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ORIGINAL ARTICLE

Detection of genital mycoplasmal infections among infertile females by multiplex PCR

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ABSTRACT

Background and Objectives: Women reproductive system is a suitable environment for growth of various pathogen and nonpathogenic microorganisms. *Mycoplasmataceae* is a family of bacteria which cause oligosymptomatic genital infections. The complications caused by these bacteria may lead to infertility in women. The aim of this study was detection of genital *Mycoplasma hominis, Ureaplasmaurealyticum* and *Mycoplasma genitalium* among infertile females who referred to the infertility clinics.

Materials and Methods: A total of 104 infertile women (in reproductive age) who referred to infertility clinics in the city of Sanandaj, Kurdistan, Iran, from February to May 2013 were selected for this study. Cervical swabs were collected from all patients. The presence of genital *Mycoplasmas* was detected by multiplex-PCR. All data were analyzed statistically.

Results: Out of 104 patients**,** 39 cases (37.5%) were infected with *U. urealyticum. Mycuplasma. genitalium* and *M. homimis* were detected in 3 (2.9%) of the infertile women. Co-infection was seen in 3.8% of the patients. There was no statistically significant difference between the infections and patient age, educational levels, literacy, situation of employment, age of first sexual intercourse, history of abortion, type of infertility and infertility duration (p value > 0.05).

Conclusions: The data showed a low percentage of infection for *M. genitalium* and *M. hominis* in the studied women while the prevalence of *U. urealyticum* was high. Despite having no symptoms of an ongoing acute inflammation of the reproductive tract, many women may have genital mycoplasmas in the cervix. We concluded that multiplex PCR using a pair of primers is a useful and cost-effective method for diagnosis of female genital infections.

Keywords: PCR, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasmaurealyticum*

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INTRODUCTION

Infertility can be defined as the lack of conception after at least one year of constant, sexual intercourse without using a contraceptive device (1,2). More than 70 million couples in the world suffer from infertility. The majority of them are residents of developing countries (3). In female, the main causes of infertility include endocrine disorders, ovulatory dysfunction,

tubal and peritoneal diseases. Uterine infections are relatively uncommon. Furthermore a great number of infertility cases still remain unexplained (4). Women reproductive system is a suitable environment for growth of various pathogen and nonpathogen microorganisms. *Mycoplasmataceae* is a family of bacteria which cause oligosymptomatic genital infections and the complications caused by these bacteria may lead to infertility in women (1).

Mycoplasma genitalium is associated with urethritis, cervicitis and endometritis, salpingitis and pelvic inflammatory disease (PID), and may be considered as a cause of infertility in women (5). *Mycoplasma hominis* also as a common commensal of the female genital tract has been associated with pyelonephritis, bacterial vaginosis, cervicitis, endometritis, PID and postpartum septicemia (6). *Ureaplasma urealyticum* is considered as the main cause of nonchlamydial, nongonococcal urethritis. Furthermore this bacterium can cause chorioamnionitis, preterm delivery, abortion, preterm birth, bacterial vaginosis and cervicitis (7). The role of these microorganisms in the etiology of infertility has been very controversial (2, 8). Studies indicate that inappropriate diagnosis, prevention and treatment of mycoplasma infections can led to chronic disease such as PID and infertility (9). The aim of the present study was to determine the prevalence of *M. hominis*, *M. genitalium* and *U. urealyticum* in infertile women by multiplex PCR.

MATERIALS AND METHODS

Patients. In this cross sectional study, 104 married infertile women aged 14 to 40 years who referred to an infertility clinic (Besat Hospital, Kurdistan University of Medical Sciences, Sanandaj, Iran) from February to May 2013 were included. The patients included 62 women with primary infertility and 42 women with secondary infertility. Primary infertility refers to women who have not become pregnant after at least 1 year of having sex without using control methods. Secondary infertility refers to women's who have been able to get pregnant at least once, but now are unable (10). The patient had no symptoms related to the genitourinary tract infections. Excluding criteria included the male factor infertility, reproductive organ infection, anatomical abnormalities, benign ovarian tumors and uterine myeloma.

All the subjects also have not been taking antibiotics from two weeks before sampling and written consent was obtained from all participants. Cervical swabs were collected from all the patients using a sterile Dacron swab (Eurotubo, Deltalab). Samples were transported to the laboratory in 5 ml Phosphate Buffered Saline (PBS).

