

INCIDENCE AND PREVALENCE OF VAGINAL INFECTIONS IN WOMEN OF REPRODUCTIVE AGE IN NORTH MACEDONIA

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ABSTRACT

In the available literature on this subject there are many studies which describe the effects of sexually transmitted infections on pregnancy and fertility of women. Because of the frequency of the infections with the atypical bacteria of the *Ureaplasma Spp.*, *Mycoplasma Spp.*, *Chlamydia Trachomatis*, as well as HPV infections in women of reproductive age, it is easy to underestimate their importance when establishing the basis of the genital health of women of reproductive age.

In this prospective analysis, conducted from 2014 to 2018 in the laboratory for HPV and Molecular diagnostics at the University Clinic of Gynaecology and Obstetrics in Skopje, North Macedonia, we analysed the results of 10,387 patients of all ages, of which 973 patients were of reproductive age. A Panel analysis was also conducted (including the above-mentioned pathogens). An HPV analysis was also conducted on 643 patients in this group. Within the group of 643 patients, there was a positive result for HPV in 26.7% of them, while in 40.9% there was a positive result for one or more pathogens on the Panel analysis of bacterial pathogens. The statistical analysis of the results showed that the most frequent of all bacterial pathogens within the Macedonian population of women of reproductive age is *Ureaplasma Spp.*, with an incidence of 33%, followed by *Mycoplasma Spp.*, with 7.8%, while *Chlamydia Trachomatis* was present in 6.4% of the cases. We should highlight that a co-infection with HPV was present in 18.5% of all the patients where there was analysis of both diagnostic procedures.

The analysis of the results in the patients co-infected with HPV and at least one bacterial pathogen on the Panel Analysis, showed a very high statistical correlation ($p < 0.001$).

Keywords: vaginal infections, reproductive health, *Ureaplasma Spp.*, *Mycoplasma Spp.*, *Chlamydia Trachomatis*, HPV, co-infections.

INTRODUCTION

According to definition provided by the WHO, reproductive /sexual health, is an integral health factor in the general health of the individual, and it is directly connected to quality of life. This implies a physical, emotional, mental, and

social wellbeing in regard to sexuality, and not only the absence of disease, disfunction or permanent incapacity. According to this definition, reproductive health warrants behaviours which are full of respect and appreciation for all kinds

of sexual bonds, as well as the capacity to experience pleasurable and safe sexual encounters, free of violence, abuse and discrimination. For this to be possible, one should first and foremost respect, practice, and protect the sexual rights and freedoms of every individual.

All of this is particularly important when family planning comes into question, as well as with female fertility. Finally, pregnancy is a well-balanced physiological act, an act which is tightly connected to the reproductive health of the mother. This is especially important, since any imbalance can result in premature labor pain and premature births. These are the labour pains which happen before 37th week of gestation, possibly resulting in premature labor. On the other hand, premature labor is responsible for the highest incidence and prevalence of neonatal morbidity and mortality across the Globe [1,2]. In the majority of these cases, the reasons for premature births are not established, although there are several risk factors [3] already established, among which: 1) poor socio-economic conditions and the mother's lack of education [4]; 2) the mother's age <20 and >35 years of age [5]; 3) physically hard labour of the mother [3]; 4) cervical incompetence, more gestations, previous abortions, previous premature births [1,3,4]; 5) the presence of urogenital infections [1]. The colonisation with pathogens and the inflammation of the mother's genital track are some of the main risk-factors associated with spontaneous premature births [5]. Epidemiological studies on the subject suggest two major factors for premature births, among which the mother's chronic stress and infections present in her genito-urinary tract. These are significant factors and are independently associated with the increased risk of spontaneous premature births. The research available on the physiology of birth suggest that neuro-endocrine and immunological processes play an important role in the physiology and the pathophysiology of term and preterm births. Still, not all women with high levels of stress and/or the presence of intravaginal infections will have a premature birth, and for now we know little about the factors which modify the tendency towards the pathophysiological changes on the level of the endocrine and immunologic system during pregnancy.

There is a vast body of research which underlines the effects of sexually transmitted gen-

ital infections and how infections, in general, affect the pregnancy and general reproductive health of women. Still, the genito-urinary infections which are not necessarily sexually transmitted, often lead to premature births. Because they are largely asymptomatic, their importance in the development of these conditions is often underestimated.

