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ORIGINAL ARTICLE

Detection of Chlamydia Trachomatis in patients with unexplained infertility: A case control study

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***Chlamydia trachomatis*, Unexplained infertility, PCR**

ABSTRACT

Our Objective*: is to detect Chlamydia Trachomatis organism in cervical swabs obtained frompatients with unexplained infertility(UI) and to compare its incidence in infertile compared to fertile females in reproductive age. The study design was a case control observational study, done in Cairo University Hospitals.* ***Methodology****: One hundred females diagnosed as unexplained infertility and 100 normal fertile females were included in the study for detection of Chlamydia trachomatis by using polymerase chain reaction (PCR) of cervical swab samples.* ***Results****: Fifteen females were positive for Chlamydia trachomatis in infertile group while only 2were positive in healthy fertile group with p value of 0.002.* ***Conclusion****: The Incidence of Chlamydia trachomatis in cervical swabs was significantly higher in unexplained infertile females compared with the control group.*

INTRODUCTION

The diagnosis of unexplained infertility can be made only after excluding common causes of infertility using standard fertility investigations, which include semen analysis, assessment of ovulation, and tubal patency test. These tests have been selected as they have definitive correlation with pregnancy. It is estimated that a standard fertility evaluation will fail to identify an abnormality in approximately 15% to 30% of infertile couples. [1]

Infertility is a worldwide health problem among couples with approximately 15% current global infertility rate, translating to one in 6 couples suffering from this condition.[2]

Chlamydia trachomatis is one of the most frequently reported cause of sexually transmited diseases (STD). It has 3 human serovars; serovar Ab, B, Ba or C, which cause trachoma (an eye infection), serovar D to K which cause pelvic inflammatory diseases (PID), ectopic pregnancy and urethritis, and serovars La to L3 which causes lymphogranuloma venereum (LGV). The *C. trachomatis* is increasingly being associated with long-term complications, such as asymptomatic endometritis, cervicitis, PID, and tubal factor infertility.

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The association between *C. trachomatis* infection and infertility has been the subject of several researchs.[3,4]

METHODLOGY

This case control study group was done between January -2014 to -November 2014 among females in reproductive age, 100 of them were infertile and diagnosed as (UI) and 100 fertile females as controls. The study was done in Cairo University hospitals, Infertility Unit, Department of Obstetrics &Gynecology.

Verbal informed consent was obtained from all participants, and procedures were previously reviewed and approved by the ethical committee of the department.

All cases were subjected to the following investigations:

- A clinical history was taken from all participants in addition to General and gynecological examination.
- Infertile women were proven as UI by the classic criteria; normal semen analysis, normal hormonal profile, normal hysterosalpingeogram, and normal ultrasound scan Demographic characteristics of the patients were noted using a preformed data collection sheet. Signs and symptoms if any, age, vital signs, blood pressure, respiratory rate, pulse, temperature, body weight, body mass index, history of IUD use & frequency of coitus per week. Physical pelvic examination was conducted by a gynecologist.

*** Specimen collection**:

Two endocervical swabbing by speculum examination were obtained from all patients by the attending gynecologist. The first swab was used before sampling to clean off the excess mucus, while the second one was rubbed and rotated several times over the endocervical cells in the cervical canal to collect samples for assay. Swabs were withdrawn from the cervical canal without touching the vaginal surface, and were placed into the Copan universal transport medium (UTM-RT, Copan Medical Diagnostic Laboratories, Hamilton, New Jersey, USA). All swabs were stored at 4°C until transported to the laboratory. The DNA extraction was performed using automated DNA extraction by MagNA Pure Compact Nucleic Acid Isolation Kit and MagNA Pure Compact system by Roche Diagnostics, Indianapolis, Indiana, USA. A 136 bp fragment of the *C. trachomatis* genome. The gene was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 (Roche Diagnostics, Indianapolis Indiana, USA). The polymerase chain reaction (PCR) was monitored by an additional PCR product of 278 bp. The master mix for real time PCR was prepared using LightCycler® Fast Start DNA Master HybProbe (Roche Diagnostics, Indianapolis, Indiana, USA). To avoid contamination, mixing of the reagents (except of the DNA template) was performed in a separate room, away from the room where DNA purification was carried out. The reaction mixture was prepared in a cold reaction tube, 15µl of the reaction mix was transferred to a light cycler 2.0 capillary. A 5 µl of template DNA were added to each capillary tube for a final reaction volume of 20 µl. One negative control was always included in each run by replacing the template DNA with water. A positive control was included in each run by replacing the template DNA with one of the control DNA from the standard row provided with the light mix kit (TIB Molbiol, Germany). The LightCycler PCR program was composed by: fast start Taq DNA polymerase activation carried out in 95°C for 10 minutes, followed by cycling: 95°C (20°C/second [s]) for 5 seconds, 55°C (20°C/s) for 5 s, and 72°C (20°C/s) for 15 s, repeated 50 times. Melting assay ended the analysis: samples were heated to 95°C (20°C/s) hold for 20 s, cooled to 40°C (20°C/s) hold for 20 s, and then heated slowly at 0.2°C/s up to 85°C, finally cooled to 40°C (20°C/s). The PCR results were obtained within 50 minutes (50 cycles and melting curve); data analysis was performed, as described in the LightCycler instrument operator's manual.

