-2 weeks after onset), which was the case with this newborn. Full recovery is expected within 3 months. Treatment with antiviral drugs and steroids is debatable, whereas examining patients with varying grades of facial palsy could lead to controversial results.

References

1. Khair AM, Ibrahim K. Idiopathic non-traumatic facial nerve palsy (Bell's Palsy) in neonates; an atypical age and management dilemma. Oman Med J 2018; 33(1): 65-68

2. Tomás-Roca L, Pérez-Aytés A, Puelles L, Marín F. In silico identification of new candidate genes for hereditary congenital facial paresis. Int J Dev Neurosci 2011; 29(4):451-60

3. Falco NA, Eriksson E. Facial nerve palsy in the newborn: incidence and outcome. Plast Reconstr Surg 1990; 85(1):1-4

4. Kirschenbaum E, Schalick WO, Faber DP, Finger S. Hector Landouzy on facial paralysis in newborn children: the case studies of a 19th-century French hospital physician. Pediatr Rehabil 2005; 8(3):180-6.

5. Shapiro NL, Cunningham MJ, Parikh SR, Eavey RD, Cheney ML. Congenital unilateral facial paralysis. Pediatrics 1996; 97(2):261-4

6. Maffei G, Magaldi L, Cassano P, Cassano M, Cella A, Magaldi R. Reversible facial nerve palsy secondary to nasal continuous positive airway pressure. J Perinat Med 2008; 36(6):550-1

7. Stew B, Williams H. Modern management of facial palsy: a review of current literature. Br J Gen Pract 2013; 63(607):109-10

8. Gupta AK, Das RR, Panda SS, Panda M. A neonate with facial asymmetry. BMJ Case Rep 2013; 2013: bcr2013200096

9. Al Tawil K, Saleem N, Kadri H, Rifae MT, Tawakol H. Traumatic facial nerve palsy in newborns: is it always iatrogenic? Am J Perinatol 2010; 27(9):711-3

10. House JW, Brackmann DE. Facial nerve grading system. Otolaryngol Head Neck Surg 1985; 93(2):146-7

11. Terzis JK, Noah ME. Analysis of 100 cases of free-muscle transplantation for facial paralysis. Plast Reconstr Surg 1997; 99(7):1905-21

12. Evans AK, Licameli G, Brietzke S, Whittemore K, Kenna M. Pediatric facial nerve paralysis: patients, management and outcomes. Int J Pediatr Otorhinolaryngol 2005; 69(11):1521-8

13. Pierick AR, Jenkins E. A Case of idiopathic acquired neonatal Bell's Palsy. J Investig Med High Impact Case Rep 2020; 8:2324709620930161

14. Sharma D, Murki S, Dhanraj G. Traumatic facial nerve palsy in newborn: A benign condition. J Clin Neonatol 2015; 4: 213-4

15. Bergman I, May M, Wessel HB, Stool SE. Management of facial palsy caused by birth trauma. Laryngoscope 1986; 96(4):381-4

16. Duval M, Daniel SJ. Facial nerve palsy in neonates secondary to forceps use. Arch Otolaryngol Head Neck Surg. 2009; 135(7):634-6

17. Toelle SP, Boltshauser E. Long-term outcome in children with congenital unilateral facial nerve palsy. Neuropediatrics 2001; 32(3):130-5.

CASE REPORT

TORSION OF A LARGE OVARIAN CYST PRESENTED AS AN ACUTE ABDOMEN: CASE REPORT

Sasho Pucakoski[1], Nadezda Spiroska[2], Andrej Nikolovski[3]

[1] General Hospital Prilep, Republic of North Macedonia
[2] University Surgery Clinic „Ss. Naum Ohridski"; Skopje, Republic of North Macedonia
[3] University Surgery Clinic „Ss. Naum Ohridski"; Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Republic of North Macedonia

Abstract

Patients with acute abdominal pain often end up in the emergency department for surgical treatment. Ovarian cysts can lead to an acute abdomen due to torsion (twisting) or rupture. In this case report we are going to present a 70 y/o female patient with left adnexal torsion, due to a presence of ovarian cyst, presented as an acute abdomen. The patient was enrolled in the emergency department with acute abdomen, nausea and vomiting. After the initial evaluation including a Computerized tomography [CT] scan, the cyst presented as a solid hypodense mass behind the front abdominal wall, with the dimensions of Anteroposterior diameter [APd] 13cm, Laterolateral diameter [LLd] 11cm and Craniocaudal diameter [CCd] 15cm. The possible differential diagnosis [DDx] included cystic tumor [TU] mass on the mesentery as well as a cystic TU on the Urogenital tract (UGT). Intraoperatively adnexal torsion due to an ovarian cyst was found. The cyst and the left adnexa were then removed. Histopathological report showed ovarian hemorrhagic infarction due to a cystic tumor and torsion in the left adnexa.

The diagnosis in such cases is often challenging because often the initial CT report can confuse the surgeon whether the mass arises from the mesentery or the urogenital tract.

