

Cytological diagnosis of genital ureaplasma urealyticum and its importance in cervical inflammation

R.A. BENDER^{1,2}, C. GUNDOGDU³

¹Department of Obstetrics and Gynecology, Biruni University School of Medicine, Istanbul, Turkey

²Department of Obstetrics and Gynecology, ³Department of Pathology, Medicana International Hospital, Istanbul, Turkey

Abstract. – OBJECTIVE: Cervical smear cytology, which is a gynecological cervical cancer screening test, can provide information about the presence of pathogenic microorganisms or the inflammation they cause. Among them, *Ureaplasma urealyticum* (Uu), which is a subspecies of *Mycoplasma* was held responsible for high-grade cervical intraepithelial lesions and malignancy due to long-lasting complicated vulvovaginitis clinic. We aimed at investigating the role of Uu in the inflammatory process of the cervix and to describe the cytological features that enable it to be recognized microscopically in cervical smear test.

PATIENTS AND METHODS: Cervical smear and mycoplasma culture data of 123 women with complicated vulvovaginitis findings were evaluated. According to the Uu culture results, women were divided into two groups: the Uu-positive (n=59) and the Uu-negative group (n=64). The groups were compared in terms of cervical smear results, macroscopic view of the cervix, and secondary cytological evaluation results.

RESULTS: The presence of inflammatory signs (83.1%) in the Uu-positive group was observed to be 83.1%, whereas 67.2% in the Uu-negative group, and the difference between the two groups was found to be significant ($p=0.04$). Besides, the difference in aggregated polymorphonuclear leukocytes (PMNL) between Uu-positive group (59.3%) and Uu-negative group (40.6%) was statistically significant ($p=0.04$). Similarly, nuclear atypia of epithelial cells in the Uu-positive group (33.9%) was observed to be higher than in the Uu-negative group (17.2%) ($p=0.03$).

CONCLUSIONS: Uu causes inflammation of the cervix and cervical intraepithelial lesions. Aggregated PMNL observed in cervical smear cytology may be one of the findings that will give clues for Uu.

Key Words:

Ureaplasma urealyticum, Cervical intraepithelial lesions, Cervical smear cytology, PAP smear test, Inflammation of the cervix.

Introduction

Genital mycoplasmas are intracellular bacteria that live in urogenital mucosal epithelial cells in humans. They are transmitted through direct contact, therefore they can be included in the category of sexually transmitted pathogens¹. *Ureaplasma urealyticum* (Uu), one of the common subspecies of genital mycoplasmas, belongs to the normal flora bacteria class¹⁻³. Uu can be found in the vagina or cervix of approximately 40-80% of sexually mature women⁴. It is suggested that Uu may cause abnormal cervical cytopathology even though it does not cause cervicitis^{2,5}. Studies^{6,7} have shown that chronic mycoplasma infections can induce chromosomal changes that may lead to the transformation of mammalian cells.

The Papanicolaou (PAP) smear test is a simple, fast, low-cost, and noninvasive screening test used in cervix cancer screening. The main advantage of this test is that it can be widely applied as a periodic routine screening method. Although it is an effective method for screening cervical malignancies, its sensitivity is lower in premalignant lesions of the cervix⁸.

The presence of microorganisms is not required to be reported in current cervical cytology reporting systems, but positive findings are added to the report if there is evidence of the presence of microorganisms on cytological examination⁹. In several developing countries, clinical examination and pap smear tests are used to diagnose cervicovaginal infections. Methods, such as PCR and cell culture, cannot be applied due to the financial burden they bring to the health system. Moreover, all cytological evaluations can also be used for the identification of pathogens¹⁰. In the literature¹¹, the most common cytological features of different microorganisms in cervical

pap smears were described. However, there is no controlled study about the presence of Uu in cervical cytological evaluation or the inflammation markers it causes.

Our study aims at investigating the role of Uu, which is commonly observed and causes abnormal cervical cytopathology, in the inflammatory process of the cervix and to define its cytological features, which enable it to be recognized microscopically in PAP smear test.

Patients and Methods

Ethical Statement

The study was approved by the Biruni University Ethics Committee (Decision date: 24.06.2022/ Decision No.: 2022/71-38). All methods were performed following the relevant guidelines and regulations by including a statement. Informed consent for the use of their medical records in scientific studies was obtained from all patients.