Statistical analysis:

All collected data were encoded and analyzed using the Statistical Package for Social Sciences version 19.0 (IBM Corp., Armonk, NY, USA). We present the frequencies and percentages for different items of nominal variables, and mean, standard deviation and range for numerical variables. We used chi-square test to compare between infertile group and the non-infertile group, We assumed there was a statistically significant when $p<0.05$.

RESULTS

Table 1: Patients and control group characters:

	Infertile (UI)	***Control***	***p-value***
Age	29.2±2.3	29.3±3.1	0.33
BMI	26.5±4.6	27.1±5.1	0.06
Temperature	37.0±0.1	36.9±0.2	0.09
Weight	78.5±12.9	76±13.1	0.12
Duration of marriage	5.5±2.2	5.3±2.3	0.44
Frequency of coitus Per week	2.2±1.2	2.1±1.1	0.47
Use of IUD(past)	5/100	7/100	0.76

Table 2: Number and percent of positive cases for *C. trachomatis* by PCR

	Infertile	***Control***	***p-value***
Positive cases	15/100	2/100	0.002
Negative cases	85/100	98/100	

DISCUSSION

The association between *C. trachomatis* infection and infertility had been the subject of several researches. A study in Iran[5] suggested the significant association between *C. trachomatis* infection and female infertility with a prevalence rate of 15.3%. The same finding was suggested by a study conducted in India, which confirmed the significant association between infertility and the duration of *C. trachomatis*[6] Furthermore, another study suggested that a positive serology screening result for *C. trachomatis* is predictive for both tubal damage, and a reduced pregnancy rate.[7] A prevalence rate of 9.6% was found in

female patients attending the infertility clinic in a study carried out in Nigeria.(8) Other prevalence rates include studies carried out in the USA (5-15% prevalence rate),(9) UK (16%),[10] Jordan (3.9%),[11] Iran (22%),[12] and Brazil (10.9%).[13]

In our study we detected high incidence of chlamydial infection in non tubal infertility, this is novel study that prove that *C. trachomatis* is playing a role in cases of unexplained infertility .

The prevelance of PCR positivity is significantly higher in UI group than control group 15% versus only 2% with p-value 0.002.

Although infertile group were proved to be patent tubes by hysterosalpigeogram,this indicates that *C. trachomatis* has many mechanisms to affect female reproduction rather than tubal block.

The *C. trachomatis* infection rate among our comparison (control) group was only 2%. This is in contrast to other studies that reported higher prevalence (6% and 8%) of C. trachomatis infection in healthy patients.[14,15].

In our study incidence among infertile women was 15% ,Some other studies conducted in other parts of the world have shown similar or even greater incidence, one example is the 16% prevalence rate found in a UK study, 40% in Jordan, and 22% in Iran.[16,17,18]

We tried to compare between two identical groups as regard patient characters, and to the best of our knowledge this is the first study comparing Chlamydial infection in unexplained infertility.

One limitation of our study is the lack of search for other causes of unexplained infertility in the studied group.

CONCLUSION

The incidence of chlamydial infection in cervical swab in unexplained infertile women is significantly higher than fertile women.

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