Bacterial vaginosis are the most prevalent infections of the lower genital tract in women of reproductive age [5,6,7]. This condition, established as a clinical syndrome, is characterised mainly by the alteration of the vaginal flora, and not by an infection with a specific microorganism. The bacterial vaginosis is characterised with significant polymicrobial proliferation, especially of the Gram negative and anaerobic bacteria and the destruction of the lactobacillus as part of the normal protective vaginal microflora [5,6]. Women with bacterial vaginosis often complain of a thin cervical mucus, pale yellow to gray, and an unpleasant smell of fish [1], however, in the majority of women, the infection is asymptomatic [7]. The presence of bacterial vaginosis during pregnancy is associated with a doubled risk of premature rupture of the membranes and premature births [7-15].

Infections with primarily asymptomatic pathogens on the other hand, among which *Ureaplasma* Spp., *Mycoplasma* Spp., *Chlamydia Trachomatis*, as well as their association with HPV infection, play a significant role in the reproductive health of women. This study elaborates on this.

MATERIAL AND METHODS

In this prospective analysis, conducted from 2014 to 2018, in the laboratory for molecular diagnostics and HPV in the University clinic of gynecology and obstetrics in Skopje, we analyzed 10,387 patients of all ages, and out of all the patients who had a Panel analysis (a PCR kit for detection of *Ureaplasma Urealyticum*, *Ureaplasma Parvum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Neisseria Gonorrhoeae*, *Chlamydia Trachomatis*, *Trichomonas Vaginalis*). For the purpose of this paper we used the result from the cohort of patients of reproductive age, especially the subgroup of those who

have both HPV typification and the Panel analysis conducted. The used result were those when the primary specimen was collected, as in 132 patients there were at least two collected specimens in two different time points, and those were removed from the analysis. Statistical analysis was completed with SPSS22, and we used descriptive statistics and Pearson's Chi Square test for independence of the statistical markers.

RESULTS

Distribution of the infections

In the cohort of 973 patients of reproductive age, there is the distribution below of infections as well as the frequency described in percent of pa-

Panel

Finding	Frequency	Percent
negative	597	61.40%
positive	376	38.60%

Distribution of the type of infection:

Panel result	negative	U.U./U.P.	M.H./M.G.	C.T.	T.V.	N.G.
Percent of subjects	61.40%	31.10%	11.30%	4.90%	1.20%	0.20%

Distribution of infections in age groups

Age group and Panel cross tabulation

		Panel		total
		negative	Positive	
Age group	number	9 _a	20 _b	29
	% in the age group	31.00%	69.00%	100.00%
	% in the Panel analysis	1.50%	5.40%	3.00%
	% of total	0.90%	2.10%	3.00%
	number	217 _a	173 _b	390
	% in the age group	55.60%	44.40%	100.00%
	% in the panel	36.70%	46.80%	40.50%
	% of total	22.60%	18.00%	40.50%
	number	239 _a	116 _b	355
	% in the age group	67.30%	32.70%	100.00%
	% in the Panel	40.40%	31.40%	36.90%
	% of total	24.80%	12.10%	36.90%
	number	127 _a	61 _a	188
	% of the age group	67.60%	32.40%	100.00%
	% of the panel	21.50%	16.50%	19.50%
	% of total	13.20%	6.30%	19.50%
Total	number	592	370	962
	% of the age group	61.50%	38.50%	100.00%
	% of the panel	100.00%	100.00%	100.00%
	% of total	61.50%	38.50%	100.00%

tients. This includes the fact that, in some patients, there was a presence of more than one pathogen.

Incidence of infections

The analysis shows a high statistical correlation among the presence of infection and the age group.

Pearson's Chi square analysis shows 25.026 with 3 s.s. and is statistically significant with the level of significance $p < 10^{-3}$.

It can be observed that the presence of infections in younger subjects is much higher. Although

the cohort of <20 years is small, the infections are much more prevalent in this age group. This can be due to the more frequent examinations requested in this age group due to symptoms in relation to the other age groups where the examinations are more frequently a result of routine control examinations without any symptoms present.

The subjects from 20-30 years have a relatively high prevalence of infections, 44% have a positive result on any of the diagnostic procedures.

