

Prevalence of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in pregnant women of Tehran by duplex PCR.

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Abstract

Background: Sexually transmitted diseases (STIs) are an important health problem. *Ureaplasma* and *Mycoplasma* are important agents producing non-gonococcal urethritis and other complications, such as pelvic inflammatory diseases and infertility. The aim of this study was to determine the prevalence of *Ureaplasma urealyticum* (*U. urealyticum*) and *Mycoplasma genitalium* (*M. genitalium*) and assessment of the infection related risk factors in pregnant women.

Materials and methods: In an 8 month cross-sectional study in 2017, urine specimens were collected from 194 pregnant women referring to Rasoul-e-Akram Hospital, Tehran, Iran for diagnosis of infection. The frequency of *U. urealyticum* and *M. genitalium* organisms in the samples and the relationship between the prevalence and risk factors of pregnant women was investigated.

Results: The prevalence of *Mycoplasma* and *Ureaplasma* was 5.6% and 11.2%, respectively. More than half of the women (88.8%) did not report any signs of STIs. As the age rises, the frequency of infections decreased. There was a significant correlation between preterm labor and infection and also between the history of genital herpes simplex infection and *U. urealyticum* infection. A positive history of abortion was found to be statistically significant with both infections.

Conclusion: In general, the prevalence of *U. urealyticum* and *M. genitalium* is similar to results of some other studies in Iran and elsewhere in the world. Further studies are recommended be conducted in target groups of pregnant women in order to obtain more accurate estimates of the prevalence of these infections as well as the causes of infections.

Keywords: *Mycoplasma genitalium*, *Ureaplasma urealyticum*, Pregnant women, PCR.

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Introduction

According to the World Health Organization (WHO), more than 448 million new cases of sexually transmitted diseases worldwide are occurring annually. According to the world's leading sources of information, worldwide sexually transmitted diseases are on the rise, and because of the contagious nature of these diseases and the complications caused by the lack of treatment and prevention, control of parasitic diseases is necessary [1-3]. *U. urealyticum* and *M. genitalium* along with other

microbial agents cause non-gonococcal urethritis and other complications of genitourinary system. *U. urealyticum*, which is part of the natural flora of the men's urethra, causes harmful consequences of pregnancy, such as infertility, spontaneous abortion, premature rupture of membranes and premature births [2,4-9].

M. genitalium is transmitted through sexual contact and is found in the genitourinary system to 80% of all women. In addition to pelvic inflammatory disease, caused infertility, postpartum septicemia, abortion, preterm labor, neonatal

weight and systemic neonatal infections [2-16]. Due to the importance of the mentioned factors in the incidence of genitourinary infection, early identification of these infections and the timely delivery of therapeutic measures, especially in high-risk individuals, are important [17]. Since host defenses and the presence of risk factors and predisposing infections play an important role in the disease and in the frequency of pathogens in the body, it is important to determine the risk factors for infection in different communities [3].

The prevalence of these two organisms among pregnant women in different countries and also in different studies in our country has been somewhat different. In a study by Hasanabad et al. [10] among 196 pregnant women, they detected a total of 15.81% of all cases had infection. The prevalence of *Chlamydia trachomatis* was 13.77%, *M. genitalium* 1.02% and both bacteria (simultaneous) 1.02% (16). In the same group of pregnant women in 2013, the prevalence of *U. urealyticum* was 21.1% and *M. genitalium* was 2.04% [10]. According to Aziz Mohammadi et al. [6] study, between 350 vaginal samples obtained from pregnant women, prevalence of *U. urealyticum* was 9.14%, *M. hominis* was 2.85%, and the simultaneous infection was 1.14% with highest frequency of infection in the age group of 45-40 years old. In the Akya et al. [2] study in Kermanshah (west of Iran) in 2011, the prevalence of *M. hominis*, *M. genitalium* and *U. urealyticum* in 235 women with cervicitis was evaluated. The PCR results showed that 82% of the cases had at least one of the above infections. The prevalence of *M. hominis* was 5.1%, *M. genitalium* 6% and *U. urealyticum* 26% and 94% had clinical symptoms. The prevalence of these infections was not significantly different from the other side of the world. On the other hand, this was directly related to increased sexual activity. In Seifoleslami et al. [9] study between 350 vaginal swab specimens obtained from fertile and infertile women, the prevalence of *M. hominis* was 3.14%, *U. urealyticum* 4.28% and both 1.42% and the frequency of both infections in infertile women were higher than that of fertile women [9].

