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Realization about *Chlamydia trachomatis* Accusation as a Principle Cause of Unexplained Infertility in Egyptian Females

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Abstract

Chlamydia species are intracellular pathogens.*Chlamydia trachomatis*(CHT) is the predominant sexually transmitted bacterial pathogen that causes pelvic inflammatory disease .The disease asymptomatic nature means delayed treatment, leading to an increased risk of complications and transmission to the other partner. Unexplained infertility (UI) is a diagnosis of exclusion. Practitioners diagnoseUI if standard infertility evaluation isnormal.There is no sufficient data about *Chlamydiatrachomatis*as a causative agent of unexplained primary infertility in Egyptian females. This work is a trial to answer the question of Is *Chlamydiatrachomatis* being claimed in unexplained primary infertility in Egyptian females?"

Patients and Methods: Samples were collected between July 2011 and December 2013. Patients were selected from females attending Gynecology clinic, Mansoura University Hospital. Standard infertility evaluation and work upwere normal for selected cases. Samples were collected from abdominal lavage during abdominal laparoscope using sterile syringe. A total of 50 patients were participated in this study; 25 complaining of UI and 25 with complaints other than infertility. After harvesting, Part (2ml)of the sample was preserved at -80°C for PCR in a micro centrifuge tube; the remaining part was used for bacteriological culture.

Results: Bacterial cultures were negative for all cases and control groups. As regard PCR, it was positive for *Chlamydia trachomatis* DNA in 4(4/25) cases of unexplained infertility group, while only one case of control group (1/25) was positive.

Conclusion: Although CHT is a big health problem and claimed in some cases of UI but in Egypt it is not statistically associated with UI. This result is due to ethically and religious habits between Egyptian women.

Keywords: Chlamydia Unexplained infertility; Pelvic inflammatory disease.

Introduction

Chlamydia species are intracellular pathogens principle infect epithelial mucosa[1].*Chlamydia trachomatis* isconsideredthe predominant sexually transmitted bacterial pathogen causing pelvic inflammatory disease[2].Itleads to two characteristic infections, one of which is chronic infectionofthe eye mucosal epithelium leading to "trachoma", and the other is of genital tract that may lead to infertility[3].Sexually active young women especially undiagnosed and untreated are more prone to in infection and complicationssuch as pelvic inflammatory disease, ectopic pregnancy, and infertility[4].Females with untreated Chlamydia infections are more related to serious reproductive complications as PID, ectopic pregnancy, tubal infertility, and chronic pelvic pain [5].

The asymptomatic nature of the disease associated with delayed treatment, so that partner infection and risk of complications are more prone to occur[6].Infertility, ectopic pregnancy and chronic pelvic pain may develop as a result of ascending genital-tract infections of endometrium, fallopian tubes and/or adjacent pelvic structures[7].In Egyptian females it is suggested increased risk of tubal factor infertility and ectopic pregnancy are associated with Chlamydia infection[8,9].About 80% of infected females are asymptomatic in nature and remains undetecteddue to effective screening programabsence[10].

In a lot of patients the cause of infertility not understoodenough .As there are heterogeneous causes of infertility and many factors responsible for reproductive success, UI is diagnosed in about 30% of couples with reproductive problem[11].Diagnosis of UI isby exclusion. It is assigned by specialists after normal standard infertility evaluation reports[12].According to Siamand Hefzy[13]about 25-30% of females in a reproductive medicine clinic are re- diagnosed with UI.

There is no sufficient data about CHT as a causative agent of unexplained primary infertility in Egyptian females. This work is a trial to answer the question of"Is *Chlamydiatrachomatis* being claimed in unexplained primary infertility in Egyptian females?"

Patients and Methods

Study Population and Clinical Specimens

Samples were collected between July 2011 and December 2013. Patients were selected from females attending Gynecology clinic, Mansoura University Hospital.Gynecological sheet were fulfilled before operation. Standard infertility evaluation and workup(hormonal, structural, immunological) were normal for selected cases.