Age group and Panel Crosstabulation

		Infections						Total
		Negative	U.U./U.P.	M.H./M.G.	C.T.	T.V.	N.G.	
A g e group	number	9	17	11	6	3	0	29
	% of age group	31.00%	58.60%	37.90%	20.70%	10.30%	0.00%	
	% of Panel	1.50%	5.70%	10.30%	12.80%	33.30%	0.00%	
	% of total	0.90%	1.80%	1.10%	0.60%	0.30%	0.00%	3.00%
	Number	217	138	40	31	0	0	390
	% Age group	55.60%	35.40%	10.30%	7.90%	0.00%	0.00%	
	% of Panel	36.70%	46.20%	37.40%	66.00%	0.00%	0.00%	
	% of total	22.60%	14.30%	4.20%	3.20%	0.00%	0.00%	40.50%
	Number	239	93	33	8	4	2	355
	% of age group	67.30%	26.20%	9.30%	2.30%	1.10%	0.60%	
	% of Panel	40.40%	31.10%	30.80%	17.00%	44.40%	#####	
	% of total	24.80%	9.70%	3.40%	0.80%	0.40%	0.20%	36.90%
	Number	127	51	23	2	2	0	188
	% of age group	67.60%	27.10%	12.20%	1.10%	1.10%	0.00%	
	% of Panel	21.50%	17.10%	21.50%	4.30%	22.20%	0.00%	
	% of Total	13.20%	5.30%	2.40%	0.20%	0.20%	0.00%	19.50%
Total	Number	592	299	107	47	9	2	962
	% of total	61.50%	31.10%	11.10%	4.90%	0.90%	0.20%	100%

The Analysis of the types of infection and the age groups:

Infection with *Chlamydia Trachomatis* are mostly prevalent among the subjects between 20 and 30 years of age. It should be noted that in 7.3% of the subjects there is more than one type of infection.

In 693 of 973 subjects there was also analysis of presence of HPV. The results were as follows:

HPV		
	Incidence	Percent
negative	514	52.80%

positive	179	18.40%
Total	693	71.20%

HPV * Panel Crosstabulation

		Panel		Total
		negative	positive	
HPV	negative			
	Number	358 _a	156 _b	514
	%of HPV	69.60%	30.40%	100.00%
	%of Panel	87.10%	55.30%	74.20%
	% of total	51.70%	22.50%	74.20%
	positive			
	number	53 _a	126 _b	179
	% of HPV	29.60%	70.40%	100.00%
% Of Panel	12.90%	44.70%	25.80%	
% of total	7.60%	18.20%	25.80%	

Total	Number	411	282	693
	% of HPV	59.30%	40.70%	100.00%
	% of Panel	100.00%	100.00%	100.00%
	% Of total	59.30%	40.70%	100.00%

The results show that there is a high statistical correlation between the presence of an atypical pathogen and HPV. Pearson's Chi Square statistics shows 88.199 with 1 s.s. and is statistically significant, with a p-value less than 10-3. It can be noted that in 70% of the subjects with a positive HPV finding there are also infections with the other examined pathogens.

Cross tabulation of HPV and the other types of infection (some patients had more than one infection determined on the Panel analysis).

It can be determined that 56.4% of the subjects have a positive finding of *Ureaplasma Spp.*, next to the infection with some of the HPV types.

		Type of infection						Total
		negative	U.U./U.P.	M.H./M.G.	C.T.	T.V.	N.G.	
HPV finding	Number	358	132	36	13	5	1	514
	% of HPV	69.60%	25.70%	7.00%	2.50%	1.00%	0.20%	
	% of Panel	87.10%	56.70%	45.00%	33.30%	83.30%	100.00%	
	% of total	51.70%	19.00%	5.20%	1.90%	0.70%	0.10%	74.20%
	Number	53	101	44	26	1	0	179
	% of HPV	29.60%	56.40%	24.60%	14.50%	0.60%	0.00%	
	% of Panel	12.90%	43.30%	55.00%	66.70%	16.70%	0.00%	
	% Of total	7.60%	14.60%	6.30%	3.80%	0.10%	0.00%	25.80%
Total	Number	411	233	80	39	6	1	693
	%of total	59.30%	33.60%	11.50%	5.60%	0.90%	0.10%	100.00%

DISCUSSION

People are, by nature, sexual beings, and the purpose of concern for sexual health should be the improvement and enrichment of life and interpersonal relationships, and not only for advising and offering medical support for the purpose of conception, and/or prevention and treatment of STDs. Reproductive health implies that people have the opportunity to engage in a pleasurable and fulfilling, yet responsible, sexual life, as well as the possibility to have children and decide when and how often they will choose to do so.

