

High Prevalence of Sexually Transmitted Infections (STIs) in Asymptomatic Greek Women

Florou Z¹, Pantelidi K¹, Fountas S¹, Skoulakis A¹, Messini C², Lachanas V³ and Petinaki E^{1*}

Abstract

Title: Sexually Transmitted Infections (STIs) varies between countries, depending on various factors (behavior, age, preventive measures etc.)

Background: Aim of this study was to determine the prevalence of various STIs in asymptomatic sexually active women in Central Greece.

Methods and Findings: From June 2014 to May 2016, a total of 188 women, negative for cervical lesion or inflammation according to cytological test, non-vaccinated for HPV and aged between 19-59 years old (mean age 34 years old), were examined. The study material included: 1. blood sample 2. sample of vaginal secretions 3. cervical swab and 4. endo-cervical brush. One hundred – ten women out 188, were found to have at least one STIs (58.5 %), while, 46 of them were positive for more than one. Among the STIs detected, *Ureaplasma parvum* predominated (34%) followed by *Candida albicans* (24.5%), high-risk HPV subtypes (23.4%), *Chlamydia trachomatis* (3.2%) and *Mycoplasma hominis* (2.1%). None woman was found to be positive for *Trichomonas vaginalis*, *Gardnerella vaginalis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Treponema pallidum* and Human Immunodeficiency Virus (HIV). The most common HPV sub-type were the 52 and 39, followed by 59, 16, 31, 33, 56, 18, 51, 58 and 35. The majority of HPV-positive women were colonised by one sub-type, while 10% of HPV-positive women were colonised with more than one.

Conclusion: No significant differences in the prevalence rate were observed between age groups. A weak positive correlation was observed between the concomitant presence of *U. parvum/M.hominis*, *U. parvum/C. trachomatis*, and HPV/ number of partners. Continued close monitoring of the prevalence of STIs in healthy individuals is important for preventing their dissemination.

Keywords: Colonization; Greece; High-risk HPV subtypes; Chlamydia; Ureaplasma; Mycoplasma

- 1 Department of Microbiology, Medical School, University of Thessaly, Larissa, Greece
- 2 Department of Obstetrics and Gynecology, University of Thessaly, Larissa
- 3 Department of Otorhinolaryngology, University Hospital of Larissa, Larissa, Greece

*Corresponding author: Efi Petinaki

✉ petinaki@med.uth.gr

Professor of Medical Biopathology-Clinical Microbiology, Head of Department of Microbiology, Medical School, University of Thessaly, Biopolis, Larissa, Greece 41222.

Tel: 241350-2508

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Introduction

It has long been recognized that the microbial community of the lower genital tract plays a vital role in maintaining the reproductive health of women [1]. The vaginal microbiota of reproductive-aged women is an ecological niche, where several aerobic and anaerobic microorganisms co-exist in a dynamic balance. The homeostasis of the vaginal ecosystem results from complex interactions and synergies among the host and different microorganisms that colonize the vaginal mucosa [2]. Changes in structure and composition are influenced by age, menarche,

time in menstrual cycle, pregnancy, infections, sexually activity, medication, and hygiene. In fertile premenopausal healthy women the vaginal ecosystem is dominated by *Lactobacillus* spp, but, a diverse array of other bacteria can be present in much lower numbers such as *Candida albicans*, *Gardnerella vaginalis*, *Chlamydia trachomatis*, *Mycoplasma* and *Ureaplasma* spp, sexually transmitted viruses (*Herpes virus*, *Human Papillomavirus*) etc.

Although in Greece, several studies have demonstrated that *C. trachomatis*, *Mycoplasma* spp and *Ureaplasma* spp etc. were causative agents for infertility and cystitis data regarding

the composition of microbiota in the genital tract of healthy-asymptomatic fertile indigenous women are few [3-8]. Considering that colonized individuals by the above described microorganisms might serve as reservoirs for spreading infections to men (urethritis etc.) and to women (cervicitis etc.) there is urgent need for their surveillance in healthy people. The aim of the present study was to determine the frequency of various sexually transmitted infections (STIs) in asymptomatic sexually active women in Central Greece in order to establish programs for prevention.