Samples were collected from abdominal lavage during abdominal laparoscope using sterile syringe. A total of 50 patients were participated in this study; 25 complaining of unexplained infertility and 25 with complaints other than infertility. Samples were referred to the laboratory immediately after harvesting, Part (2ml)of the sample was preserved at -80°C for PCR in a micro centrifuge tube, and the remaining part was used for bacteriological culture. The bacterio-scopic examination was carried out using Gram stain of uncultured samples.

Samples were cultured on Blood agar, McConkey agar and chocolate agar (incubated at 10% co2) and incubated at 37oC for 24 hours.

DNA Extraction

Frozen samples were thawed to be at room temperature, and then centrifuged at 10000 rpm for 15 min.The supernatant was used for DNAextraction. Chlamydia DNA was extracted using GeneJet Genomic DNA purification kit (Fermentas). The Extracted DNA was used for amplification.

PCR Amplification

A major outer membrane protein (MOMP) gene of CHT conserved region was used as primer pair. This 180 bp primeris common to all serotypes of CHT(Sigma). Primers sequences are: Sense; 5' GCC GCT TTG AGT TCT GCT TCC 3'. Antisense; 5' GTC GAA AAC AAA GTC ACC ATA GTA 3 [14]. The reagents were Dream Taq Green PCR master mix kit(Sigma-Aldrich).Volume of amplification mixture was 50UL; each primer (0.5uM), 25Ul Readymade master mix, 10Ul DNA and completed with water. Negative and positive controls were used.

The PCR reaction tubes were mixed well and centrifuged for 5 seconds in Ependorf centrifuge. The tubes were processed for 35 cycles in the thermal cycler. The start was 95°C for 1 min followedby cyclic amplification. This was followed by sequential incubations at 94°C for 1 min,60°C for 1 min, and72°C for 2 min. After this process the tubes were preserved for 7 min at72°C.Theproducts were kept frozen till loading onto 1.5% agarose gel for visualization on Electrophoresis.The samples were then run in parallel to the DNA marker (Sigma)and visualized on U.V. trans illuminator to detect the presence of specific band by its fluorescence at bp 180.

Statistics

Description by percentage was used. Chi-square test was used to test for significant association between any two groups. P value was considered significant if less than 0.05.These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS 16).

Results

Intra-operative abdominal lavage samples were obtained using sterile syringe. Samples were collected between July 2011 and December 2013. Patients were selected from females attending Gynecology clinic, Mansoura University Hospital.There were a total of 50 cases, 25 patients complaining of unexplained infertility and 25 control group with complaints other than infertility. Bacterial cultures were negative for all cases and control groups.

Lower abdominal pain, discharge character (odour and colour) and clinical signs (Cervicitis) were with no statistically significant value. Wherever intra-operative laparoscopic findings were statistically significant (p=0.0001)(Table 2).

As regard PCR, it was positive for Chlamydia trachomatis DNA in 4(4/25) cases of unexplained infertility group, while only one case of control group (1/25) was positive. As shown in the Table 3 PCR results had no statistically significant value.

Table 1: Demographic data

Group		N	Mean	**Std. Deviation**	**P-value**
Age	**1**	**25**	**27.2800**	**3.72514**	**0.5**
	2	**25**	**27.9600**	**4.08738**	
Marriage	**1**	**25**	**3.7600**	**1.12842**	**0.03***
	2	**25**	**7.6400**	**2.72152**	

Table 2: Clinical Data

Parameters		Groups		Totals	*P*-value
		Infertile group	Fertile controls		
a. Complaint	No complaint	0	25	25	0.0001*** (a)
	With complaint	25	0	25	
b. Discharge	No discharge	7	3	10	0.364 (b)
	White	8	11	19	
	Yellow	9	7	16	
	Green	1	3	4	
	Brown	0	1	1	
c. Odour	No odour	14	15	29	0.5 (a)
	Malodorous	11	10	21	
d. Cervicitis	Absent	20	16	36	0.345 (a)
	Present	5	9	14	

e. PID	Absent	24	17	41	0.02* (a)
	Present	1	8	9	
f. LAB	Absent	19	13	32	0.139 (a)
	Present	6	12	18	
g. Laparoscopic findings	Normal	25	0	25	0.0001*** (a)
	Pathological	0	25	25	
h. PCR	Negative	21	24	45	0.3 (a)
	Positive	4	1	5	

