Association between genital mycoplasma and cervical squamous cell atypia

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Abstract Objectives: To investigate the existence of an association between genital mycoplasma infections and cervical squamous cell atypia.
Study design: Prospective cross-sectional study.
Setting: Department of Obstetrics and Gynecology, in collaboration with the Clinical Pathology and Pathology Departments, Faculty of Medicine, Benha University, Egypt.
Patients and methods: Three-hundred women were scheduled into two equal groups. The control group included 150 women with negative Pap smear for cervical atypia. The study group included 150 women with cervical squamous cell atypia proved by Pap smear. Swabs obtained from endocervix and posterior vaginal fornix were subjected to culture for detection of genital mycoplasma using Mycoplasma IST2. Outcome measures were the rates of cervicovaginal infection with genital mycoplasma in both groups, and estimation of the co-occurrence of genital mycoplasma and cervical squamous cell atypia.
Results: Using Mycoplasma IST2 kit genital mycoplasmas were positive in (49.33% vs. 28.67%) of cases in study and control groups, respectively. Ureaplasma urealyticum was isolated more frequent than mycoplasma hominis and mixed mycoplasma infection. U. urealyticum colonization was demonstrated in women with HSIL (57.5%) significantly more frequent compared to women with LSIL (36.59%), ASCUS (30.43%), and with normal cytology (21.33%); P = 0.019.
Conclusion: Ureaplasma urealyticum was present significantly more often in women with cervical cytological atypia, suggesting the existence of an association between cervicovaginal infections with U. urealyticum and precancerous lesions of the uterine cervix.

1. Introduction

Cervical cancer is an important cause of morbidity and mortality among females worldwide, more so in developing countries. Fortunately, cervical carcinoma is a both preventable and treatable disease (1). The primary prevention eliminates the
possibility of having the cervical cancer by knowing the etiological factors and how to remove them. However, the secondary prevention relies on population screening for early detection and appropriate management of its precursor lesions, cervical intraepithelial neoplasia (CIN) (2). The epidemiological studies show that early age at first intercourse and multiple sexual partners increase the risk of development of CIN. These observations suggest that agents or factors associated with or transmitted by sexual intercourse may have a role in the etiology of CIN (3).

The human papillomavirus (HPV) is one of the most common sexually transmitted pathogens and is strongly associated with preneoplastic and neoplastic lesions of the uterine cervix. It has been postulated that HPV infection alone may not be sufficient to promote cervical carcinogenesis and that other cofactors could be implicated (4). Factors that may have a role in this progression include early onset of sexual activity (<16 years), high parity, multiple sexual partners, smoking, pregnancy, oral contraceptives, immunosuppressive, vitamin deficiency, low socioeconomic status and infection with sexually transmitted diseases (STDs), such as bacterial vaginosis (BV), chlamydia trachomatis and trichomonas vaginalis (4–6).

Mycoplasma species are the smallest free-living, cell wall-deficient microorganisms. Genital mycoplasmas, represent a species frequently found in the lower genitourinary tracts of sexually active healthy men and women. The most prevalent genital mycoplasmas are M. hominis, U. urealyticum, and M. genitalium. In evaluating the role of these organisms in human diseases, their high prevalence among asymptomatic women must be taken into account (7,8). Many authors have reported that these organisms could play important roles in genital tract pathologies such as bacterial vaginosis (9), pelvic inflammatory diseases, infertility (10), and several adverse pregnancy outcome, e.g., preterm delivery, premature rupture of membranes, and chorioamnionitis (11).

As genital mycoplasma is similar to CIN in epidemiologic features, we investigated the existence of an association between genital mycoplasma and the development of CIN. Limited publications reported such associations are available (6,12–14).

2. Patients and methods

2.1. Study population

This is a prospective cross-sectional study finally recruited 300 women, among those attending the Gynecology Outpatient Clinics of Benha University Hospital. The study was conducted in collaboration with the Departments of Clinical Pathology and Pathology, Faculty of Medicine, Benha University, Alkhalubia, Egypt, between July 2011 and June 2012. The study protocol was approved by the Local Ethics Committee. All women gave a written informed consent before the study commenced.

