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Occurrence of *Ureaplasma parvum* and *Ureaplasma urealyticum* in Women with Cervical Dysplasia in Katowice, Poland

The aim of this study was to evaluate the occurrence of genital mycoplasmas, especially *Ureaplasma parvum* and *Ureaplasma urealyticum*, in women with atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesions (LSIL) and high grade squamous intraepithelial lesions (HSIL), compared to women with normal cytology living in Katowice, Poland. Two sterile swabs were used to obtain material from the posterior vaginal fornix of 143 women with squamous intraepithelial lesions and 39 healthy women: first for general bacteriology, second for detection of urogenital mycoplasmas using Mycoplasma IST2 kit. From each positive Mycoplasma IST2 culture DNA was isolated and PCR was performed for identification of *U. parvum* and *U. urealyticum*. Mycoplasma IST was positive in 34.1% cases. Urogenital mycoplasmas were demonstrated in women with HSIL significantly more often compared to women with LSIL, ASCUS, and with normal cytology. DNA of *U. parvum* was demonstrated in majority of Mycoplasma IST2-positive cases, *U. urealyticum* DNA-only in 9 (4.9%). Predominance of 3/14 serovars of *U. parvum* was demonstrated. *U. urealyticum* biovar 2 was present more often in women with squamous intraepithelial lesions.

Key Words : *Mycoplasma hominis; Ureaplasma urealyticum; ASCUS; LSIL; HSIL*

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INTRODUCTION

Frequent colonization of female and male genitourinary tract by ureaplasmas sometimes hinders evaluation of these microorganisms as infectious agents. Although *Ureaplasma urealyticum* was divided into two biovars and new species *Ureaplasma parvum* was described, additional studies are required to determine differences in pathogenicity and respective role of these microorganisms in diseases. Detection of ureaplasmas is possible by characteristic growth on appropriate media and urease activity, but species identification of *U. urealyticum* and *U. parvum* must be demonstrated by molecular methods. Differentiation between *U. parvum* and *U. urealyticum* is very important, especially for correct interpretation of laboratory results and evaluation of pathogenicity.

U. parvum includes small genome strains: 0.75-0.76 Mpb (former biovar 1 of *U. urealyticum*-serovars 1, 3, 6, and 14). *U. urealyticum* includes serovars 2, 4, 5, and 7-13 (former biovar 2) (1-4). Cervical dysplasia is connected with infection with high-oncogenic types of human papillomavirus (HPV). Disruption in function of immune mechanisms and inflammation might be initiated by co-occurrence of bacterial pathogens, including ureaplasmas. The influence of ureaplasmas on cytokine concentrations was confirmed with cell cultures in vitro and in vivo, using animal models and human cervico-vaginal samples (5, 6). Ureaplasmas can also act on progression of cervical dysplasia, classified with the Bethesda System 2001 classification scheme as atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL) and cancer (7).

The aim of this study was to evaluate the occurrence of genital mycoplasmas, especially *U. parvum* and *U. urealyticum*, in women with ASCUS, LSIL and HSIL, compared to women with normal cytology in Katowice, southern Poland.

MATERIALS AND METHODS

One hundred and eighty two non-pregnant, menstruating, sexually active women (mean age 39.5 yr), with similar socio-economic status, who attended Department of Medical Microbiology of Medical University of Silesia in Katowice for microbiological diagnosis, were included in this study. They did not use oral or vaginal contraceptives and antibiotics/antimycotics within at least 4 weeks before examination. Cervical swabs were taken from all studied women and tested for cytology. All cytology results were interpreted by

two pathologists into normal, ASCUS, LSIL, HSIL, squamous cell carcinoma (SCC) according to 2001 Betheda System (7). All women gave informed consent for this study. This study was approved by Bioethical Committee of Medical University of Silesia NN-6501-246/04.

Two sterile swabs were used to obtain material from the posterior vaginal fornix: first for general bacteriology was cultured on the following agar plates: Columbia blood, MacConkey, Chapman, Thayer-Martin and Sabouraud, respectively for streptococci, lactobacilli, Gram-negative rods, staphylococci, gonococci and yeasts. BV-bacterial vaginosis was diagnosed by Nugent and Amsel criteria (8, 9).

Isolated microorganisms were identified with morphological, biochemical and serological characteristics. Second swab for detection of urogenital mycoplasmas was cultured using Mycoplasma IST2 (bioMérieux, Marcy-L'etoile, France), according to manufacturer's instructions. For isolation of DNA, each culture in logarithmic phase of growth was centrifuged 15.000 *g* for 30 min in 4°C, pellet was twice washed with PBS and incubated with buffer containing proteinase K. Isolation of DNA was performed with appropriate columns, using DNeasy Tissue Kit, Qiagen. Cultures of mycoplasmas in urea-arginine broth and DNAs were stored at -70°C until used. Two cervical swabs were taken from each of 182 studied women for the determination of *Chlamydia trachomatis* with AMPLICOR *C. trachomatis*, Roche Molecular Systems, U.S.A., and high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 by Amplicor HPV, Roche Molecular System U.S.A., respectively.