In a study by Karamsatji [18] in Tehran, 125 women with abortion and 250 patients in terms of age, education and occupation were compared with the case group. The bacteria were isolated with more percentage of people with abortion, with *M. hominis* in 14.4% and *U. urealyticum* isolated in 31.2% of patients with abortion. While those in the control group were 7.2% and 19.2%, respectively [18]. The meta-analysis of 29 studies carried out by Ahmadi et al. [19] from different regions of Iran. The average prevalence of genitourinary Mycoplasma was 11.1% in men and 12.8% in women and significantly higher in infertile men compared to fertile individuals [19]. In the Bayraktar et al. [7] study, frequency and antimicrobial susceptibility of *M. hominis* and *U. urealyticum* were evaluated in 100 pregnant women (50 symptomatic). *M. hominis* was detected in 5% and Ureaplasma was found

in 27% of cases. Twelve registered women had abortion history, of which 66.7% had infections with one or both bacteria. Also, 40.7% of women with infections had a history of low birth weight. This study showed the relationship between abortion and birth weight with a history of these infections [7]. In Campos investigation in Brazil frequency of *M. genitalium* and *M. hominis* were evaluated in peripheral blood and vaginal swab specimens of 302 healthy women with results of 31.8% and 28.1% respectively. Increased levels of IL-1 β were associated with Mycoplasma infection. Sexual behavior was also associated with increased Mycoplasma and other genital tract infections [3].

The golden standard for the detection of these microorganisms is culture under specific laboratory conditions. Nowadays, due to the slow growth and incontinence of non-culture, molecular diagnostic methods such as DNA Hybridization and Nucleic Acid Amplification Test (NAAT) as low sensitive and high sensitivity methods has been raised [9,20,21]. Molecular methods such as polymerase chain reaction (PCR) and lipase chain reaction (LCR) provide the possibility that, with a high sensitivity and specificity, these factors can be found in less invasive samples such as urine samples or through genitourinary swabs [8,22]. Multiplex PCR methodology, due to its high sensitivity and specificity, the ability to simultaneously detect more than one organism, save time and cost, and reduce the probable contamination of samples, has particular importance among various diagnostic methods [23,24]. The aim of this study was to determine the prevalence of infections caused by two *U. urealyticum* and *M. genitalium* infections in a group of pregnant women using Duplex PCR method. However, the risk factors of infection with these organisms were also evaluated and finally, the relationship between risk factors with the frequency of organisms was determined.

Materials and Methods

This cross sectional study was conducted in Rasoul-E-Akram hospital in Tehran during 2015.

Study Population

The sample size was calculated based on the prevalence of *U. urealyticum* and *M. genitalium* in pregnant women reported in previous studies. Using the appropriate formula, 196 samples were obtained. After evaluation, 210 pregnant women with no underlying conditions were referred to the women's ward of the hospital. The research samples were enrolled into the study after obtaining informed consent. From all of the above samples, 10 cc of urine were collected over a period of 8 months. Simultaneous demographic data were recorded by two questioners in the questionnaire and their clinical findings were recorded. Samples were tested at the Laboratory of Infectious Diseases Research Center of Iran University of Medical Sciences.

PCR Assays

After precipitation of urine specimens, DNA extraction was performed and in the next stage samples were tested by Master Mix of Sinaghan Company of Iran by Duplex PCR. The primer sequence was used based on previous studies (24). 3 µl of DNA of two bacteria *U. urealyticum* and *M. genitalium* were added as positive control and distilled water as negative control.

Using the specific primers for each bacterium (Table 1) the infection was investigated. Initial denaturation at 95°C for 5 min, 35 cycles including: separation of the two strands at 95°C for 40 s connection to the single-stranded DNA primers at 52°C for 40 latest new fields in 72°C for 40 s and in the final elongation for 8 minutes at 72°C was performed. Then samples are loaded into agarose gel, the band of interest with dock Gel were studied (Figure 1).

Data Analysis

The results of the instances of each with respect to the information contained in the questionnaire, was analyzed using SPSS version 16.

The frequency of these organisms was determined by descriptive statistics and its association with the underlying profile and risk factors of infection in pregnant women by analytical statistics including chi-square test with significant level of p<0.05.

Results

In terms of age, 3.1% of participants were under age 20, 52.6% between the ages of 20 and 29, 41.3% between 30-39 years old and 3.1% older than 40 years of age. In