Material and Methods

Clinical setting

The study was prospectively conducted between June 2014 - May 2016 in Thessaly, a rural Greek area with a population of 1.200.000 inhabitants; the protocol was approved by the Ethics Committee of the University Hospital of Larissa, Greece (N 3425).

Study population

A total of 2128 indigenous women, all residents of Thessaly-Central Greece, aged from 19-59 years old, sexually active, not pregnant, not vaccinated for HPV and with no medical or surgical treatment during the study period, were informed of the objectives of the study and were invited to participate. Two hundred-sixteen women (216), which agreed to participate, provided written informed consent, and completed a questionnaire stating reasons for attendance, age, number of lifetime sexual partners, history of sexually transmitted infections (STIs) and recent/current antibiotic treatment. The first visit was organized to obtain a Pap smear test and women who had a positive Pap smear test were excluded from the study. Subsequently, women with normal Pap smears were included and various clinical samples were taken from them.

Sample collection

The study material collected included: Blood sample for detection of antibodies against *Treponema pallidum* and Human Immunodeficiency Virus (HIV).

Sample of vaginal secretion for *Gardnerella vaginalis*, *Candida* spp and *Trichomonas vaginalis* analysis

Cervical swab for detection of *Neisseria gonorrhoeae* - endocervical brushes, for the molecular detection *C. trachomatis*, *U. urealyticum* *U. parvum*, *M. hominis*, *M. genitalium* and high-risk HPV subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59).

Collection of blood samples, vaginal secretion and endocervical brushes was made by medical doctors at the Department of Gynecology of the University Hospital of Larissa. All biological materials collected were appropriately transported and stored until processing.

Detection of STIs

All vaginal secretions and cervical swabs were placed in Stuart transport medium and were sent to the Department of Microbiology, where a smear, Gram stain, and culture on appropriate media were performed. Antibodies against

T. pallidum and HIV were detected by ARCHITECT i2000SR immunoassay analyzer. DNA extracted from clinical samples was tested for the presence of microorganisms (*M. hominis*, *M. genitalium*, *C. trachomatis*, *U. urealyticum*, *U. parvum* and high-risk HPV subtypes) by Real Time PCR using commercial kits (Sacace Biotechnologies)

Statistical analysis

Prevalence of STIs was estimated. Potential correlations between the coexistence of the STIs, as well as between the presence of STIs and number of sexual partners were explored by using Spearman's correlation coefficient. Furthermore, the studied population was divided into four age groups according to the biological alterations in women's reproductive system (19-29, 30-39, 40-49 and 50-59 years old). One-way Anova was used to compare the prevalence of STIs among the four age groups. SPSS 20 statistical software (IBM, Chicago, IL, USA) was used for statistical analysis. P values lower than 0.05 were considered as statistically significant.

Results

Of the 216 women who agreed to take part in the study, 188 with normal cervical cytology were recruited; the demographic characteristics, as well as the reproductive and sexual history of these women are shown in **Table 1**. None of the women tested was found to be positive for *T. vaginalis*, *G. vaginalis*, *M. genitalium*, *U. urealyticum*, *T. pallidum* and HIV. However, 110 of them (58.5%) were found to be positive to any of the following STIs (*U. parvum*, *Candida albicans*, *C. trachomatis*, *M. hominis* and high-risk HPV subtypes); in forty-four women more than one pathogens were detected. The predominant microorganisms were *U. parvum* (34%), *Candida albicans* (24.5%) and some high-risk HPV subtypes (23.4%) **Table 2**.

