Evaluation of the prevalence of sexually transmitted bacterial pathogens in Northern Cyprus by nucleic acid amplification tests, and investigation of the relationship between these pathogens and cervicitis

Kuzey Kıbrıs'ta cinsel yolla bulaşan bakteriyel patojenlerin prevelansının nükleik asit amplifikasyon testleri ile incelenmesi ve bu patojenlerin servisitle olan ilişkilernin araştırılması

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Abstract

Objective: To evaluate the prevalence of pathogens, Chlamydia trachomatis, Neisseria gonorrhoea and Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, and Ureaplasma parvum in women via multiplex-polymerase chain reaction (PCR)-deoxyribonucleic acid (DNA).

Materials and Methods: Cervical swabs of 273 women in reproductive age who underwent gynecologic examination in our outpatient clinic were evaluated using the multiplex-PCR-DNA method. The presence of cervicitis, contraceptive methods, marital status, and the number of partners were evaluated.

Results: One hundred six (39%) of the 273 women had at least one bacterium, 25 women (9.8%) had two bacteria, and three women (1%) had three bacteria. U. urealyticum was the most frequently encountered bacterium (13.9%), followed by M. hominis (12.8%), U. parvum (12.4%), C. trachomatis (5.4%), M. genitalium (2.9%), N. gonorrhoea (2.5%), and T. vaginalis (0.3%). Bacterial infection was detected more frequently in women aged <25 years, single, who had multiple partners, and clinically diagnosed with cervicitis. The cervicitis rate was 39% in our study. M. genitalium was significantly more frequent in women with cervicitis than in women without cervicitis (5.6 vs. 1.2%, p=0.005). C. trachomatis and N. gonorrhoea, which are often associated with cervicitis, were comparable in women with and without cervicitis.

Conclusion: Women with clinically diagnosed cervicitis or even with a normal-appearing cervix should be tested using multiplex-real-time PCR-nucleic-acid-amplification tests on suspicion of such an infection. M. genitalium is an emerging bacterial agent for cervicitis along with C. trachomatis and N. gonorrhoea.

Keywords: Chlamydia, neisseria, trichomonas, mycoplasma, ureaplasma

Öz

Amaç: Kadınlarda multipleks-polimeraz zincir reaksiyonu (PCR)-deoksiribonükleik asit (DNA) ile Chlamydia trachomatis, Neisseria gonorrhoea ve Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum ve Ureaplasma parvum patojenlerinin görülme sıkalığı değerlendirilerek ve klinik olarak servis tanısı konulan kadınlarda bu bakterilerin rolünü incelenmesi.

Gereç ve Yöntemler: Polikliniklikle alınan servikçili swabs, multipleks-PCR-DNA yöntemiyle incelendi. Servis tanısı konulan kadınlarda bu bakterilerin rolünü incelendi. Servis varlığı, kontraseptif yöntemler, evlilik durumu ve partner sayısı değerlendirildi.

Bulgular: Iki yüz yetmiş üç kadınnın %39’u en az bir bakteri tespit edildi. En sıklık sırasına göre M. hominis (%12.8), U. parvum (%12.4), C. trachomatis (%5.4), M. genitalium (%2.9), N. gonorrhoea (%2.5), T. vaginalis (%0.3) oranlarında tespit edildi. Genitalium daha sık tespit edildi. Cervicitis tespit edilen kadınlarda M. genitalium’in prevalansı %5.4 ve cervicitis olmayan kadınlarda %1.2 olarak belirlendi (p=0.005). C. trachomatis ve N. gonorrhoea, cervicitis tanısı konulan kadınlarda comparable olarak tespit edildi.

Sonuç: Kadınlarda cervicitis tanısı konulmuş veya cervicitis tanısı olmayan normal-appear cervix’lerde tespit edilen patojenlere yönelik multiplex-polimeraz zincir reaksiyonu testleri kullanılarak cervicitis patojenlerinin prevalansı değerlendirilmeli ve klinik olarak servis tanısı konulan kadınlarda bu bakterilerin rolü araştırılmalıdır.