terms of education, 83.3% had undergraduate education, 5.6% primary education, 24.5% secondary education and 53.6% secondary school. College education was also 16.3%. 11.2% of women with genital history had 24.5% history of abortion and 9.2% had preterm labor history. In this study, 40.3% of the subjects each year regularly visited the relevant centers for the screening of sexually transmitted diseases, of which 17.3% were referred to the doctor only when there was a sign or indication of sexually transmitted disease. 36.2% of the participants had clinical symptoms of infection. Vaginal secretion was the most common symptom with 14.8%, followed by pelvic pain (8.7%), urinary frequency (7.7%) and blood in urine (3.1%) and burning in the urine with 2% of other clinical symptoms. Blood transfusion was higher in women with co-infection of both organisms. In this study, 22 (11.2%) patients had *Ureaplasma urealyticum* infection, 11 (5.6%) had *Mycoplasma genitalium* infections and 5 (2.6%) had infection with both organisms. To determine the relationship between MG and UU with the variables studied, Chi-square test was used with a confidence level of 0.05. The highest incidence of infection was 52.6% for Mycoplasma and 54% for Ureaplasma in the age group of 20-29 years old and the frequency of these factors decreased with age. There was a significant correlation between the history of genital herpes and *Uu* infection. Six out of 22 people with Ureaplasma infection had a history of sexually transmitted diseases. A positive history of abortion was associated with *Mycoplasma genitalium* infection. Also, a positive history of abortion with *Ureaplasma urealyticum* infection was also statistically significant (Table 2). The results of statistical analysis showed that the presence of

Organism	Primer	Nucleotide sequence (5'→3')	Length of the piece
<i>Ureaplasma urealyticum</i>	F	5'-GCAATCTGCTCGTGAAGTATTAC-3	167 bp
	R	5'-GAAACGACGTCATAAGCAACT-3	
<i>Mycoplasma genitalium</i>	F	5'-AGTTGATGAAACCTTAACCCCTTG-3	320 bp
	R	5'-CATTACCAGTTAAACCAAAGCCT-3	

Table 1. Primer sequences used in this study

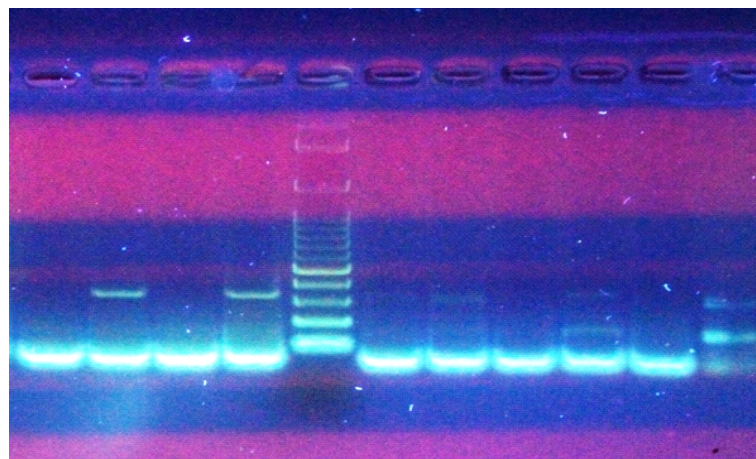


Figure 1. Agarose gel bands of samples

Presence of Infection	<i>U r e a p l a s m a urealyticum</i>		P Value	<i>M y c o p l a s m a genitalium</i>		P Value	<i>Mycoplasma genitalium and Ureaplasma urealyticum (concurrent)</i>		P Value
	Yes	No		Yes	No		Yes	No	
Abortion History									
Yes	10	38	<0.05	7	41	<0.05	4	48	<0.05
No	12	136		4	144		1	147	

Table 2. Relationship between the history of abortion and infection with both studied organisms in the Chi square test

any of these two organisms was bilateral and significant (P <0.05) with co-infection with other organisms.

Discussion

The present study was conducted to investigate the frequency of *U. urealyticum* and *M. genitalium* and correlation of them with risk factors in pregnant women. In the present study, 36.2% of the participants had clinical symptoms of infection. According to Bayraktar et al. [7] study in Turkey in 2006 to 2007, half of the pregnant women participating had clinical symptoms that the difference in the prevalence of symptoms could be attributed to adverse health conditions due to the social and cultural situation of the participants, which in that section has existed. In this study, the prevalence of *U. urealyticum* was 11.2%, *M. genitalium* 5.6% and infections with both organisms 2.6%.

Similar studies have been conducted on the prevalence of these infections in different regions of the world. Ahmadi et al. [19], in the systematic review of several years of internal articles; A high level of heterogeneity was observed for both men ($I^2=92.4\%$; $P<0.001$) and women ($I^2=93.3\%$; $P<0.001$) found that the prevalence of urogenital *Mycoplasma* in the male population was 11.1% and in female was 12.8%. Prevalence of these bacteria was significantly higher in infertile men compared with that in fertile men. The findings are similar to the results of Aziz Mohammadi et al. [6] study in 2015. In this study, the prevalence of *U. urealyticum* was 9.14%, which is consistent with the result of the present study.