ПРИКАЗ НА СЛУЧАЈ

ТОРЗИЈА НА ГОЛЕМА ЦИСТА НА ЈАЈНИК ПРЕЗЕНТИРАНА КАКО АКУТЕН АБДОМЕН: ПРИКАЗ НА СЛУЧАЈ

Сашо Пуцакоски[1], Надежда Спироска[2], Андреј Николовски[3]

[1] Општа болница Прилеп, Република Северна Македонија
[2] Универзитетска клиника за хируршки болести „Св. Наум Охридски", Скопје, Република Северна Македонија
[3] Универзитетска клиника за хируршки болести „Св. Наум Охридски"; Универзитет „Св. Кирил и Методиј" во Скопје, Медицински факултет, Република Северна Македонија

Извадок

Пациентите со акутна абдоминална болка често завршуваат на одделот за ургентни состојби, за хируршки третман. Цистите на јајник може да се манифестираат како акутен абдомен поради настаната торзија или руптура на тие цисти. Во овој приказ на случај прикажуваме 70 годишна пациентка со торзија на лева аднекса, поради присуство на циста на левиот јајник, која се презентираше како акутен абдомен. Пациентката беше примена на одделот за Ургентни состојби со симптоми на акутен абдомен, гадење и повраќање. После иницијалната евалуација која вклучуваше компјутерска томографија [КТ], цистата се презентираше како солидна хиподензна маса зад предниот абдоминален ѕид со димензии на Антеропостериорен дијаметар [АПд] 13 см, Латеролатерален дијаметар [ЛЛд] 11 см и Краниокаудален дијаметар [ККд] 15 см. Диференцијално дијагностички [ДДг] во предвид се земени следните дијагнози: цистична туморска [ТУ] маса која потекнува од мезентериум, како и цистична ТУ маса со потекло од урогенитален тракт [УГТ]. Интраоперативно најдовме торзија на левата аднекса поради присуство на циста на левиот јајник. Цистата заедно со левата аднекса беа отстранети. Хистопатолошкиот извештај покажа хеморагичен инфаркт на јајникот, поради цистичен тумор и торзија на левата аднекса. Поставувањето на точна дијагноза во вакви случаеви е честопати предизвикувачка, поради тоа што се случува иницијалниот КТ извештај да го доведе до забуна хирургот за тоа дали масата потекнува од мезентериум, или од урогенитален тракт.

Introduction

According to the National Institute of Health research results, 5% to 10% of women in the United States will require surgical exploration for an ovarian cyst during their lifetime. Of those cysts 13% to 21% will be malignant[1].

However the prevalence in postmenopausal women is 14% to 18%, with an yearly incidence of 8%. It was reported that 30% to 54% of postmenopausal ovarian cysts will persist for years [2,3].

Most of the ovarian cysts are asymptomatic, with the cysts being discovered incidentally during ultrasonography or routine pelvic examination in the patients. Some cysts, however, may have a range of symptoms, which sometimes can be severe, such as: discomfort or pain in the lower abdomen due to local organ obstruction, severe pain from rupture or torsion (twisting) of the adnexa[4.]

Case presentation

A 70 years old female patient presented in the emergency department with a severe diffuse abdominal pain which started earlier that day. She was previously diagnosed with atrial fibrillation, treated with oral anticoagulant therapy for 8 years. She underwent Caesarean section 40 years ago.

Physical examination revealed tenderness in the peri umbilical area and the lower abdomen.

Complete blood analysis presented with abnormal findings as of: Leukocytes 21 (3.5 - 10.0 x 10^9/L), with Neutrophils 8.4 (2.0 - 8.0 x 10^9/L), Lymphocytes 0.06 (1.2 - 3.2 x10^9/L), C- reactive protein [CRP] 115.5 (0.0 - 5.0 mg/L), Serum Iron [Fe] 2.0 (6.6 - 28.3 umol/L). Computerized tomography, according to the radiography report (Native series) showed larger hypodense cystic TU formation, in some places with a thickened wall in the periumbilical area with possible connection to the mesentery and/or left adnexa. The TU suppressed the intestines. Its dimensions were as follows: APd 13 cm, LLd 11 cm, CCd 15 cm (Figure 1,2,3).
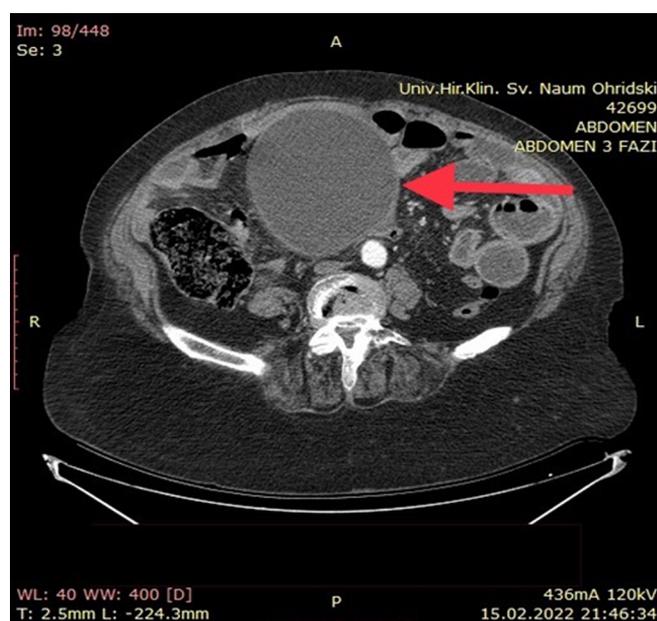