Keywords: Chlamydia, Neisseria, Trichomonas, Mycoplasma, Ureaplasma

Address for Correspondence/Yazışma Adresi: Onur Güralp, MD, Carl von Ossietzky Oldenburg University Faculty of Medicine, Obstetrics and Gynecology, Klinikum AöR, Oldenburg, Germany

Phone: +49 441 403 22 88 E-mail: dronur@hotmail.com ORCID ID: orcid.org/0000-0002-3517-3046

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Introduction

Every year, more than 1 million people are infected with sexually transmitted diseases (STDs)(1). *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* are the very well-known sexually transmissible pathogens, whereas *Mycoplasma genitalium* has recently gained importance in the pathogenesis of cervicitis(2). These bacteria are either asymptomatic or present themselves with mild symptoms, which may easily be overlooked(1). These bacterial STDs may lead to tubal infertility and extrauterine pregnancy as well as chronic pelvic pain, which is associated with a severe socioeconomic burden(3). Besides *Mycoplasma hominis* and *Ureaplasma urealyticum*, *Ureaplasma parvum* may be commensally colonized in the cervix. However, some authors suggest that such colonization may be associated with poor obstetric outcome, postpartum sepsis, and neonatal infections(3). The serologic diagnosis or traditional culture media may not be sufficient for diagnosis(2,4). In some cases, the presence of multiple agents makes it even more difficult to diagnose the actual agents(4). For that reason, due to their high sensitivity and specificity for the diagnosis of STDs, as well their ability to diagnose more than one pathogen at once, multiplex real-time polymerase chain reaction (PCR) nucleic acid amplification tests (NAAT) have gained popularity over conventional microbiologic culture methods(4-6). In this study, we aimed to evaluate the prevalence of pathogens including *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*, *M. hominis*, *M. genitalium*, *U. urealyticum* and *U. parvum* in women via multiplex PCR DNA tests, and to assess the role of these bacteria in women with clinically diagnosed cervicitis who were admitted to our outpatient clinic in Near East University for gynecologic examinations.

Material and Methods

In this study, the cervical swabs of 273 women in reproductive age who were admitted for gynecologic examinations with symptoms of vaginal discharge or who asked for a screening of sexually transmitted infections without any symptoms to the outpatient clinic of Near East University, Department of Obstetrics and Gynecology, between 2014 and 2016, were examined using the multiplex PCR DNA method. The results were retrospectively evaluated. The study was approved by the Ethics Committee of Near East University on March 31st, 2016 (number: 2016/36-266). Informed and signed consent was obtained from all participants. Inclusion criteria were apparently healthy, sexually active women aged >18 years without pelvic pain or fewer, who were not pregnant, and had not received antibiotic recently for a gynecologic infection. Women with pelvic inflammatory disease (PID) were excluded from the study. All women had a gynecologic examination, and the presence of cervicitis, contraceptive methods, marital status, and the number of partners were documented.

Cervicitis was described as the presence of purulent or mucopurulent discharge and/or hyperemic, edematous and friable (bleeding even with a light touch of a cervical swab) cervix(7,8). The cervical swabs from participants were taken by gynecologists using a single-use speculum with the manufacturer’s kits and were sent to the genetic laboratory.

Nucleic Acid Isolation Procedure

Nucleic acid isolation was performed in accordance with the manufacturer’s instructions (GeneAll RibospinTM vRD). Swab samples were obtained from the cervix and then transferred to the Medical Genetics Laboratory of Near East University Hospital. The following steps were performed: Centrifugation at 5000 rpm for 15 minutes, addition of buffer (VL, 500 µL), incubation for 10 min at 25 °C, addition of buffer (700 µL RB1), and vortexing. Preparation of the spin column. Removal of residual buffer by centrifugation of the mixture at 12,000 g. Addition of nuclease-free H2O. Re-centrifugation at over 10,000 g for 60 seconds. The purified nucleic acid was kept at 4 °C for direct analysis and kept at -70 °C for subsequent analysis.