Although all the women included in the study were negative according to cytological diagnosis for cervical lesions, forty-four of them (23.4%) were found to be positive for some high-risk HPV subtype **Table 2**. The most common HPV sub-types were 52 and 39 (6/22 and 6/22 respectively), followed by 59 (5/22), 16 (4/22), 31 (4/22), 33 (3/22), 56 (3/22), 18 (2/22), 51 (2/22), 58(1/22), 35 (1/22). Twenty-six out of 44 HPV-positive women

Table 1 Demographic and sexual characteristics of women included Characteristics Total (n=198 women).

Age (years) Median (Interquartile range)	34 years (19-59)
Education	n %
Elementary school	14 (7.4%)
High school	116 (61.7%)
University	58 (30.8%)
Age at 1st sexual intercourse (years)	
Median (Interquartile range)	22 (16-32)
Number of Sexual partners	n %
<3	92 (49%)
≥ 3	96 (51%)
(Median (Interquartile range):	3 (0-6)
Pregnancies	n %
Yes	98 (52%)
No	90 (48%)

Table 2 Prevalence of various STAs in all women as well as in the four age groups.

	Total positivity n=188	19 -29 years n=82	30 – 39 years n=46	40 - 49 years n=36	50-59 years n=24
STIs	110 (58.5%)	56 (68.3%)	26 (56.5%)	18 (50%)	10 (41.7%)
<i>U.parvum</i>	64 (34%)	38 (46.3%)	14 (30.4%)	8 (22.2%)	4 (16.7%)
<i>C.albicans</i>	46 (24.5%)	26 (31.7%)	8 (17.4%)	8 (22.2%)	4 (16.7%)
High-risk HPV	44 (23.4%)	24 (29.3%)	12 (26.1%)	4 (11.1%)	4 (16.7%)
<i>C. trachomatis</i>	6 (3.2%)	6 (7.3%)	0 (0%)	0 (0%)	0 (0%)
<i>M.hominis</i>	4 (2.1%)	2 (2.4%)	0 (0%)	2 (5.6%)	0 (0%)

Table 3 Spearman's rho and p values for the correlations between the coexistence of the HPV, *Ureaplasma*, *Mycoplasma*, *Chamydia* and *Candida*.

	HPV	<i>Ureaplasma</i>	<i>Mycoplasma</i>	<i>Chamydia</i>	<i>Candida</i>
HPV		0.080 (p=0.443)	-0.082 (p=0.435)	0.043 (p=0.684)	-0.081 (p=0.439)
<i>Ureaplasma</i>	0.080 (p=0.443)		0.205* (p=0.047)	0.253* (p=0.014)	0.009 (p=0.932)
<i>Mycoplasma</i>	-0.082 (p=0.435)	0.205* (p=0.047)		-0.027 (p=0.798)	-0.084 (p=0.421)
<i>Chamydia</i>	0.043 (p=0.684)	0.253* (p=0.014)	-0.027 (p=0.798)		-0.103 (p=0.322)
<i>Candida</i>	-0.081 (p=0.439)	0.009 (p=0.932)	-0.084 (p=0.421)	-0.103 (p=0.322)	

Table 4 Spearman's correlations of number of partners with HPV, *Ureaplasma*, *Mycoplasma*, *Chamydia* and *Candida*.

	Spearman's rho	P
HPV	0.251*	0.018*
<i>Ureaplasma</i>	0.136	0.207
<i>Chlamydia</i>	0.069	0.523
<i>Mycoplasma</i>	0.020	0.852
<i>Candida</i>	- 0.103	0.341

*Correlation is significant at the 0.05 level.

(59%) were colonised by one type, while, eighteen of them (41%) were colonised with more than one HPV sub-type.

The presence of *U. parvum* was significantly correlated with the co-existence of *C. trachomatis* (Spearman's r: 0.253 / p: 0.014) and *M. hominis* (Spearman's r: 0.205 / p: 0.047). No other significant correlation was found between the presence HPV and the other STAs studied **Table 3**. Furthermore, the presence of high-risk HPV sub-types was significantly correlated with the number of partners (r: 0.251/ p:0.018) **Table 4**.