(a) Means P-value estimated by Fisher exact test, (b); means P-value estimated by Chi-square, PID; means pelvic inflammatory disease and LAB; means lower abdominal pain

Table 3: Laboratory findings

Parameters		Groups		Totals	P-value
		Infertile group	Fertile controls		
PCR	Negative	21	24	45	0.3 (a)
	Positive	4	1	5	

(a) Means P-value estimated by Fisher exact test, PCR; means polymerase chain reaction

Discussion

Chlamydia trachomatis infection is the mostly reported sexually transmitted infection in the United States. Female pelvic inflamm U.S.are Recommended for chlamydia screening (grade B recommendation)[4].According to CDC [16]due to asymptomaticdisease,sexually active young women <25 years should routinely screened for CHT infection, with prompt treatment for infected cases, and timely treatment of sex partners to prevent re-infection. Disease burden is under estimated due to asymptomatic nature leading to unreported or non-diagnosed cases.Because untreated chlamydia can persist, data of reporting cases are affected by screening activity[16].

The Practice Committee of the American Society for Reproductive Medicine (ASRM) has published guidelines for a standard infertility evaluation. It includes a semen analysis, assessment of ovulation, a hysterosal Pingogram, and, if indicated, laparoscopy. When the results of a standard infertility evaluation are normal, practitioners assign a diagnosis of unexplained infertility [12].

Marrazzo et al.[17]showed that NAAT can detect the presence of lower number of Chlamydia elementary bodies than non-NAAT.

Klausner and Hook[22]stated that although NAATs are expensive, the high sensitivity may allow them to be more cost-effective than other Chlamydia tests. This is due to ability of PCR to multiply a specific sequence of DNA 105 – fold or greater. The lower limit of detection of PCR is in the range of 1 to 10 elementary bodies (Ebs) but EIA requires 10,000 Ebs and that of culture is 10 to 100 Ebs [18].

The aim of this study was to evaluate the role of *Chlamydia trachomatis* as a cause of unexplained primary infertility in Egyptian females. This study was conducted on 50 female patients who were selected from those attending Outpatient Gynecology Clinic, Mansoura University Hospital. Twenty five consulting for infertility complaints whenever the other 25 complaining of gynecological problems otherthan infertility. Informed consent was obtained from all patients.A standard genitourinary physical examination and Standard infertility work up of each patient were performed. Samples were collected from abdominal lavage during abdominal laparoscope using sterile syringe. Samples were referred to the laboratory immediately after harvesting, Part (2ml)of the sample was preserved at -80°C for PCR in a microcentrifuge tube, and the remaining part was used for bacteriological culture.

The ages of patients in the current study ranged from 21 – 36 years, with a mean age of 27.28 years for patient group and 4.09 years for control group. In this study Discharge Colour, Odour, Cervicitis was with no statistically significant difference in correlation with UI. On the other hand PID and Laparoscopic findings correlation was statistically significant.

Diagnosis of *Chlamydia trachomatis* infection using PCR technique was proved in 4/25 (16%) of all cases with no statistically significant difference. This may be due to ethically and religious habits between Egyptian women. This is in co-ordnance withSiam and Hefzy[13] and Rashidi et al.[19] whofound that no significant differences among fertile and infertile women for *Chlamydia trachomatis* infection using PCR technique.BébéaranddeBarbeyrac[20] concluded that the diagnosis of *Chlamydia trachomatis* is best made by using nucleic acid amplification tests, because they perform well and do not require invasive procedures for specimen collection. Also, Pavlin et al.[21] reported that PCR is rapid and sensitive technique.

In addition to theprevious stated advantages of PCR technique, in this study it can be used to detect all serotypes of CHT, because the MOMP gene of CHT from which primer was selected is from the conserved region of published sequence [14].

From this study we can conclude that although *Chlamydia trachomatis* is a big health problem and claimed in some cases of UI but in Egypt it is not statistically associated with UI. This result is due to ethically and religious habits between Egyptian women.

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