Sexual and reproductive health is a very intimate subject, and thus many people have issues with finding adequate information connected to it. The stigmatization of sexual and reproductive health, especially sexually transmitted infections, leads to improper conditions and services, even an unpleasant treatment of the individual themselves, also in some of the countries belonging to the Western world and Europe.

Our analysis of the available data of a large cohort of patients, provided us with results congruent to world trends in regard to the distribution by age of the HPV infections and atypical pathogens. The analysis showed that there is a very high statistical correlation in the incidence and prevalence of the infections and the particular age group. It can be observed that among younger patients, the incidence and prevalence of these infections is higher, which is congruent with the trends of the sexual behaviors of this population. Although the cohort of patients below age 20 is smaller, it is evident that the infections are highly prevalent in this age group. This can be due to the more frequent examinations warranted in this age group and initiated by the presence of symptomatic infections compared to the overall number of patients in this age group. The examinations requested in the other age groups, however, is more often due to regular check-ups, without any symptoms present. The cohort of patients in the age group of 20-30 years of age have a relatively high prevalence of infections, with 44% of them being positive to at least one of the examined pathogens.

During the analysis of the pathogens themselves, it was confirmed that among the female population of reproductive age in North Macedonia, the most prevalent infections are those with *Ureaplasma Spp.* at 31.1%, *Mycoplasma Spp.* with

11.3% while *Chlamydia Trachomatis* is present in only 4.9% of the cases. The highest prevalence of *Chlamydia Trachomatis* was found in the 20-30 years old age group. Worldwide this is the leading infection among the women of reproductive age [6,15]. Polymicrobial infections are present only in 7.3% of the overall cohort of patients.

What is of interest is that the high statistical correlation between the co-infections with HPV, in which there was a high predictive value of the presence of HPV and some of the analyzed atypical pathogens. In 70% of patients with an HPV infection, there is presence of at least one of the other analyzed pathogens. This is especially important when detecting the association between different pathophysiologic mechanisms, also implied in the early cervical ripening and the premature births.

Finally, the goal of all research in this domain is to allow a safe, available, and acceptable method for regulation of fertility, according to the personal preference of every woman and every man, as well as the available health system resources and services which will allow each woman to have a safe pregnancy and birth, and allow each couple to have healthy offspring.

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Резиме**ИНЦИДЕНЦИЈАТА И ПРЕВАЛЕНЦИЈАТА НА ВАГИНАЛНИТЕ ИНФЕКЦИИ КАЈ ЖЕНИТЕ ВО РЕПРОДУКТИВНА ВОЗРАСТ ОД СЕВЕРНА МАКЕДОНИЈА**

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Во литературата се среќаваат повеќе трудови што укажуваат на ефектите кои сексуално преносливите генитални инфекции ги имаат врз бременоста и врз фертилитетот, односно врз репродуктивното здравје на жените во РС Македонија. Кај инфекциите со атипични бактерии од типот на *Ureaplasma* spp, *Mycoplasma* spp., *Chlamidia trachomatis*, како и кај инфекциите со HPV кај жените во репродуктивна возраст, може да дојде до потценување на нивното значење при утврдувањето на гениталното здравје на жените во репродуктивна возраст.

Во проспективната анализа спроведена од 2014 до 2018 година во лабораторијата за HPV и молекуларна дијагностика при Клиниката за гинекологија и акушерство со Скопје, беа анализирани 10387 пациентки од сите возрасти, од кои кај 973 жена во репродуктивна возраст беше изработена панел-анализа на горенаведените патогени. Од нив, кај 643 беше изработена и HPV-типизација. Од нив, кај 26,7 % постоеше позитивен резултат за HPV додека кај 40,9 % имаше позитивен резултат на панелот за бактериски патогени. Освен анализите спроведени по возрастни групи и по индикација за прегледот, како и HPV-типизацијата, се покажа дека најфреквентен патоген од бактериските причинители се *Ureaplasma* spp. со 33 %, потоа *Mycoplasma* spp. со 7,8 %, додека *Chlamidia trachomatis* беше присутна кај 6,4 % од случаите. Треба да се нагласи дека коинфекцијата со HPV беше присутна во 18,5 % од анализираните случаи каде што имаше наод за двете дијагностички процедури.

Анализата на резултатите кај пациентките со коинфекција на HPV и позитивен наод на панел-анализата покажа висока статистичка зависност ($p < 001$).

Клучни зборови: вагинални инфекции, репродуктивно здравје, *Ureaplasma* spp, *Mycoplasma* spp., *Chlamidia trachomatis*, HPV, коинфекции