PCR identification of ureaplasmas was done according to Kong et al. (3), using termocycler Genius (Techne, U.K.) and *Taq* PCR Master Mix Kit (Qiagen). Primers UMS- 57/ UMA222 and UMS-170/UMA263 were used for identification of *U. parvum* (326 bp) and *U. urealyticum* (476 bp), respectively. Amplified products were visualized under UV light after electrophoresis in 2% agarose gel, containing ethidium bromide (Table 1). The reference strains from American Type Culture Collection: *U. urealyticum* ATCC 27618 and *U. parvum ATCC* 27815T were used as positive controls.

Statistical analysis was performed by chi-square test ($P<0.05$ was statistically significant).

RESULTS

According to cervix cytology results, the following subgroups were identified: 67 women with ASCUS, mean age 40.5 yr; 49 women with LSIL, mean age 36.6 yr; 22 with HSIL, mean age 34.1 yr; and 39 with normal cytology, mean age 39 yr; SCC was diagnosed in 5 cases. Presence of urogenital mycoplasmas was confirmed by Mycoplasma IST2 in 62/ 182 cases 34.1%. Women with HSIL showed the presence of urogenital mycoplasmas significantly more often than women with LSIL ($P<0.001$), ASCUS ($P=0.005$) and normal cytology ($P<0.001$). In 58 out of 62 Mycoplasma IST2-positive cases DNA of *U. parvum* was demonstrated while in 9 cases (4.9%) *U. urealyticum* was detected. In 5 cases both DNAs-*U. parvum* and *U. urealyticum* were detected (Table 2). We detected fewer instances of *U. urealyticum* compared to *U. parvum*, however, in all but one cases, *U. urealyticum* biovar 2 strains were isolated from birth canal of women with squamous intraepithelial lesions (Table 2). Among isolated strains of *U. parvum* predominance of 3/14 serovar was demonstrated (Table 3).

Urogenital mycoplasmas were detected significantly more often in HPV-positive women, compared to HPV-negative women, 57.5% and 18.3%, respectively. All HSIL and cancer cases were HPV-positive. Co-occurrence of selected vaginal microorganisms, including HPV and urogenital mycoplasmas, is presented in Table 4. All women included in this study were *C. trachomatis*, *Neisseria gonorrhoeae* and BV-negative.

DISCUSSION

Commercial mycoplasmal kits showed good correlation with PCR results in detection of *Ureaplasma* strains, although it is impossible to differentiate *U. parvum* and *U. urealyticum* using these commercial kits (10, 11). On top of that, urea-

Table 1. Identification of *U. parvum* and *U. urealyticum* and subtyping of *U. parvum* by PCR

Primer pairs	Sequence 5′-3′	Specifity	Amplicon size
UMS57 UMA222*	YAAATCTTAGTGTTCATATTTTTTAC GTAAGTGCAGCATTAAATTCAATG	*U. parvum*	326 bp
UMS170 UMA 263†	GTATTTGCAATCTTTATATGTTTTCG TTTGTTGTTGCGTTTTCTG	*U. urealyticum*	476 bp
UMS83 UMA269*	TACTGTAGAAATTATGTAAGATTGC CCAAATGACCTTTTGTAACTAGAT	*U. parvum* serovar 1	398 bp
UMS125 UMA269*	GTATTTGCAATCTTTATATGTTTTCG CCAAATGACCTTTTGTAACTAGAT	*U. parvum* serovar 3/14	442 bp
UMS54 UMA269*	CTTAGTGTTCATATTTTTTACTAG CCAAATGACCTTTTGTAACTAGAT	*U. parvum* serovar 6	369 bp

*94°C/3 min, 35 cycles 95°C/30 sec; 58†(55)°C/30 sec, 72°C/60 sec, 72°C/7 min.

Table 2. Occurrence of urogenital mycoplasmas in study groups (number and percentage of positive cases)

Pathogens	ASCUS		LSIL		HSIL		Cancer		Normal cytology	
	n	%	n	%	n	%	n	%	n	%
U. parvum	19	28.4	7	14.3	7	31.8	2	40.0	7	17.9
U. urealyticum	0	0.0	2	4.1	0	0.0	0	0.0	1	2.6
U. parvum+*U. urealyticum*	0	0.0	2	4.1	1	4.5	0	0.0	0	0.0
U. parvum+*U. urealyticum* +*M. hominis**	0	0.0	1	2.0	1	4.5	0	0.0	0	0.0
U. parvum+*M. hominis**	4	6.0	1	2.0	5	22.7	0	0.0	1	2.6
U. urealyticum+*M. hominis**	0	0.0	0	0.0	1	4.5	0	0.0	0	0.0
Total	23	34.3	13	26.5	15	68.2†	2	40.0	9	23.1

*strains of *M. hominis* always accompanied ureaplasmas; †occurrence of urogenital mycoplasmas in women with HSIL was significantly higher compared with those with LSIL P=0.001, ASCUS P=0.005 and normal cytology P<0.001.
ASCUS, atypical squamous cells of undetermined significance; LSIL, low grade squamous intraepithelial lesions; HSIL, high grade squamous intraepithelial lesions.