Figure 1 .   Axial CT scan of the abdomen showing the cystic tumor (arrow)
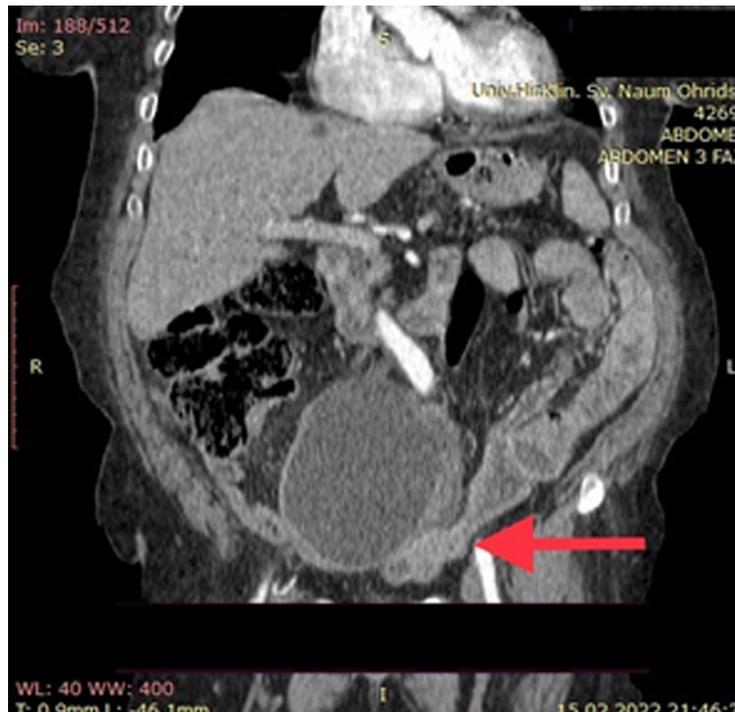
Figure 2 .   Possibly connected to the left adnexa and/or mesentery (arrow)
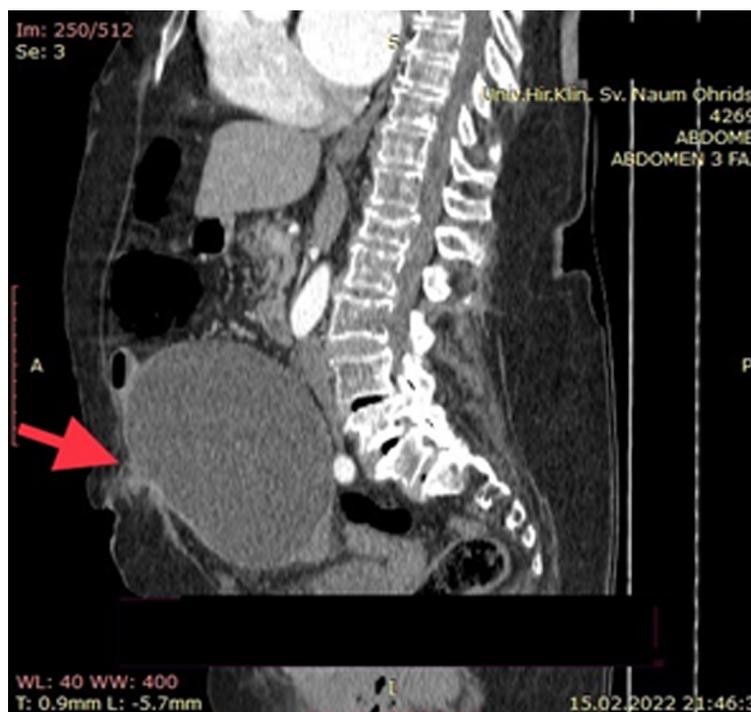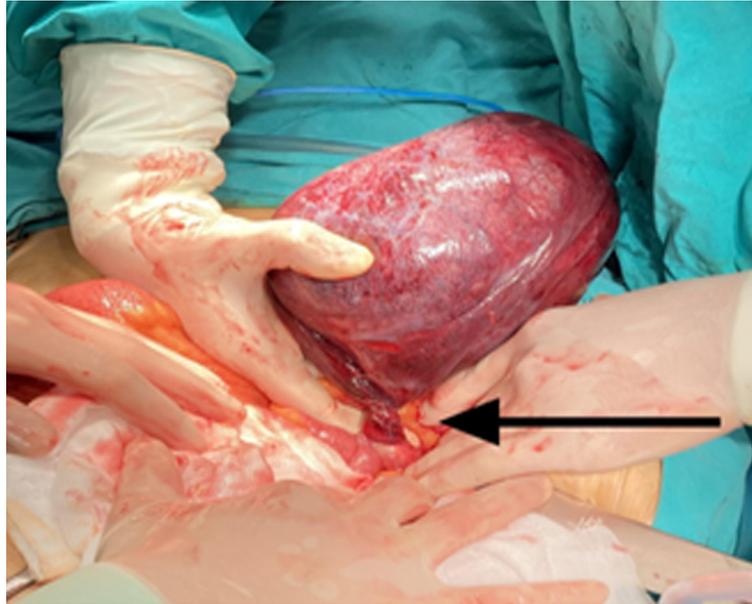