Table 2 shows the prevalence of genital agents in the four age groups. One-way Anova showed no significant differences in the prevalence rates of STIs among the age groups (p>0.05).

Discussion

Several studies have shown that the prevalence of various STIs among healthy-individuals varies worldwide, depending on the geographical region, age, sexual behavior, education, pregnancy, and previous history of STIs in husbands emphasizing that there is necessity for a local systematic surveillance of STIs [9-17].

According to our data, 58.5% of women were carriers of STIs, while the most common microorganisms detected were *U. parvum*, followed by *C. albicans* and HPV. As mentioned above, the prevalence of *U. parvum* reached to 34%, two fold higher than that recently described in Northern Greece (34 versus 16.13%); this difference could be explained by the fact that the samples of the present study were analyzed by Real-Time PCR which is more sensitive than the traditional techniques used in the former study [7]. Regarding the role of *U. parvum* in the pathogenicity of genital infections, previous studies have shown that genital

ureaplasmas including *U. urealyticum* biovar 2 and *U. parvum* are considered natural inhabitants of the lower urogenital tract of humans as they are often isolated from healthy individuals [18]. Although their clinical significance has not been proven, several studies have demonstrate their participation in recurrent miscarriage, infertility, pelvic inflammatory disease, orchitis, epididymitis, prostatitis and non-gonococcal urethritis. Published data lack indisputable conclusions and doubts still exist as to whether these Ureaplasmas are pathogens or mere co-factors associated with genital infections [19]. Indeed, the co-existence of *U. parvum* either with *C. trachomatis* or *M. hominis* in our study verifies that its presence play significant role on mixed infections of the genital tract [20].

Although the study population included women free of clinical symptoms high prevalence of *C. albicans* (24.3%) was detected. The absence of clinical signs was well correlated with the very low microbial load, since the microorganism was isolated after two or three days incubation. It is well known that *C. albicans* is member of the vaginal normal flora; in the cases where the composition of genital flora changes (antibiotic therapy etc) the microorganism overgrows resulting to vaginal mycosis.

However, the most interesting finding of the present study is focused on the prevalence of high-risk HPV sub-types (23.4%) in women with normal cytology. Various sub-types were identified, emphasizing the need for introduction of the 9-valent HPV vaccine that includes the sub-types 6, 11, 16, 18, 31, 33, 45, 52, and 58. Interesting finding of the present study was the predominance of HPV52. Although HPV52 is among the most frequent high-risk sub-types associated with significant cervical pathology, in a previous study in Slovenia it was identified as

the fourth most frequently HPV sub-type in women with normal cytology, whereas, in a recent study conducted in China it was among the most common sub-types within 16, 58, 51, and 39 [21, 22]. Our results are in concordance with recent studies where high percentage of women with normal cytology was found to be positive for any high-risk HPV sub-type whereas, it is clear that the sexual behavior plays significant role [17,23,24]. Cervical HPV colonization appears to occur frequently within a few years of sexual debut [25,26]. Persistence of cervical HPV colonization for 10-15 years presents a real risk of developing precancerous lesions of the cervix, which can progress to invasive cervical cancer. The new 9-valent HPV vaccine was introduced in Greece on July 2017. So, the results of our study will be the basis for the evaluation of future changes in the epidemiology of HPV infection in Central Greece, particularly in view of the upcoming 9-valent HPV vaccine.

In conclusion, high prevalence of STIs was observed in healthy women in Central Greece. [27,28]. As STIs pose a public health

burden, continued surveillance, monitoring of the prevalence of STIs in healthy individuals, is important for preventing their dissemination.

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None to declare

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The protocol was approved by the Ethics Committee of the University Hospital of Larissa, Greece (N 3425). Formal consent was obtained from all participants.

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