Table 3. Subtyping of isolated *Ureaplasma parvum* strains (n=58)

Study groups	Number of strains				Total (n=58)
	Serovar 1 (n=5)	Serovar 3/14 (n=17)	Serovar 1+3/14 (n=28)	Not typable (n=8)	
Normal cytology	0	2	5	1	8
ASCUS	2	6	11	4	23
LSIL	1	4	5	1	11
HSIL	1	5	6	2	14
Cancer	1	0	1	0	2

ASCUS, atypical squamous cells of undetermined significance; LSIL, low grade squamous intraepithelial lesions; HSIL, high grade squamous intraepithelial lesions.

Table 4. Co-occurrence (%) of urogenital mycoplasmas and selected vaginal microorganisms (percentage of positive cases)

Pathogens	HPV‡	*Lactobacillus* spp.	GBS*	*Candida* spp.	Gram (-) facultative rods
U. parvum-positive (n=58)†	67.2	86.2	10.3	6.9	6.9
U. urealyticum-positive (n=9)†	88.9	100	11.1	11.1	11.1
U. parvum-negative *U. urealyticum*-negative *M. hominis*-negative (n=120)	25.8	83.3	20.0	9.2	14.2

*GBS-group B streptococci; †in 5 cases both *U. parvum* and *U. urealyticum* DNAs were detected; ‡HPV, human papillomavirus.

arginine broth used in *Mycoplasma* IST is suitable for isolation of mycoplasmal DNA (12). This is why in the case of PCR detection we only used samples positive in *Mycoplasma* IST2. Our results concerning colonization of 32% of tested women with *Ureaplasma* spp. is in accord with other authors, who demonstrated 20-50% colonization (6, 13-18). However, Abele-Horn et al. in 1997 (19) described 70% positive results. Recently published studies from Eastern Poland show 29.8% occurrence of ureaplasmas in non-pregnant women suffering from urogenital diseases (14). In the case of pregnant women in Central Poland this ratio is 26.3% (6). Our finding of *M. hominis* (7.7%) is in agreement with our previously described observations as well as with other authors (13, 15, 17). Domination of *U. parvum* (86.6%) among isolates in our study as well as prevalence of serovar 3/14 was in concordance with others (2, 12, 19-22). Co-occurrence of different mycoplasmal DNAs and presence of different serovars of *U. parvum* in the same sample demonstrated in our study is in accord with other authors (2, 19, 20).

Only limited publications are available about relations of ureaplasmas with ASCUS, LSIL and HSIL. Lukic and coworkers in 2006 (18) described variations in occurrence of *Ureaplasma* spp. depending of cytological results: in ASCUS -27%, LSIL -35% and HSIL -45%, compared to women with normal cytology, 19%. The authors concluded that ureaplasmas are an important co-factor for HPV. In our study we demonstrated significantly more frequent occurrence of urogenital mycoplasmas in the group of women with HSIL: DNA of HPV was detected in each women in this group. In our previous studies more frequent isolation of urogenital mycoplasmas in the group of women with LSIL infected with HPV, compared with HPV-negative women, was demonstrated (13). In the present paper we demonstrated that infection with high-risk HPV types often accompanied *U. urealyticum* 88.9%, less frequently *U. parvum* 67.2%, and rarely it was confirmed in women without mycoplasmal infection 25.8%. Several studies demonstrated higher frequency of isolation of *U. parvum* compared with *U. urealyticum*. In our study the same results were obtained, although percentage of isolated *U. urealyticum* was very low. In the present work occurrence of *U. urealyticum* (biovar 2) was 4.9%, but was demonstrated in all but one woman with squamous intraepithelial lesions. Similar results of low percentage of *U. urealyticum* were demonstrated by Japanese authors in preterm birth group 4.8% (22).

In many papers *U. urealyticum* is indicated as the cause of pathology: in the group of preterm birth women and those with pelvic inflammatory diseases (19), men with nongonococcal and nonchlamydial urethritis (23, 24). Newborns infected with *U. urealyticum* were subject to more frequent and longer therapeutic procedures supporting respiration, needed more frequent surfactant and antibiotic administration (21). However, controversial data are published as to the role of mycoplasmas in pathogenesis of bronchopulmonary dysplasia and chronic lung disease in newborns (14, 20, 25, 26).

Although our study groups were relatively small, significantly higher occurrence of *U. urealyticum* in women with squamous intraepithelial lesions suggests a possible role of this bacterium in pathology. Further investigation using larger groups of patients are required to demonstrate possible interactions of microorganisms infected female genital tract and their role in progression of cervical dysplasia.

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