Figure 3 .   CT Sagital presentation of the mass (arrow)

Indication for emergency laparotomy was set.

Intraoperatively a huge central abdominal cyst arising from the left adnexa was found with a moment of torsion (twisting) in the left adnexa. The mass was then removed together with the left adnexa. Ovarian artery and tubal ligature were made (Picture 1).

Picture 1 .   Twisted left ovarian cyst

In the postoperative period the intestinal function was reestablished. The patient was discharged on day 6 post operation. Seroma of the surgical wound occurred at first check up. It was treated in an outpatient set-up. The pathology report described a cystically changed ovarium with massive oedema, congestion, extensive hemorrhage, and polymorphonuclear infiltration. Ovarian infarction, due to torsion (twisting) of the cyst occurred.

There was no presence of atypical or malignant cells reported in the Report.

Discussion

Large intra abdominal cystic lesions can present with certain diagnostic challenges and difficulty in the setting of straight diagnosis due to the image overlapping of different abdominal entities. In some cases the cystic lesion can be recognized to arise from a certain organ in the abdominal cavity. Therefore the diagnosis is more straightforward. Occasionally the presentation of these large cysts can mislead and cause diagnostic difficulties[5].

Large abdominal masses can compress local organs, such as intestines and lead to obstruction and potential necrosis of these organs[6]. Therefore the need for urgent surgery in such masses sometimes is necessary in order to avoid irremediable changes of the intestines.

Female patients admitted to emergency department with symptoms of an acute abdomen, nausea and vomiting, should always be examined for intestinal pathologies as well[6]. In our case, the intestines and other surrounding organs were non-compromised.

Detorsion of a twisted ovarian cyst is preferable management in younger patients, in order to salvage ovarian function and preserve its fertility[7].

Most of the cysts are benign, but there are a few that are malignant and they're presenting with a very low rate of survival. In such cases

the diagnosis should be obtained surgically, while the biopsy and aspiration can often be harmful[8]. Age factor is important in terms of increased malignancy rate possibility. Median age for ovarian carcinoma was found to be 63 years[9]. Sharma et al. included 186 postmenopausal women who underwent a surgical evaluation for ovarian cyst 10 cm or larger. Malignant process was found in 13%, which indicates that the larger the cyst gets, the bigger the chance for it being malignant is[10]. Knowing these facts, the clinicians should try to balance the risk of surgery for what may be a benign mass and the risk of a delay of diagnosis in potential malignancy[8].

Conclusion

A giant ovarian cyst is a rare condition and management is challenging, mainly because of the difficulty to set the correct diagnosis upfront.

Occasionally it can present as acute abdomen due to a cystic torsion (twisting). No matter the diagnostic challenges, surgery is the mainstay of its treatment in order to avoid compromise and gradual worsening of the patient's condition. Even more when the patient presents with an acute abdomen, surgery is indicated no matter the origin of the cyst lesion.

References

1. NIH consensus conference. Ovarian cancer. Screening, treatment, and follow-up. NIH Consensus Development Panel on Ovarian Cancer. JAMA. 1995;273(6):491-7. PMID: 7837369.

2. Modesitt SC, Pavlik EJ, Uealnd FJ, DePriest PD, Kryscio RJ, Van Nagell JR. Risk of malignancy in unilocular ovarian cystic tumor less than 10centimeters in diameter. Obstet gynecol 2003, 102:594-599

3. Greenlee RT, Kessel B, Williams CR, Riley TL, Ragard LR, Hartge P, et al. Prevalence, incidence, and natural history of simple ovarian cysts among women >55 years old in a large cancer screening trial. Am J Obstet Gynecol 2010; 202(4):373.e1-9.

4. Bottomley C, Bourne T. Diagnosis and management of ovarian cyst accidents. Best Pract Res Clin Obstet Gynaecol. 2009; 23(5):711-724.

5. Yacoub JH, Clark JA, Paal EE, Manning MA. Approach to cystic lesions in the abdomen and pelvis, with radiologic-pathologic correlation. Radiographics 2021;41(5):1368-1386.