Polymerase Chain Reaction

PCR was conducted for detecting the STD panel. The fast track diagnostic urethritis plus real-time PCR kit was used for analysis, which examines *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *M. hominis*, *M. genitalium*, *U. urealyticum*, and *U. parvum*. The DNA amplification reactions were performed using Qiagen Rotor-gene Q. After the DNA amplification, the results were interpreted according to the given fluorescence trace of the positive samples. The results were examined using the data supplied by the manufacturer.

Statistical Analysis

Continuous parametric variables are given as mean and standard deviation. Categorical variables are expressed as number or percentage. T-test or analysis of variance were used for the comparison of parametric variables. Categorical variables were compared using the chi-square ($\chi^2$) test. Statistical calculations were performed using Statistical Package for Social Sciences (SPSS 15.0, Chicago, IL, USA). P<0.05 was accepted as significant.
Results

A total of 273 women were included in this study. The demographic and clinical features of the patients are given in Table 1. The mean age of the women was 31.03±9.20 years. The study group consisted mainly of married women (70%), with a single partner (85%); 39.5% of the women had cervicitis. One hundred six (39%) of the 273 women had at least one bacterium, 25 women (9.8%) had two bacteria, and 3 women (1%) had three bacteria. Among the 273 women, U. urealyticum was the most frequently encountered bacterium in the cervix (13.9%), followed by M. hominis (12.8%), U. parvum (12.4%), C. trachomatis (5.4%), M. genitalium (2.9%), N. gonorrhea (2.5%), and T. vaginalis (0.3%). The infection rates according to the age, marital status, number of partners, the presence of cervicitis, and type of contraceptive method are presented in Table 2. Bacterial infection was detected more frequently in women aged <25 years, those who were single, who had multiple partners, and clinically diagnosed with cervicitis. Bacterial infection was detected less frequently in women who used a condom as a contraceptive method. M. hominis was the most commonly seen bacterium in women aged under 25 years. M. hominis and U. urealyticum were significantly more common in women with multiple partners. The cervicitis rate

Table 1. The demographic and clinical features of the patients

|                                | Number of women | percent (%) |
|--------------------------------|-----------------|-------------|
| **Age groups**                 |                 |             |
| <25 years                      | 83              | 30.40       |
| 26-30 years                    | 78              | 28.57       |
| 31-35 years                    | 48              | 17.58       |
| >36 years                      | 64              | 23.44       |
| **Marital status**             |                 |             |
| Single                         | 81              | 29.67       |
| Married                        | 192             | 70.33       |
| **Number of partners**         |                 |             |
| One                            | 233             | 85.35       |
| Multiple                       | 40              | 14.65       |
| **Deliveries**                 |                 |             |
| No                             | 122             | 44.69       |
| Yes                            | 151             | 55.31       |
| **Vaginal discharge**          |                 |             |
| Absent                         | 231             | 84.62       |
| Present                        | 42              | 15.38       |
| **Cervicitis**                 |                 |             |
| absent                         | 165             | 60.44       |
| present                        | 108             | 39.56       |
| **Contraceptive method**       |                 |             |
| Condom                         | 17              | 6.23        |
| Withdrawal (coitus interruptus)| 160             | 58.61       |
| Oral contraceptives            | 63              | 23.08       |
| Intrauterine device            | 33              | 12.09       |

Chi-square test, p<0.05 was accepted as statistically significant

Table 2. The infection rates according to the age, marital status, number of partners, vaginal mucopurulent discharge, presence of cervicitis and type of contraceptive method