Study Design

The data of 123 women who applied to the Medicana International Istanbul Hospital Gynecology outpatient clinic between April 2018 and May 2022, whose ureaplasma culture and liquid-based smear material were taken simultaneously, were scanned retrospectively. Data of sexually active women aged 22 to 50 years with symptoms of complicated vulvovaginitis, HIV-negative, and who had not received any oral contraceptive, antibiotic, immunosuppressive, or topical therapy for at least one month were included in the study. Menopausal patients and pregnant women were excluded from the study. According to the results of *Ureaplasma* culture, women were divided into two groups. Uu breeding women were determined as Uu-positive group (n=59), while others were determined as Uu-negative group (n=64).

For ureaplasma culture, samples were retrieved from the ectocervix and endocervix using a sterile swab and transferred into tubes containing a transport medium. The cell culture analysis of the collected material was performed using the Mycoplasma IST 2 kit. Growth of 10^4 >CCU mycofast/ml was considered positive for Uu in the samples.

After obtaining liquid-based cytology samples, Papanicolaou staining was performed routinely in the pathology laboratory of our hospital and evaluated with a light microscope. In the cytomorphological evaluation, it was primarily checked whether the taken samples were suffi-

cient. Sample adequacy was determined by the presence of endocervical cells and the appropriate number of epithelial cells.

According to the Bethesda system, smear results and a macroscopic view of the cervix during the examination were compared in two groups. As the appearance of the cervix on examination, normal, erosive, and vascularized appearances were determined. Cervical smear cytology results were classified into the following categories according to the current Bethesda system: (1) negative, (2) atypical squamous cells of undetermined significance (ASC-US), (3) atypical glandular cells (AGC), (4) atypical squamous cells-cannot exclude high-grade intraepithelial lesion (ASC-H), (5) low-grade squamous intraepithelial lesion (LSIL), (6) high-grade squamous intraepithelial lesion (HSIL), (7) squamous cell carcinoma, (8) adenocarcinoma *in situ*, and (9) invasive adenocarcinoma⁹.

At the same time, previous PAP smear tests of the women were reevaluated by independent pathologists. In the secondary evaluation, the presence of unexpected pathological parameters that ureaplasma infection may cause in the host cell and nucleus in cervical cytology was investigated. The cytological evaluation was held considering polymorphonuclear leukocytes (PMNL) density ≥ 10 PMNL/epithelial cells in at least 10 high-powered fields (hpf)¹¹. Furthermore, the scattered localization of the evaluated PMNLs or the formation of aggregates – namely polyballs – were also considered as subtitles of inflammation markers (Figure 1). According to the maturation index, the hormonal status of cervical cells (dominance of intermediate or superficial cells) and the presence of döderlein bacilli were evaluated as basic flora and host characteristics. Nuclear contour irregularity, hyperchromasia, and nuclear enlargement were determined as the criteria for

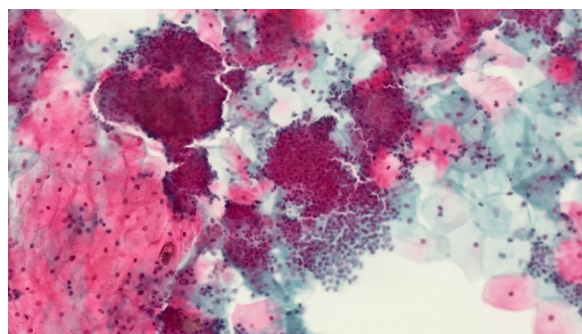


Figure 1. Aggregated polymorphonuclear leukocytes "Polyballs".

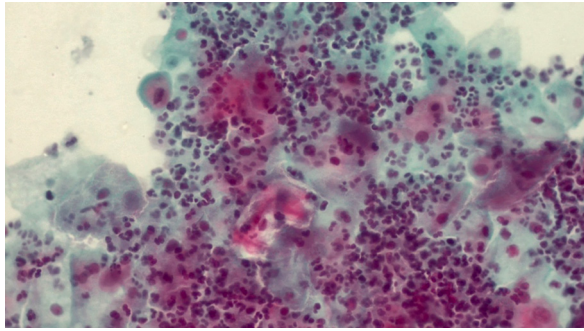


Figure 2. Nuclear alteration (irregular nuclear contours and hyperchromasia).

nuclear atypia in epithelial cells (Figure 2). The two groups were also compared in terms of these parameters.