6. Duran A, Duran FY, Cengiz F, Duran O. Intestinal Necrosis due to Giant Ovarian Cyst: A Case Report. Case Rep Surg. 2013;2013:831087.

7. Dasgupta R, Renaud E, Goldin AB, Baird R, Cameron DB, Arnold MA, et al. Ovarian torsion in pediatric and adolescent patients: A systematic review. J Pediatr Surg 2018;53(7):1387-1391.

8. Ross EK, Kebria M. Incidental ovarian cysts: When to reassure, when to reassess, when to refer. Cleve Clin J Med 2013;80(8):503-514.

9. Sharma A, Gentry-Maharaj A, Burnell M, et al; UK Collabora-

tive Trial of Ovarian Cancer Screening (UKCTOCS). Assessing the malignant potential of ovarian inclusion cysts in post-menopausal women within the UK Collaborative Trial of Ovarian Cancer Screening (UKC-TOCS); A prospective cohort study; BJOG 2012; 119:207-219

10. Ghezzi F, Cromi A, Bergamini V, Uccella S, Siesto G, Franchi M, Bolis P. Should adnexal mass size influence surgical approach? A series of 186 laparoscopically managed large adnexal masses. BJOG. 2008;115(8):1020-1027.

SK. The dichotomy of relative humidity on indoor air quality. Environ Int 2007;33:850–857.

REVIEW

DRY EYE DISEASE AND RISKS OF URBAN AIR POLLUTION – LITERATURE REVIEW

Natasha Trpevska-Shekerinov[1], Andrijana Petrushevska[1], Emilija Gjoshevska-Dashtevska[1], Toni Shekerinov[1], Jana Nivichka-Kjaeva[1]

[1] *University Clinic for Eye Diseases; Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Republic of North Macedonia*

Abstract

Air pollution has a major impact on health, and it particularly affects the mucous membranes of the respiratory tract and the eyes. The ocular effects of chronic, long-term exposure to high levels of air pollution are still unclear. The increase in air pollution levels can be associated with an increase in the instability of the tear film. The aim of this paper is to evaluate and discuss the available data about chronic eye diseases in regions with high air pollution. Furthermore, the review also offers a certain understanding of the link between chronic dry eye disease (DED) and air pollution. Materials and methods: Specific keywords (dry eye, air pollution, and urban) were used to search the medical databases of PubMed and Medline. This research technique led to obtaining 103 papers, dating from 1995 to 2021. Out of those, 15 were used as the basis of this paper. Results: The pathophysiological mechanisms of oxidative stress and ocular surface inflammation involve the selective binding of environmental agents to ocular surface membrane receptors, leading to the activation of proinflammatory signaling pathways with changes in the extracellular stromal matrix and consequent occurrence of inflammation of the ocular surface with epithelial defects. Conclusions: Dry eye disease, pollution, and eye allergy overlap, but their presentations can be different. Future advancements in monitoring technology and the development of modern, non-invasive diagnostic methods will help prove the link between air pollutants and DED. The points should be aimed at preventing the global risks of antigenic stimulation of „urban eye".

ПРЕГЛЕД НА ЛИТЕРАТУРА

СУВО ОКО И УРБАНОТО ЗАГАДУВАЊЕ НА ВОЗДУХОТ КАКО РИЗИК ФАКТОР- ПРЕГЛЕД НА ЛИТЕРАТУРА

Наташа Трпевска-Шекеринов[1], Андријана Петрушевска[1], Емилија Ѓошевска-Даштевска[1], Тони Шекеринов[1], Јана Нивичка-Ќаева[1]

[1] *Универзитетска клиника за очни болести; Универзитет „Св. Кирил и Методиј" во Скопје, Медицински факултет, Република Северна Македонија*

Извадок

Загадувањето на воздухот има големо влијание врз здравјето, а особено влијае на мукозните мембрани на респираторниот тракт и очите. Окуларните ефекти од хроничната, долготрајна изложеност на високи нивоа на загадување на воздухот сè уште се нејасни. Зголемувањето на нивото на загаденост на воздухот може да биде поврзано со зголемување на нестабилноста на солзниот филм. Целта на овој труд е да се евалуираат и дискутираат достапните податоци за хроничните очни болести во регионите со високо загадување на воздухот. Понатаму, прегледот нуди и одредено толкување за врската помеѓу хроничната болест на суво око и загадувањето на воздухот. Материјали и методи: Специфични клучни зборови (суво око, загадување на воздухот и урбано) беа користени за пребарување на медицинските бази на податоци на PubMed и Medline. Пронајдовме 103 трудови, кои датираат од 1995 до 2021 година. Од нив, 15 беа користени како основа на овој труд. Резултати: Патофизиолошките механизми на оксидативниот стрес и воспалението на окуларната површина вклучуваат селективно врзување на агенсите од средината за рецепторите на окуларната површина што доведува до активање на проинфламаторните сигнални патишта со промени во екстрацелуларната стромална матрица и последователна појава на воспаление на окуларната површина. со дефекти на епителот. Заклучок: Суво око, загадувањето и алергијата на очите се преклопуваат, но нивните презентации може да бидат различни. Идните достигнувања во технологијата за следење и развојот на современи, неинвазивни дијагностички методи ќе помогнат да се докаже врската помеѓу загадувањето на воздухот и сувото око. Мерките треба да бидат насочени кон спречување на глобалните ризици од антигенска стимулација на „урбаното око".

