

Rate of *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* in Infertile Females and Control Group

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ABSTRACT

Infertility in female is one of the most important sequela of genital infection with *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. In the present study the frequency of these bacteria was studied in 125 infertile female by direct and indirect immunofluorescence tests and culture method and compared with 250 normal population. *Mycoplasma hominis* was isolated from 32 (35.6%) of infertile females compare with 18 (7.2%) of normal population. *Ureaplasma urealyticum* was isolated from 41 (32.8%) of infertile females compare to 48 (19.2%) of normal population. *Chlamydia trachomatis* was detected by direct IF in 11 (8.8%) of infertile and 2 (0.8%) control group. The antibody titer against D-K serotypes of *Chlamydia trachomatis* was also measured in both groups of infertile and normal population and a positive titer of 1/16 and above was detected in 26 (20.8%) of infertile cases and in 8 (3.2%) of control group. The rate of *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* in case and control groups was significant (respectively $P < 0.0001$, $P < 0.0001$, $p = 0.0018$).

INTRODUCTION

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Female genital tract is a suitable environment for growth of various pathogen and nonpathogen microorganisms. Some of the organisms such as *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Listeria monocytogenes* and *Neisseriae gonorrhoeae* upon localization and colonization at the appropriate anatomical site may cause various pathological disorders like cervicitis, vaginitis, urethritis, endometritis, salpingitis and bartholinitis. The pathological disorders might lead to pelvisitis, ectopic pregnancy and infertility. It was reported that some of these isolated microorganisms produce noraminidase like substances which showed to induce certain disorders in blastocyst of murine model, and many cause a similar pathological disorders in human (1, 6, 10, 11-14). The following hypothesis are proposed in regard to attachment of the organisms to sperm in genital tract:

1. The attachment of bacteria to sperm might cause unusual metabolism in sperm.
2. Attachment of bacteria to sperm might cover the region of sperm membrane, which is essential for sperm identification and attachment.
3. Attachment of bacteria to sperm might inhibit sperm penetration to the ovum.

The above mentioned hypothesis would initiate the concept that some of the microorganisms present in vagina cause disorder in sperm function which might lead to infertility in females. The role of infectious agent in infertility is not only due to creation of certain disorder in sperm function, but also infection in different parts of the female genital tract might induce infertility due to various reasons (4, 7, 9, 17, 20).

The purpose of this study was to investigate the rate of Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum in infertile females and comparison with control group.

A serial dilution of each serum sample was prepared and added to different acetone fixed antigens, after 30 minutes of incubation in 37°C in moist condition, slides were washed with PBS, for 10 minutes and then dried at room temperature. Then the labeled anti-human FITC conjugate was added to each slide and incubated at 37°C in moist condition for 30 minutes. The slides were washed with PBS and PBS containing Evans blue, slides were dried at ambient temperature and a drop of buffered glycerol solution was added to each. Slides were overlaid with a cover slip, and were observed under fluorescence microscope (2, 3, 16).

RESULTS

In this study 125 samples from infertile females and 250 samples from control group (Healthy volunteers) were collected during the years 1996-1998. The samples were processed by culture and serological test. The results are as follow:

Table 1 shows frequency distribution of females on the basis of age, signs of disease and level of education in case and control groups. In regard of the profession of the subjects under investigation, out of 125 cases, 107(85.6%) were housewives and 18 (22.4%) were employed and in 250 control group, 93(37.2%) were housewives and 157 (62.8%) were employed.

Table 2 and 3 shows rate of *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and antibody titers against D-K serotypes of *Chlamydia trachomatis* in case and control groups.

DISCUSSION

The rate of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Chlamydia trachomatis* in infertile females and control group are shown in Tables 1-3 and indicated as follow:

- The presence of *Mycoplasma hominis* in 32 (35.6%) and in control group 18 (7.2%) reported the rate of these bacteria in healthy %, respectively (8, 17).
- The presence of *Ureaplasma urealyticum* in infertile % and in control group 48(19.2%)

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Samples

By using speculum, 4 sterile cotton swabs were used to collect specimens. One of the swabs was transferred to PPLO Broth medium, the two other were used for prepare samples for Gram stain and direct fluorescence test, the forth swab was kept at -70° C for further validation. Then the samples were transported to laboratory for further tests. Five ml of blood sample was collected antichlamydial antibodies. The media containing s through membrane filter inoculated in to urea and at 37°C with 5% CO₂ and sample was transferred confirmation (2, 5, 15). Serum samples were checked chlamydia antigens: 1- A, B, BI, C; 2- D, E, F, G, H, I, J, K; 3- L1, L2, L3.

Table 1. Frequency distribution of females on the basis of age, signs of disease and level of education in case and control groups

Age	Case group	Control group
	N = 125	N = 250
<20	12 (9 . 6)	13 (5 . 2)
21 - 24	27 (21 . 6)	61 (24 . 4)
25 - 29	38 (30 . 4)	70 (28)
30 - 34	29 (23 . 2)	59 (23 . 6)
35 - 39	17 (13 . 6)	37 (14 . 7)
> 40	2 (1 . 6)	10 (4)
Signs of disease		
Spotting	20 (16)	—
Discharge	61 (48 . 8)	—
Itching and burning	32 (25 . 6)	—
Urine frequency	23 (18 . 4)	—
Pain beneath abdomen	43 (34 . 4)	—
Painful intercourse	39 (31 . 2)	—
Painful menstrual cycle	61 (48 . 8)	—
Spotting after sexual contact	5 (4 . 7)	—
STD of sex partner	3 (2 . 4)	—
Self STD	5 (4)	—
Level of education		
Illiterate	11 (8 . 8)	19 (7 . 6)
Primary education	31 (24 . 8)	43 (17 . 2)
High school education	64 (53 . 6)	107 (42 . 8)
Higher education	16 (12 . 8)	81 (32 . 4)

($P < 0.0018$). It was reported the rate of these bacteria in healthy persons 39.5% and 45%, respectively (17,20).

-The obtained results from the study of the samples for detection of *chlamydia trachoma* particles by the method of direct immunofluorescence indicates that in infertile females 11(8.8%) and in control group 2(0.8%) were positive ($P < 0.0001$). It was reported the rate of *Chlamydia trachomatis* by direct immunofluorescence in infertile females 18.8% (3).

In this study specific antibody titer equal or more than 1/16 against serotypes D to K of *Chlamydia trachomatis* was observed in 26(20.8%) of infertile females and 8 (3.2%) of control group ($P < 0.0001$). Our results is nearly the same as reported (18, 19).

Z statistical analysis test with 99% confidence showed that the rate of *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* case and control group was significant.

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Table 2. Frequency distribution of case and control groups on the basis of bacteria detection

Bacteria	Case group		Z	P
	N = 125			
	Control group			
	N = 250			
Mycoplasma hominis	32 (25 . 6)	18 (7 . 2)	4. 94	<0.0001
Ureaplasma urealyticum	41 (32 . 8)	48 (19 . 2)	2. 92	0.0018
Chlamydia trachomatis (Direct - IF)	11 (8 . 8)	2 (0 . 8)	3. 99	<0.0001

Table 3. Frequency distribution of females on the basis of specific antibody titers against Chlamydia trachomatis in case and control groups

Antibody titers	Case group				Control group				Z	P
	N = 125				N = 250					
	Positive n = 26				Positive n = 8					
Against chlamydia	1/128	1/64	1/32	1/16	1/128	1/64	1/32	1/16		
Number	0	10	6	10	0	2	2	4	5.58	p<0.0001
Percentage	0	8.8	4.8	8.8	0	0.8	0.8	1.6		

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