|                                | No detectable bacteria |          | Bacteria positive |          | P      |
|--------------------------------|------------------------|----------|-------------------|----------|--------|
| **Age groups**                 | n (%)                  | n (%)    |                   |          |        |
| <25 years                      | 42 (50.60)             | 41 (49.40)| 0.09              |
| 26-30 years                    | 50 (64.10)             | 28 (35.90)|                  |
| 31-35 years                    | 30 (62.50)             | 18 (37.50)|                  |
| >36 years                      | 45 (70.31)             | 19 (29.69)|                  |
| **Marital status**             |                        |          |                   |          |        |
| Single                         | 36 (44.44)             | 45 (55.56)| <0.001**          |
| Married                        | 131 (68.23)            | 61 (31.77)|                  |
| **Number of partners**         |                        |          |                   |          |        |
| One                            | 155 (66.52)            | 78 (33.48)| <0.001**          |
| Multiple                       | 12 (30.00)             | 28 (70.00)|                  |
| **Parity**                     |                        |          |                   |          |        |
| No                             | 65 (53.28)             | 57 (46.72)| 0.02*             |
| Yes                            | 102 (67.55)            | 49 (32.45)|                  |
| **Complaint**                  |                        |          |                   |          |        |
| Asymptomatic                   | 147 (63.64)            | 84 (36.36)|                  |
| Vaginal mucopurulent discharge | 20 (47.62)             | 22 (52.38)| 0.05*             |
| **Cervicitis**                 |                        |          |                   |          |        |
| Absent                         | 110 (66.67)            | 55 (33.33)| 0.02*             |
| Present                        | 57 (52.78)             | 51 (47.22)|                  |
| **Contraceptive method**       |                        |          |                   |          |        |
| Condom                         | 16 (94.12)             | 1 (5.88) | 0.03*             |
| Withdrawal (coitus interruptus)| 93 (58.13)             | 67 (41.88)|                  |
| Oral contraceptives            | 36 (57.14)             | 27 (42.86)|                  |
| Intrauterine device            | 22 (66.67)             | 11 (33.33)|                  |

Chi-square test, p<0.05 was accepted as statistically significant
was 39% among the 273 women in our study. Among women with cervicitis, M. genitalium was significantly more frequent in women with cervicitis than in those without cervicitis (5.6% vs. 1.2% \( p<0.005 \)). C. trachomatis and N. gonorrhoea, which are often associated with cervicitis, were comparable in women with and without cervicitis. In 55 (33%) of 165 women with no clinical cervicitis, at least one bacterium was detected, and 15 (9%) women had at least one of the bacteria known to be associated with cervicitis, such as C. trachomatis, N. gonorrhoea, M. genitalium or T. vaginalis. By contrast, among 108 women with clinical cervicitis, the rate of the bacteria known to be associated with cervicitis was 14.9% (9% vs. 14.9%; \( p=0.133 \)). The rates of simultaneous infections with multiple bacteria were comparable between women with and without cervicitis (9.3% vs. 11.5%, \( p=0.565 \)).