Introduction

Dry eye disease is a chronic condition. Being very common, it affects millions of people worldwide and is one of the most common causes for patients to visit ophthalmologists[1]. It is a multifactorial disease, and incidence can be significantly increased by climatic conditions, the environmental pollution, traffic, extreme temperatures, and relative humidity. Studies prove that there is a correlation between low relative humidity and the increased prevalence of DED. Namely, air-conditioned offices and vehicles, extremely hot air, and air pollution with high levels of PM particles lead to tear film instability.

The National Eye Institute and Industry of workshop on Clinical Trials in Dry Eyes held in 1995 proposed a global definition for this entity, as disorder of the tear film due to tear deficiency or excessive tear evaporation, which cause damage to the interpalpebral ocular surface, associated with ocular discomfort. Both of these factors lead to the damage of the interpalpebral ocular surface, which is associated with ocular discomfort[2].

According to epidemiological studies, more than 6 % of people over the age of 40 suffer from dry eye, with the prevalence increasing to 15 % when it comes to adults age 65 and older. Several environmental factors have been linked to DED, including high altitude, wind, air pollution, allergens, adjuvants, temperature, relative humidity (RH), and UV radiation[3].

Air pollution has a major impact on health, and it particularly affects the mucous membranes of the respiratory tract and the eyes.

It appears that the contamination related to traffic emissions is associateded with DED and other allergic diseases; thus, lowering pollution levels caused by exhaust gas is necessary to mitigate this health problem.

The ocular effects of chronic, long-term exposure to high levels of air pollution are still unclear. The increase in air pollution levels can be associated with an increase in the instability of the tear film.

It is considered that the abnormalities of the ocular surfaces caused by high levels of air pollution are subtypes of the dry eye disease[2]. Furthermore, the rise in temperature, which results from the ongoing climate changes, causes high rates of tear evaporation in patients with DED. Global warming contributes to the prolongation of the allergy season and leads to the development of allergy-triggered DED. Since environmental conditions like these can't be bypassed, prevention strategies must be created with those suffering from the disease taken into account.

There aren't any large studies on the link between air pollution and DED while focusing on multiple air pollutants. On the other hand, there is an important knowledge gap when it comes to how environmental factors (especially air pollution along with weather conditions) affect the frequency of detrimental changes to the eye.

Main inductors contains air pollution, exposure to pollutants: particulate matter (PM10, PM2.5), in-

creased ozone and nitrogen oxides (O3, NOx), increased allergenic bio particles, chronic use of preserved eye drops, reduced enzymes – nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, pollen-associated lipid mediators (PALMs) and allergic proteins. In fact, reactive oxygen radicals (ROS) present on the PM particles can act anti-oxidative barrier of the tear film[2,3].

The lacrimal gland reduces the production of mucin and increases tears evaporation, which reduces the protective barrier and facilitates the flow of antigens.

According to the World Health Organization, the air quality in the countries of the Western Balkans is terrible. The air in each country of this region contains large quantities of harmful PM 2.5 particles; in fact, the concentration of PM 2.5 and PM 10 particles is often 10 times the allowable level. The biggest polluters in this part of the world are coal power plants and old cars[4].

The aim of this paper is to evaluate and discuss the available data about chronic eye diseases in regions with high air pollution. Furthermore, the review also offers a certain understanding of the link between chronic dry eye disease and air pollution.

Materials and methods

In this paper we were focused on recent DED-related studies to identify the urban eye allergy syndrome associated with DED and perennial conjunctivitis (PAC). We are presenting update about some hypothetical mechanisms of changes in the structure of allergen with acceleration of immune reaction of conjunctiva. The studies discussed in this paper suggest objective diagnostic methods for DED. Specific keywords (dry eye, air pollution, and urban) were used to search the medical databases of PubMed and Medline. This research technique led to obtaining 103 papers, dating from 1995 to 2021. Out of those, 15 were used as the basis of this paper.