Statistical Analysis

The data obtained in the study were analyzed with SPSS (version 25, IBM Corp., Armonk, NY, USA). Results were evaluated as mean \pm standard deviation or frequencies (percentages). In group comparisons, normally distributed parameters were evaluated with the paired sample *t*-test, and non-normally distributed parameters were evaluated with the Wilcoxon sign test. Data with a *p*-value of <0.05 and a 95% confidence interval were considered statistically significant. The minimum number of samples was determined as 8 with 80% power.

Results

The women included in the study were divided into two groups according to the ureaplasma

culture results. The group with growth in culture was called as Uu-positive group, whereas the group with no growth in culture was called as Uu-negative group. Age, body mass index (BMI), preferred contraception method, presence of leukorrhea, and smoking were evaluated as demographic data for both patient groups (Table I). There were no statistically significant differences within the demographic data.

In the comparison between the groups, there was no significant difference between the macroscopic view of the cervix and cervical smear results. Re-evaluation of cervical cytology revealed that the presence of inflammatory signs was found in 83.1% of the Uu-positive group while was 67.2% of the Uu-negative group and the difference between these two groups was statistically significant ($p=0.04$). Moreover, aggregated PMNL ratio was 59.3% in Uu-positive group and 40.6% in Uu-negative group ($p=0.04$). Nuclear atypia of epithelial cells was found higher in the Uu-positive group than in the Uu-negative group ($p=0.03$). Accordingly, nuclear atypia of epithelial cells was seen in 20 (33.9%) patients in the Uu-positive group and 11 (17.2%) patients in the Uu-negative group in the re-evaluation of the liquid-based smears of the patients. The hormonal status of the cervix and the presence of Döderlein bacillus ratios in liquid-based smears of both groups were similar (Table II).

Discussion

In routine gynecology outpatient evaluation of the cervix, speculum examination, and cervical smear examination are generally preferred, even

Table I. Demographic characteristics for *Ureaplasma urelyticum* positive group and *Ureaplasma urelyticum* negative group.

	<i>Ureaplasma urelyticum</i> positive group n = 59	<i>Ureaplasma urelyticum</i> negative group n = 64	<i>p</i> -value
Age (mean \pm SD*)	35.03 \pm 6.01	35.37 \pm 6.69	0.76
BMI* (mean \pm SD)	24.49 \pm 3.76	25.01 \pm 3.44	0.42
Contraception method			
Condom	25 (42.4%)	24 (37.5%)	0.71
IUD*	11 (18.6%)	10 (15.6%)	0.81
Tubal ligation	0	1 (1.6%)	0.33
Coitus interruptus	14 (23.7%)	19 (29.7%)	0.54
None	9 (15.3%)	10 (15.6%)	0.95
Leukorrhea	57 (96.6%)	62 (96.9%)	0.93
Smoking	24 (40.7%)	17 (26.6%)	0.17

*SD: Standart deviation; *BMI: Body Mass Index; *IUD: Intrauterine device.

What are the genital ureaplasma findings in cytology?

Table II. Cervical Demographic characteristics for *Ureaplasma urelyticum* positive group and *Ureaplasma urelyticum* negative group.

	<i>Ureaplasma urelyticum</i> positive group n = 59	<i>Ureaplasma urelyticum</i> negative group n = 64	p-value
Macroscopic view of the cervix			
Normal	55 (93.2%)	53 (82.8%)	0.10
Erosion	3 (5.1%)	10 (15.6%)	0.07
Increased vascularity	1 (1.7%)	1 (1.6%)	0.95
Cervical smear result			
Negative	57 (96.6%)	61 (95.3%)	0.72
ASCUS*	2 (3.4%)	1 (1.6%)	0.60
LSIL*	0	2 (3.1%)	0.17
Presence of inflammatory signs	49 (83.1%)	43 (67.2%)	0.04
Scattered PMNL*	25 (42.4%)	17 (26.6%)	0.08
Aggregated PMNL (“Polyballs”)	35 (59.3%)	26 (40.6%)	0.04
Nuclear atypia of epithelial cells	20 (33.9%)	11 (17.2%)	0.03
Hormonal status of cervix			
Estrogen effect	30 (50.8%)	27 (42.2%)	0.36
Progesterone effect	29 (49.2%)	37 (57.8%)	0.36
Presence of Döderlein bacillus	55 (93.2%)	61 (95.3%)	0.70

*ASCUS: Atypical squamous cells of undetermined significance; *LSIL: Low-grade squamous intraepithelial lesion; *PMNL: polymorphonuclear leukocyte.

in complicated vulvovaginitis. Although pathologists using Bethesda and other classification systems for cervical cytological evaluation often add information on existing pathogens to their reports, these systems do not have an additional evaluation set related to this⁹. According to the data in our study, the results of the macroscopic view of the cervix and cervical smear results were similar in both groups and did not provide any additional data on women with Uu growth in culture. Therefore, we aimed at determining the cytological changes that may be caused by this common intracellular microorganism.