**Discussion**

In our study, 39% of women had at least one bacterium. Among the 273 women, *U. urealyticum* was the most frequently encountered bacterium in the cervix (13.9%), followed by *M. hominis* (12.8%), *U. parvum* (12.4%), which showed a balanced distribution. The detection rate of bacteria was reported to vary between 30.7 and 49% in previous screened populations.\(^{(4,5)}\) Yamazaki et al.\(^{(10)}\) screened 799 Korean women and detected at least one bacterium in 49% of women. Contrary to our study, *U. parvum* was the most frequently (32.5%) found bacterium, followed by *U. urealyticum* (3.5%) and *M. hominis* (1%). Lee et al.\(^{(4)}\) screened 304 women and detected bacteria in 36.5%, most frequently *U. urealyticum* (14.5%), followed by *M. hominis* (13.8%). In south Italy, Del Prete et al.\(^{(8)}\) screened 1272 women and detected at least one bacterium in 30.7% of women. The most commonly detected bacterium was by far *U. parvum* (25.9%). In our study, we detected the bacterial colonization of *U. parvum* with a rate of 12.4%. Yamazaki et al.\(^{(10)}\) reported high detection rates of *U. parvum* as 41.7%. Yamazaki et al.\(^{(10)}\) suggested that the high prevalence of the latter two bacteria might be attributed to the region, culture, and tendency to nightlife. Camporiondo et al.\(^{(4)}\) performed a screening study in 309 Italian women and detected no *C. trachomatis, M. genitalium* or *N. gonorrhoea*, but *U. parvum* (28.8%), *M. hominis* (3.9%) and *U. urealyticum* (4.5%). McIver et al.\(^{(11)}\) evaluated 175 sexually active Australian women and detected *U. parvum* (53%), *M. hominis* (7.4%), and *U. urealyticum* (3.4%) in descending order. Simultaneous infection rates with *U. parvum + M. hominis, U. urealyticum + M. hominis* and *U. urealyticum + U. parvum* were 7.4%, 1.1%, and 2.9%, respectively. In our study *U. urealyticum, M. hominis*, and *U. parvum* were among the most commonly detected bacteria in the cervix. They are accepted as genital commensalistic organisms and found in healthy women.\(^{(10)}\) Routine screening and treatment of the latter three bacteria are controversial.\(^{(10)}\) Some authors suggest that colonization with *U. urealyticum* and *U. parvum* in high density is associated with non-specific cervicitis,\(^{(12)}\) whereas others suggest that there is not enough evidence to suggest that these bacteria cause cervicitis or PID.\(^{(13)}\) However, several studies in pregnant women showed that the presence of these bacteria in amniotic-fluid or membranes might be associated with preterm labor, preterm premature rupture of membranes (PPROM), and neonatal infections.\(^{(14-24)}\) Abele-Horn et al.\(^{(21)}\) showed that *U. urealyticum* was associated with preterm labor. Kataoka et al.\(^{(22)}\) evaluated 877 pregnant women under 11 gestational weeks (GW) and detected the prevalence rates of *U. urealyticum, M. hominis*, and *U. parvum* as 52.0%, 11.2%, and 8.7%, respectively. Despite the higher prevalence of *U. urealyticum* in the latter study, *U. parvum* had a stronger association with late abortion and preterm labor compared with *U. urealyticum*.\(^{(22)}\) One hundred eighty-four pregnancies complicated with preterm labor and PPROM were evaluated in a prospective study and coinfection with *M. hominis, and U. urealyticum* was shown to be associated with poorer pregnancy outcomes compared with infection with *U. urealyticum* alone.\(^{(24)}\) In another study, vaginal *U. urealyticum* and *U. parvum* colonization were also shown to be associated with choioamnionitis in pregnancies under 28 GW complicated with PPROM.\(^{(24)}\) Rumyantseva et al.\(^{(25)}\) recently evaluated 1773 women and observed that the isolation rates of *U. parvum* and *M. hominis* in women with bacterial vaginosis were significantly higher in women with altered vaginal microflora compared with women with normal vaginal flora. Chlamydia is known to be the most common STD,\(^{(26,27)}\) and it is one of the major organisms causing cervicitis and PID, even if it is asymptomatic. In our study, the prevalence of *C. trachomatis* was 5.4%, which was more frequent than other sexually transmissible bacteria such as *M. genitalium* (2.9%), *N. gonorrhoea* (2.5%) and *T. vaginalis* (0.3%). Chlamydia prevalence, along with the prevalence of other STDs, may vary according to the age, race, region, and socioeconomic status.\(^{(26,28)}\) The prevalence of chlamydia was reported as 0.6% in Australia,\(^{(11)}\) 2.6% in the Netherlands,\(^{(29)}\) 2.3% in China,\(^{(30)}\) and as high as 14.2% in South Africa.\(^{(31)}\) The prevalence of chlamydia in the United States of America (USA) was reported to be 4.2% in the general population, but as high as 10% in Mexicans living in the USA.\(^{(26)}\) In a systematic review and meta-analysis, the prevalence of chlamydia in Europe and developed countries such as Canada, Australia, and New Zealand was reported as 3.0-5.3%\(^{(28)}\), which is also concordant with the values in our study. In the present study, the prevalence of gonorrhea was 2.5%. Gonorrhea is the second most common sexually transmitted bacterial infection following chlamydia.\(^{(29)}\) According to the World Health Organization report in 2012, the global gonorrhea prevalence varies between 0.3% and 1.7%.\(^{(11)}\) In our study, the prevalence of gonorrhea was significantly over the global average. Nevertheless, the prevalence of STD may vary according to the country or even to the region in the same country. Kim et al.\(^{(5)}\) evaluated 799 Korean women and detected no gonorrhea, whereas Lee et al.\(^{(9)}\) evaluated 304 Korean women and detected gonorrhea in 3.3% of the screened population, which was even higher than...
in our study group. Gaydos et al. (32) evaluated a group of 324 women comprising mainly young African-American women in Baltimore, USA, and detected the rate of gonorrhea as 4.6%. The high rates of gonorrhea in our study may be attributed to the presence of nightclubs in our region. In our study, the rate of M. genitalium infection was 2.9%. The global M. genitalium infection rate is reported as 1.6-2.2% (33). Nevertheless, prevalence rates as high as 19.2% have also been reported (32). Nowadays, since the widespread use of NAATs in recent years, M. genitalium counts as one of the most important bacteria, following N. gonorrhoeae and C. trachomatis, causing cervicitis (34). The prevalence of T. vaginalis was 0.3% in our study. T. vaginalis is the most common non-viral sexually transmissible infection in the USA and may cause urethritis in men and women, and vaginitis and cervicitis in women (35). It is hard to determine the true prevalence of T. vaginalis because the feedback is not so efficient as with other STD pathogens. According to the Centers for Disease Control and Prevention, the prevalence of T. vaginalis in non-Hispanic women in the USA is 1.8% (2). Moreover, in hitherto literature, prevalences of T. vaginalis as low as 0.1% have been reported (3).