DNA extraction and PCR amplification. DNA extraction of the specimens was carried with High pure PCR template Preparation Kit (Roche Co, Germany Cat.No.11796828001) in accordance with the instructions of the suppliers. Multiplex-PCR was used for detection of genital Mycoplasmas. The utilized primers which were capable of detecting *M. hominis, M. genitalium* and *U. urealyticum* simultaneously were as follows: 16 S rRNA forward primer sequence: MyUu F5-TGGAGTTAAGTCGTAACAAG-3, and reverse primer sequence: MyUu R5-CTGAGATGTTTCACTTCACC-3 (7). PCR reaction was performed using the PCR master mix (Ampliqon Co, Skovlunde, Denmark, Cat. No. 180301) in 25µl final volume for each sample including: 12.5 µl master mix 2X (1.5 mM magnesium chloride,15mM deoxy nucleotide triphosphate,1.25 U Taq DNA polymerase), 5 µl DNA template, 1µl of each primer pair in a total volume of 25µl. The PCR reaction was performed using a GenAmp PCR system (Corbiet, Sydney, Australia) according to the following program: pre denaturation for 5 minutes at 95ºC followed by 30 cycles each containing denaturation at 94ºC for 30 seconds, annealing at 56ºC for 30 seconds and extension at 72ºC for 60 seconds, followed by final extension at 72ºC for 5 minutes. The *M. genitalium* (ATCC: 33530), *M. hominis*(ATCC: 15056) and *U. urealyticum* (ATCC: 29557) extracted DNA, as well as sterile double-distilled water were used as positive and negative controls for PCR reaction, respectively. PCR products were electrophoresed in a 1.5% agarose gel, followed by staining by SYBER green (CinaGen Co, Tehran, Iran) and visualizing under an UV transilluminator.

Statistical analysis. Statistical analysis was conducted to determine how many samples were positive for each bacterium, as well as for those positive for 2 or 3 bacterial isolates. In order to observe relation between these variables and the presence of an infection in our patients, we used the chi-square test. A p value <0.05 was considered statistically significant. The tests were performed using SPSS for windows

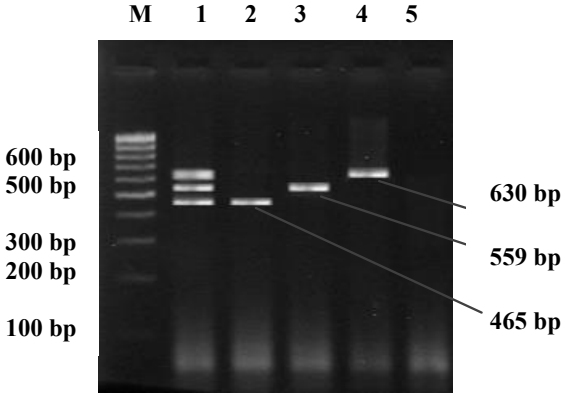

Figure1- Agarose gel electrophoresis of PCR amplified products.

Fig. 1. Lane 1 shows positive control for *M. genitalium*(465bp), *U. urealyticum*(559bp) and*M. hominis* (630bp). Lane 2 shows 465 bp *M. genitalium*. Lane 3 shows 559 bp *U. urealyticum.* Lane 4 shows 630 bp *M. hominis* amplification product. Lanes 5 shows the negative control showing no Mycoplasma infection and Lane M is the DNA size marker (100 bp DNA ladder, SM#333).

version 16.0 (SPSS Inc, Chicago, IL).

RESULTS

The length of PCR products were 630, 559 and 465 bp for16 SrRNA gene of *M. hominis* and *U. urealyticum* and *M .genitalium*, respectively (Fig. 1). The studied group age was 14-40 (The mean age was 29.2 years old). In 40 patients (38.4%), there were at least one infection. Thirty nine cases (37.5%) were co-infected with *U. urealyticum*. *M. genitalium* and *M. hominis* were detected from 3(2.9%) of the patients (Table 1). *U. urealyticum* was detected in a decisive majority of patients.

The patients characteristics have been summarized in Table 2. In terms of socio-economic indicators (age, literacy, situation of employment, age of the first sexual intercourse), their history of abortion, type of infertility(primary and secondary), duration of infertility and Mycoplasma infections. No significant difference was observed between infected and uninfected individuals (P value >0.05).

DISCUSSION

Mycoplasma and Ureaplasma are agents of sexual transmitted diseases. They are considered to be a threat to community health (11). Most of these infections are not diagnosable due to lack of symptoms, the antibacterial effect of sperm, the high possibility of contamination with other urethral organisms and the difficulty of culturing (11, 12). Numerous researchers have attempted to study the association between genital Mycoplasmal infections and infertility. Several epidemiological reports have documented the presence of *M. hominis*, *M. genitalium* and *U. urealyticum* in infertile women (13, 14). Also in vitro studies have shown that sperm samples infected with these Mycoplasmas undergo detrimental changes in sperm count, sperm velocity and motility parameters (15). The pregnancy success rate of in vitro fertilization (IVF) might be reduced as a result of prior mycoplasma colonization of the female and male genital tract (11).