The participants were nonpregnant, premenopausal, sexually active women, and had not received any oral contraceptive, antibiotic, immunosuppressive or topical therapy for at least one-month prior. Women with history of diagnosed genital tract malignancy, total abdominal hysterectomy or current vaginal bleeding were excluded.

Participants were scheduled according to cytology report into two equal groups. The control group included 150 women with negative Pap smear for cervical atypia. The study group included 150 women with cervical squamous cell atypia recruited according to the order of diagnosis by Pap smear. The cytological report used the 2001 Bethesda System (15) and identified 3° of cervical atypia: atypical squamous cells of undetermined significance (ASCUS); low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous lesion (HSIL).

2.2. Sample collection and processing

Sample collection and processing were performed during the postmenstrual period. Patients were instructed to avoid vaginal douching 48 hours before sample collection. The patient was placed in a lithotomic position. Cervix was exposed through introduction of a nonlubricated sterile Cusco speculum into the vagina.

2.2.1. Papanicolaou (Pap) smear

Sample was collected from the uterine cervix by rotating the Ayre spatula 360 around the uterine ectocervix for scraping of the transformation zone. The sample was then smeared on a glass slide and immediately fixed by spraying with cytospray (ethyl alcohol) then sent to the cytopathology laboratory for staining and reading through the optical microscope.

2.2.2. Bacteriological specimen

Sterile swabs were used to obtain discharge from the posterior vaginal fornix and endocervix for the diagnosis of the presence or absence of genital mycoplasma (M. hominis and U. urealyticum) by mycoplasma IST-2 kits (bioMe® rieux, Marcy-L’etoile, France). The mycoplasma IST2 is a test for the identification of urogenital mycoplasmas by cultivation, biochemical identification, indicative enumeration and antibiotic susceptibility testing to (doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, pristinamycin, azithromycin, clarithromycin, and ciprofloxacin) and interpreted according to manufacturer’s instructions. Samples were considered positive for M. hominis and U. urealyticum for values ≥10^4 color changing unit (CCU) per milliliter of the specimen.

The outcome measures of this study were the rates of cervicovaginal genital mycoplasma infections in the studied population and the co-occurrence of genital mycoplasma infections and cervical cytological atypia.

2.3. Statistical analysis

Data obtained were statistically analyzed using Statistical Package for Social Sciences (SPSS, Chicago, USA) software version 15.0 for Windows. Results were expressed as range, mean ± SD, numbers and percentages. Means were compared using the unpaired student’s t test while proportions were compared using the chi-square test (χ²). P value of less than 0.05 was considered of statistical significance.

3. Results

Table 1 shows that there were no statistically significant differences between both groups as regards social and demographic data of participants at time of study enrollment.
Fig. 1 shows colonization of 39% of the study group with *U. urealyticum*, 5% with *M. hominis* and 5% with both pathogens.

Fig. 2 shows colonization of 21% of the control group with *U. urealyticum*, 3% with *M. hominis* and 4% with both pathogens.

Fig. 3 shows that the study group was subdivided according to the grade of cytological cervical lesions into three subgroups; ASCUS (46%), LSIL (27.67%) and HSIL (26.33%).

Table 2 shows that *U. urealyticum* was demonstrated in women with HSIL significantly more often compared to women with LSIL, ASCUS, and with normal cytology. However, *M. hominis* did not demonstrate such association.

4. Discussion

Although, evidence of HPV is found in approximately 80% of cervical carcinomas, epidemiological studies have
Table 2 Occurrence of genital mycoplasmas in study and control groups (number and percentage of positive cases).