Results

The pathophysiological mechanisms of oxidative stress and ocular surface inflammation involve the selective binding of environmental agents to ocular surface membrane receptors leading to the activation of pro-inflammatory signaling pathways with changes in the extracellular stromal matrix and consequent occurrence of inflammation of the ocular surface with epithelial defects[3].(Figure 1)
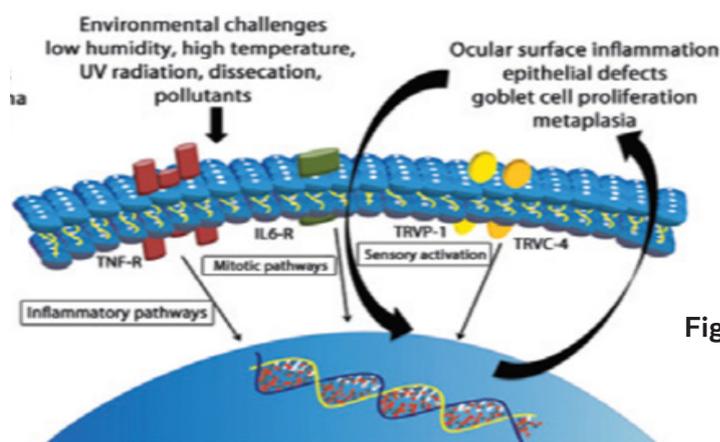

Fig. 1. Pathophysiological mechanisms of DED at the cellular level

Source: Alves M. et al., Arq Bras Oftalmol. 2014

There is increasing evidence that the dry eye disease leads to inflammation of the ocular surface and is partially responsible for ocular surface epithelial diseases and irritation symptoms that develop. Inflammation may be caused by different factors, including desiccation, hyperosmolarity, alteration in the tear film composition, and micro trauma of the eyelids. Whatever the initial etiology of DED, once it develops, inflammation becomes the core mechanism of ocular surface disease.

Exposure to ozone exacerbates the adverse effects of allergic reactions by enhancing the production of the IgE. Current studies show that patients with chronic allergic or atopic tendencies may have less tolerance for additional environmental antigen stimulation[5].

Antioxidative defenses in the ocular surface occur in the form of tear proteins such as lactoferrin and S100A proteins and enzymes like superoxide dismutase (SOD), peroxidase, catalase, and mitochondrial oxidative enzymes. An imbalance between the level of reactive oxygen species (ROS) and the action of protective enzymes will lead to oxidative damage and possibly inflammation[5].

The manifestations of the pseudo-allergic form of irritating conjunctivitis include the following eye problems as immediate effects of exposure: watering, burning, redness, irritation, itching, stinging, chronic discomfort, dry eye symptoms, foreign body sensation, blurred vision, increased sensitivity to light, inflammation of the cornea, and worsening of allergic symptoms, which can be minimal, middle or chronic[6]. (Figure 2, 3)

Fig. 2.   Figure 2, 3 Manifestations of the pseudo- allergic form of irritating conjunctivitis

The latest articles describes the tear film as an interactive hydrated mucin gel with the presence of lipids and proteins distributed throughout the gel. Eye moisture, which is of optical and physiological importance, is maintained by the secretion of lipids and aqueous solution and the mucous layer. Lipids pre-

vent evaporation and stabilize the tear film, and any disruption of the lipid secretion process will result in increased evaporation and destabilization of the tear film and consequentially lead to DED.

Kjaeregaard et *al*. confirm that there is a link between high humidity and

the stability of the tear film, emphasizing that air in a room should be dry, cool, and with a humidity level of 40%[7].

In their study, Hwang et al. point out that high ozone levels and low humidity are the main causes of DED in the Korean population[8].

Another study showed that outpatient visits for conjunctivitis significantly increase during periods of higher levels of air pollution and the presence of PM particles in the air[9] (Fu et al.).

Furthermore, the authors of the study suggest a complexity of the clinical picture of DED, with symptoms of the condition including not only those related to the dry eye disease, but eye infections and irritation, dry cough, itching of the nose, fluctuation vision, etc. Analyses confirm that PM particles present on the surface of the eye are more damaging for eyes affected by the dry eye disease than they are for healthy eyes, suggesting that poor air quality might be a factor in the development of DED.

Discussion

Recognizing that insufficient tear production and increased evaporation are two different components of DED that interact with the condition differently can lead to a significant progress in the management of this disease[10].

Identifying the presence of volatile film instability in the various stages of DED and understanding that the thickness of the lipid layer can determine the stability of the tear film can also improve the treatment plans of individuals with the condition.

Evaluations of the effects of exposure to pollution in patients with irritating and perennial conjunctivitis (PAC) contain evaluation of the conjunctiva (hyperemia, hyperplasia, chemosis); evaluation of the tear film lipid layer; ocular surface staining and corneal involving; corneal sensation; Schirmer test and Tear film break up time; cultures from the ocular surface or associated tissue and serum antibody biomarkers and/or other serological tests[11,12].

 The diversity of the existing clinical forms of DED and other conjunctival and corneal diseases that may have similar symptoms and clinical presentations leads to the conclusion that diagnosing DED is not easy and that it should be done by obtaining different information with various procedures.