The prevalence of *U. urealyticum* and *M. hominis* was shown to be in an equal range as reported by Miron *et al*. (Romania- 2013), Günyeli (2011- Turkey), Verteramo*et al*. (2013 - Italy) and

Table1- Detection of genital Mycoplasmas by using multiplex PCR

PCR Results	Positive	Percent
M. genitalium	3	2.88
U. urealyticum	39	37.5
M. hominis	3	2.88
M. genitalium+U. urealyticum	1	./96
M. genitalium + M. hominis	1	./96
U. urealyticum + M. hominis	1	./96
M. genitalium + U. urealyticum + M. hominis	1	./96
Total positive patients	40	38.46
Total negative patients	64	61.54
Total	104	100

Table 2. The characteristics of *M. genitalium, M. hominis* and *U.uralyticum*- infected patients

Total	**Prevalence of at least one infection n subjects/n total**	**P**	***Mycoplasma hominis* n subjects/n total**	***Mycoplasma genitalium***	***Ureaplasma urealyticum***
	40/104		3/104	3/104	39/104
Age distrubition					
<23 years old(34)	12		3	2	12
23-31 years old (41)	16	0.736	0	1	15
>31 years old(26)	12		0	0	12
Literacy					
Illiterate(13)	6		0	0	5
Low – literate(52)	19		2	2	19
Diploma(32)	12	0.975	1	1	12
Higher edacatione(7)	3		0	0	3
Situation of employment					
Unemployed (4)	3	0.126	0	0	3
Employee (100)	37		3	3	36
Age on first intercourse					
(14-27)years old (88)	34	0.932	3	3	33
>27 years old (16)	6		0	0	6
History of Abortion					
Abortion (20)	6	0.387	1	0	5
Not Abortion (84)	34		2	3	34
Duration of infertility					
(1-3)years old (58)	22		2	2	22
(4-6)years old (24)	9	0.693	1	1	8
(7-9)years old (9)	5		0	0	5
>9 years old (13)	4		0	0	4
Type of infertility					
Primary(62)	24	0.950	1	1	23
Secondary (42)	16		2	2	16

Zdrodowska *et al*.(2006 - Poland)(11, 16-18). There were no prominent differences in infection rates beside discrepancy in study population being geographically and socially different in each report. The infertile women in this study had no symptoms of acute infection of the genital tract, so the low prevalence of infection with *M. hominis* and *M. genitalium* was normal (20). High percentage of *U. urealyticum* infection may be related to the age and sexual activity the selected group of young women. Low prevalence of *M. hominis* infections may probably be due to the lack of any patients suffering from bacterial vaginosis (19, 20).

Mycolplasma genitalium was shown to be associated with infertility due to fallopian tube abnormalities (21). The frequency of this bacterium in the infertile women was almost similar to those reported by Tomusiak *et al.*(2013- Poland) and

Gowin *et al.* (2011-Turkey) (22,23); but lower than those observed by Grześko *et al*. (24). In a few studies performed on women with tubal factor infertility, *M. genitalium* has not been isolated (25, 26).

Since the prevalence of *M. genitalium* is higher in women with PID, it's presence is always indicative of pathologic conditions (27). Different rates of infection indicate discrepancy in sampling and other condition. However, more studies with large study group are needed to evaluate the rate of *M. genitalium* infection.

Detection of Mycoplasmas is very difficult and time consuming in many countries. Meanwhile numerous reports documented the role of these bacteria in women infertility and introduced them as true pathogens to the medical community. Obviously, adequate detection of these bacteria is urgently needed. An obstacle to diagnosis is high cost of Mycoplasmas culture. This study used multiplex PCR with a pair of primers in order to detect, in less than 8 hours, without interference from other microorganisms present in the sample.

In conclusion, our data showed a low rate of *M. genitalium* and *M. hominis* infections and high prevalence of *U. urealyticum*. These results could be a reflection of the regional and social conditions (muslim women with limited partners) which reduce bacterial infection in this population. In overall, this study results were in the same range of the reviewed articles. Despite having no symptoms of an ongoing acute inflammation of the reproductive tract, many women may have genital mycoplasmas in the cervix. We concluded that multiplex PCR method using a pair of primers is a useful and low cost method for diagnosis of genital infections in women.

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