The diagnosis of cervicitis is made clinically and by microscopy. For microscopic diagnosis, >30 PMNL/hpf in gram staining or 10 (≥ 10 PMNL/epithelial cell) in cervical cytological evaluation in at least 10 hpf suggest cervicitis^{11,12}. In our study, we determined the presence of signs of inflammation as PMNL density ≥ 10 PMNL/epithelial cells in at least 10 hpf in cervical cytology.

The fact that the presence of inflammation signs in the study was statistically significant in the Uu-positive group, shows that Uu infection triggers the inflammatory process in the cervix, although it does not cause cervicitis².

Furthermore, the fact that aggregated PMNLs were observed more in the Uu-positive group in cervical cytology indicates that the aggregated PMNL view may be an important criterion in revealing the suspicion of Uu in cytological evaluation.

Accumulating evidence reports that the cervical inflammatory process that starts for any reason, especially in the long term, is associated with high-grade cervical intraepithelial lesions and malignancy¹³. According to the results of studies^{5,14} related to mycoplasma species and oncogenesis in the literature, Uu can cause cervical cytopathology and oncogenesis. In a survey conducted by Zhang et al⁶, the mechanism causing cervical cytopathology and malignant transformation has been reported to be the aberrant expression of oncogenes and tumor suppressors. Chronic infection of mycoplasmas leads to more irreversible expression of cancer-related genes¹⁵. Overall, Uu infections, whose treatment is delayed and which become chronic due to failure to diagnose, may cause malignant transformation in the cervix due to long-term cervical inflammation.

According to these results, the inflammatory process caused by long-term untreated Uu in the cervix may lead to high-grade cervical intraepithelial lesions and malignancy. Cell culture and PCR are diagnostic tests that are difficult to be accessed due to financial difficulties. For this reason, the diagnosis and treatment of the infection are delayed in many patients and become complicated. However, when the cervical smear test, which is commonly used during the routine gynecological examination, is re-evaluated for cytology aggregated PMNL, positive findings

may be found for the suspicion of Uu. As this will reduce the number of patients requiring PCR and culture, the use of tests will become more effective.

According to our findings, when the cervical cytology preparations were re-evaluated, there was no significant difference between the presence of normal flora bacteria (presence of Döderlein bacillus) and the hormonal status of the cervix in both groups. This indicates that these women have similar flora and hormonal characteristics for infections such as cervicitis and vulvovaginitis. On the other hand, the absence of cervicovaginal screening results for the Human Papilloma Virus, which causes cervical cytopathology, is one of the limitations of the study.

Conclusions

Ureaplasma urealyticum, which is a subspecies of mycoplasma strain, invades the urogenital mucosal epithelial cells and is a high cost and difficult to diagnose pathogen despite it is seen frequently.

Inflammation process in cervical mucosa caused by *Ureaplasma urealyticum* may give rise to high grade cervical intraepithelial lesions and malignancy in cervix. Therefore, diagnosis and treatment of *Ureaplasma urealyticum* has a great importance.

In these circumstances, our study shows that aggregated polymorphonuclear leukocytes (“polyballs”) seen on re-evaluation of the cervical cytology which is regularly used on routine gynecologic examination might be a specific finding for *Ureaplasma urealyticum* existence. In addition, randomized controlled trials which could evaluate the recent findings on cervical smear cytology for *Ureaplasma urealyticum* are necessary.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were per-

formed by Rukiye Ada Bender and Cemal Gundogdu. The first draft of the manuscript was written by Rukiye Ada Bender and Cemal Gundogdu, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available due to the hospital's patient data policy, but are available from the corresponding author on reasonable request.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Biruni University Ethics Committee (Decision date: 24.06.2022/Decision No.: 2022/71-38).

Informed Consent

Informed consent for the use of their medical records in scientific studies was obtained from all patients.

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