Cervicitis

In our study, the cervicitis rate was 39% among 273 women, which is similar to the 41% among 324 women reported by Gaydos et al. (32). Gaydos et al. (32) detected that C. trachomatis and M. genitalium were associated with cervicitis; however, only M. genitalium had a significant association with cervicitis in multiple regression analysis. In our study, the M. genitalium infection rate was significantly elevated in women with clinical cervicitis compared with women without cervicitis, which supports recent data about the importance of M. genitalium as an emerging pathogen of cervicitis. The clinical diagnosis of cervicitis is not always suggestive for a sign of bacterial infection. It has been shown that no infectious pathogen is detectable in cervicitis is not always suggestive for a sign of bacterial infection. There was no significant difference regarding simultaneous infection with multiple bacteria between women with and without cervicitis.

Table 3. Studies about the detection rates of infectious agents causing vaginitis and cervicitis

| Agent          | Present Study % | Lee et al. (4) | Kim et al. (5) | McIver et al. (11) | Camporiondo et al. (6) | Del Prete et al. (9) | Choe et al. (39) | Gaydose et al. (32) |
|----------------|-----------------|----------------|---------------|--------------------|------------------------|----------------------|------------------|-------------------|
| U. urealyticum | 13.9            | 14.1           | 7.6           | 6.1                | 4.5                    | 5.1                  | 40.3             | -                 |
| M. hominis     | 12.8            | 13.8           | 9.9           | 13.7               | 3.9                    | 5.9                  | 14.9             | -                 |
| U. parvum      | 12.4            | -              | 42            | 57                 | 28.8                   | 24.9                 | 52.7             | -                 |
| C. trachomatis | 5.4             | 3.0            | 1.1           | 0.6                | -5.9                   | 1.8                  | 4.0              | 11.1              |
| M. genitalium  | 2.9             | 0.3            | 1.0           | 1.3                | -0.3                   | 0.3                  | 4.0              | 19.2              |
| N. gonorrhoeae | 2.5             | 3.3            | 0.0           | 0.0                | -0.1                   | 0.1                  | 1.5              | 4.6               |
| T. vaginalis   | 0.3             | 2.0            | 0.1           | 4.0                | 1.3                    | 1.4                  | 1.0              | 15.3              |
Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Near East University in 31.03.2016 with the number of 2016/36-266.

Informed Consent: Informed and signed consent was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: O.G., Data Collection or Processing: O.G., B.K., Analysis or Interpretation: O.G., A.B., E.O.B., Literature Search: O.G., M.S.S., Writing: O.G.

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