New, non-invasive imaging methods include the following: optical coherence tomography (AS- OCT) for tear meniscus; corneal biopsy for evaluating the thickness and structure of the cornea; corneal topography for measuring the tear break-up time and tear meniscus height; meibomian gland imaging and osmolarity testing, which measure the adverse effects that exposure to high levels of air pollution has on the ocular surface; and using the Inflame Dry Detector (RPS) to detect the production of the inflammatory MMP- 9 cytokine marker.

Findings about the link between inflammation with reduced secretion of tears and subsequent damage to the ocular surface have led to a proposal for a unified concept of DED[13].

Air pollutants may create temporary eye irritations, but eye redness typically clears up when pollution levels decrease. However, people living in areas where the air contains many pollutants are three to four times more likely to develop an eye condition called dry eye syndrome. Four years of monitoring the air after the conclusion of the New York State Department of Environmental Conservation's (NYSDEC's) Tonawanda Community Air Quality Study showed a reduction in the concentrations of benzene and other air pollutants within the Tonawanda community[14].

The interactions between different microenvironment components during the development of DED need to be investigated as well. The ultimate goal of DED management is to revert the ocular surface and tear film to their normal homeostatic state. A wide range of therapeutics is available in treating DED, but evaluating whether certain treatment interventions can interfere with the functions of other components and cause unrelated complications or exacerbate DED is crucial[15].

Ophthalmologists need to check the environmental histories of patients with dry eyes and allergic conjunctivitis. Environmental manipulations, such as increasing local humidity and decreasing the exposure to air pollution, and encouraging frequent blinking hydration, the use of sunglasses, and the finding of ways to increase indoor humidity and making the necessary lifestyle changes (AQI) are vital in preventing DED[14].

Conclusion

Dry eye disease, pollution, and eye allergy overlap, but their presentations can be different.

Future advancements in monitoring technology and the development of modern, non-invasive diagnostic methods will help prove the link between air pollutants and DED. The points should be aimed at preventing the global risks of antigenic stimulation of "urban eye".

The best way to alleviate the effects of air pollution on the eyes is to avoid the same, but this is not always possible. A reasonable prevention strategy would be to keep the eyes well lubricated with artificial tears that don't contain preservatives and to keep an eye on cleaning the caps and eyelashes after long-term exposure to high levels of pollution.

References

1. Zemanová M. Dry Eye Disease A review. Cesk Slov Oftalmol. 2021 Winter;77(3):107–119.

2. Lamp MA. Report of the National institute / industry workshop on Clinical Trials in Dry Eyes. CLAO J. 1995; 21(4):221-32

3. Cha SH, Lee JS, Oum BS, Kim CD. Corneal epithelial cellular dysfunction from benzalkonium chloride (BAC) in vitro. Clin Exp Ophthalmol 2004;32(2):180-184.

4. Wolf J, O'Neill NR, Rogers CA, Muilenberg ML, Ziska LH. Elevated atmospheric carbon dioxide concentrations amplify Alternaria alternata sporulation and total antigen produc-

tion. Environ Health Perspect. 2010;118(9):1223-8.

5. Alves M, Novaes P, Rocha EM. Is dry eye an environmental disease? Arq Bras Oftalmol 2014;77(3):193-200

6. Seen S, Tong L. Dry eye disease and oxidative stress. Acta Ophthalmol 2018;96(4):e412-e420.

7. Kjæregaard SK, Hempel-Jørgensen A, Mølhave L, et al. Eye trigeminal sensitivity, tear film stability, and conjunctival epithelium damage in 182-non-allergic, non-smoking Danes. Indoor Air 2004;14:200–207.

8. Hwang SH, Choi YH, Paik HJ, Wee WR, Kim MK, Kim DH. Potential importance of ozone in the association between outdoor air pollution and dry eye disease in South Korea. JAMA Ophthalmol2016;134(5):503-510.

9. Fu Q, Mo Z, Lyu D, Zhang L, Qin Z, Tang Q, et al. Air pollution and outpatient visits for conjunctivitis: A case-crossover study in Hangzhou. China Environ Pollut 2017; 231(Pt 2): 1344-1350.

10. Perry HD, Donnenfeld ED. Dry eye diagnosis and management in 2004. Curr Opin Ophthalmol 2004;15:229–304.

11. Sullivan BD, Crews LA, Messmer EM, Foulks GN, Nichols KK, Baenninger P, et al. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. Acta Ophthalmol 2014.

12. Choi H, Lee SB. Nonseasonal allergic conjunctivitis in the tropics: experience in a tertiary care institution. Ocul Immunol Inflamm 2008; 16(4):141-5.

13. Stern ME, Beuerman RW, Fox RI, et al. A unified theory of the role of the ocular surface in dry eye. Adv Exp Med Biol 1998;438:643–651.

14. Foulks GN. Determinants of Tear Film Stability. American Academy of Ophthalmology Annual Meeting October 15–18 Chicago The Castroviejo Lecture; 2005.

15. Wolkoff P, Kjæregaard SK. The dichotomy of relative humidity on indoor air quality. Environ Int 2007;33:850–857.