One of the studies that have been carried out on the abundance of these organisms in our country was Hasanabad et al. [16] research. He determined the prevalence of *M. genitalium* in pregnant women in 2010, about 1.02% [16]. In Hasanabad et al. [10] re-study in 2013, the prevalence of *U. urealyticum* was 21.1% and *M. genitalium* frequency was 2.04%. These results are different from the results and indicate a decrease in the prevalence of *U. urealyticum* and a clear increase in *M. genitalium* infection in pregnant women in this study. Although Haghghi studies and colleagues were referred to the pregnant women referred to hospital, due to socio-cultural differences and the geographical dispersion of infectious diseases, the results of these studies cannot be compared with the findings. Some studies that measure the prevalence of these infections have a different target

population than pregnant women who have been in the hospital, and the findings from these studies cannot be generalized to other target groups such as pregnant women. In the Akyu et al. [2] study in Kermanshah in 2011, the prevalence of *U. urealyticum* in non-pregnant women with 26% cervicitis was reported and that was more than double the prevalence of the study. This increase was due to the sampling of a referral risky population that have been in the treatment center and all of which have clinical signs of cervicitis and 82% of cases have at least one of the studied infections. However, in this study, the frequency of *M. genitalium* was 6%, which is similar to the findings of our study. Other researches that have a different target group with the population of pregnant women referring to the hospital include the study of Seifoleslami et al. [9] and the research of Karamsatji [18] in Tehran.

According to Seifoleslami et al. [9] study in 2013, the frequency of *Ureaplasma* was 4.28%, which is significantly lower than the present study. This result is due to differences in the sampling of the two studies, so that in the study of Seifoleslami et al. [9], infertile women referred to the infertility center and also, volunteers without a history of infertility and illness were enrolled in the study, which have different characteristics and individual conditions than those reported by women referring to the hospital. Therefore, due to the difference in the risk factors of infection in the two groups, the results of the infection are also different. Karamsatji [18] study was performed in two groups including abortion women and women were matched with the first group. For these reasons, *U. urealyticum* was 31.2% in patients with abortion, which is much higher than the present result. Of course, the study of *U. urealyticum* in women with no history of abortion was 19.2%, which raises other reasons.

The prevalence of *U. urealyticum* in pregnant women in the present study is significantly lower than the frequency estimated by the Bayraktar et al. [7] study in 2006 to 2007. It means that the frequency of *U. urealyticum* was 27% in this study. Reducing the frequency of infection in this study can be seen in the presence of social conditions prone to genital tract infection, especially sexual relations and the lack of attention to personal hygiene and the improvement of the environment, which existed in the target population of the study in Turkey in 2006 to 2007. The prevalence of *M. genitalium* in the present study

shows a significant decrease in the prevalence of *Campylobacter* in healthy women in Brazil, which contradicts existing assumptions about the prevalence of this bacterium in a population with a history of infection and abortion [3]. In addition to determining the prevalence of two *U. urealyticum* and *M. genitalium* infections, the relationship between the frequency of these infections and the risk factors associated with infection was also studied. Among the findings of this study, there is a correlation between the infections of *U. urealyticum* with a history of genital disease in pregnant women participating in the study. This case has been confirmed in some other studies [12,13]. In the Akya et al. [2] study in Kermanshah, high incidence of genital infections, including *U. urealyticum*, was seen in women with cervicitis.

The results of Seifoleslami et al.'s [9] study also showed that the prevalence of *U. urealyticum* in infertile women is higher. Increasing the prevalence of this bacterium in infertile men was also confirmed in the systematic review conducted by Ahmadi et al. [19]. On the other hand, the association between *U. urealyticum* and the history of genital herpes has been rejected in some studies [14,15].

In the present study, any infection of *M. genitalium* or *U. urealyticum* is associated with a history of abortion. The role of genital *Mycoplasma* and *Ureaplasma* in the emergence of spontaneous abortion can be seen in our country and most of the previous studies in different countries. In the study of Karamsatji [18] in Tehran, *M. hominis* and *U. urealyticum* were significantly differentiated in patients with abortion. Bayraktar et al. [7] study also revealed the association of abortion with these infections [7]. In Ren et al. [4] work and in 33.3% of aborted fetal tissue samples, the presence of *U. urealyticum* was confirmed by PCR method. Also, in Cheng et al. [5] *U. urealyticum* positive culture was reported in 50% of aborted embryo tissue samples and cervical samples of women with spontaneous abortion.

Conclusion

Overall, the prevalence of *U. urealyticum* and *M. genitalium* is similar to results of other studies in our country and elsewhere in the world. Because of the high importance of the health status of pregnant women as one of the most important high-risk groups in the community and also because of the importance of the complications of these two bacteria, it is recommended that similar studies be conducted in target groups of pregnant women in our country, due to get more accurate estimate of the prevalence of these infections, as well as the causes of infections. Although various studies have been conducted on the association of *Ureaplasma* with abortion, but due to the significant percentage of *Ureaplasma* contamination in adult women, commenting on this relationship cannot be conclusive. It is usually recommended screening of pregnant women (especially those with a high risk pregnancy and those with a history of abortion) for these

two infections. Therefore, with the appropriate treatment, the likelihood of the occurrence of repeated abortions decreases.

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