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Group</th>
<th>Study group (Squamous cell abnormalities) (n = 150)</th>
<th>Control group (Negative for intraepithelial lesions) (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ASCUS (n = 69)</td>
<td>LSIL (n = 41)</td>
</tr>
<tr>
<td>M. hominis</td>
<td></td>
<td>2 (2.9%)</td>
<td>3 (7.32%)</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td></td>
<td>21 (30.43%)</td>
<td>15 (36.59%)</td>
</tr>
<tr>
<td>M. hominis + U. urealyticum</td>
<td></td>
<td>3 (4.35%)</td>
<td>2 (4.88%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>26 (37.68%)</td>
<td>20 (48.79%)</td>
</tr>
</tbody>
</table>

Data expressed as number (percentage); $X^2$ = Chi-square test

* Significant difference as $P < 0.05$.

demonstrated that STDs other than HPV have a role in the pathogenesis of cervical cancer. The associations between cervical cancer and bacterial vaginosis (BV), chlamydia trachomatis or trichomonas vaginalis have been established (4–6). Concomitant infections with human papillomavirus and various mycoplasma and Ureaplasma species in women with normal cervical cytology have been reported (14). However, the knowledge about the specific role of these pathogens in the natural history of CIN is limited.

There are several mechanisms by which the genital mycoplasma could be implicated in the progress of cervical dysplastic and neoplastic lesions either indirectly by increasing the susceptibility of the inflamed epithelium to carcinogens or directly through suppression of cell mediated immunity, which allow the pathogen to persist and predispose the host for colonization by other pathogens (16). Toll-like receptors (TLRs) 1, 2, and 6 on the surface of antigen presenting cells (APCs) have evolved to recognize conserved products unique to myco-

plasma metabolism. This specificity allows to detect the presence of infection and to induce activation of inflammatory and antimicrobial innate immune responses that lead to a Th2-type polarization of the immune response and the secretion of IL-4, IL-5, IL-10 and IL-13. These cytokines are antagonists to Th1 cytokines (TNF-α, IL-2, IFN-γ, IL-6, and IL-12); imbalance between both types of cytokines disturbs the cellular and humoral immune response (17) resulting in depressed apoptosis and excessive cellular proliferation (18). In addition it was reported that Mycoplasma infections induce in vitro chromosomal changes and cell transformation throughout gradual progressive chromosomal loss and Robertsonian translocations (19).

The current study, reported a significantly higher colonization rate with U. urealyticum of the study group compared to control group (39.33% vs. 21.33%, respectively; $P = 0.001$). This is in agreement with other studies, which demonstrated 32–35% colonization with U. urealyticum in women with cervical atypia compared to 19–29.8% and 26.3% in nonpregnant and pregnant women with normal cervical cytology (6,12,13,20). Nevertheless, these results were lower than the 70% positive results described by Abele-Horn et al. in 1997 (21). The current study reported low rates of M. hominis (5.33% and 3.33%) and combined M. hominis and U. urealyticum (4.67% and 4%) in study and control groups, respectively. These conclusions are in agreement with those described by other authors (13).

U. urealyticum colonization was demonstrated in women with HSIL (57.5%) significantly more often compared to women with LSIL (36.59%), ASCUS (30.43%), and with normal cytology (21.33%). Although limited publications are available to investigate such association, all confirmed the results of the current study and concluded that U. urealyticum is an important cofactor interacting with HPV in the development of pre-cancerous and cancerous lesions of the uterine cervix (6,13).

Identification of genital mycoplasma was performed using mycoplasma IST2 kits. These kits would be an easier, faster (i.e. results are available within 48 h) and more practical alternative to culture. It provides information about pathogenic colonization density with a titer of $\geq 10^4$ CCU per milliliter of the specimen, and has the advantages of a shelf life of up to 12 months (22). Also, the IST2 kit provides information on antibiotic susceptibilities of the genital mycoplasmas, but the IST2 kit cannot determine resistance patterns for each organism separately in cases of mixed mycoplasma infections. Some authors reported that mycoplasma IST2 kits exhibited a good correlation with polymerase chain reaction (PCR) results in the detection of Ureaplasma strains, but PCR is still needed to differentiate U. parvum and U. urealyticum (13). The main limitations of this study are the small number of subjects examined, and the lack of correlation of G. mycoplasma infections with HPV infections and cervical histopathology.

5. Conclusions

U. urealyticum was present more often in women with cervical cytological abnormalities, suggesting the existence of an association between cervicovaginal infections with U. urealyticum and precancerous lesions of the uterine cervix.
Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

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