ORIGINAL ARTICLE

Estimation of *chlamydia Trachomatis*Antibodies amongst Infertile Women after Assisted Reproduction Technology Failure in Alsir Abu Elhassan Fertility Center Khartoum,Sudan

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ABSTRACT

Background: *Chlamydia trachomatis* is the leading cause of bacterial sexually transmitted infection (STI) globally. The WHO estimates 131 million new C. trachomatis genital infection cases occur annually.
Aim: To detect *Chlamydia Trachomatis* Antibodies among infertile women who had Assisted Reproduction Technology (ART), find the relation between seropositivity and age, duration of mirage,failure ofART, and severity of the disease.
Methods: This was a case-control study conducted in Elsir Abu El-Hassan Fertility Center, Khartoum,Sudan, from October 2018 - May 2019. Ninety women were enrolled(Sixty women with ART failure (cases) and thirty women had successful ART (control)); their ages ranged from 20 to 50 years with 31 years mean. Anti-*Chlamydia Trachomatis* Antibodies were tested in serum specimens through enzyme-linked immunosorbent assay (ELISA). Data was collected and analyzed with the SPSS program, version 21.
Results: Out of the total of 60 infertile women with recurrent ART failure, 4(6.7%) were positive for Chlamydiae IgG, while all the control group was negative. Regarding IgM antibodies, All participants were negative. Out of total IgG positive, 75% belonged to (30-40) age group, and 50% were married for less than five years.75% of them committed ART one time. Statistical analysis revealed no relation between IgG seropositivity and ART succeed(P =0.1) (having kids (P=0.3), age (P=0.4), duration of marriage(P=0.2), and number of ART (P=0.8).
Conclusion: Seroprevalence of *Chlamydia Trachomatis* infection is prevalent among infertile women with recurrent ART failure;there is no relation between Chlamydia infection and ART's recurrent failure.
Keyword: *Chlamydia Trachomatis*, ELISA, ART, Infertile women

INTRODUCTION

Genital infection with *Chlamydia trachomatis*, the most common reportable disease in the United States, can lead to severe sequelae among women, including pelvic inflammatory disease (PID), tubal factor infertility, ectopic pregnancy, and chronic pelvic pain[1].

About 8% of U.S. women and 15% of Swedish women have reported a PID diagnosis in their lifetimes[1]. *C. trachomatis* infection's potential to cause serious sequelae, chlamydia screening and treatment programs have been implemented in many countries. Despite ongoing control efforts,this has raised several fundamental questions about C. trachomatis infection's natural history. For example, if *C. trachomatis*infections were not detected and treated through a control program, what proportion would result in sequelae. This influences the overall potential benefit of the program and its cost-effectiveness. An even more critical consideration maybe the timing of tubal inflammation and damage relative to the acquisition of infection. This timing affects the likelihood that diseases can be detected and treated by a control program before developing symptomatic PID or the development of tubal damage that could ultimately lead to infertility or ectopic pregnancy. Contributing to observed increases in chlamydia case rates is likely to increase repeat infections, common in some populations. Thus, another fundamental question is how harmful repeated *C. trachomatis* infections lead to sequelae[(1).] Persistent tubal infections by *C. trachomatis* are also a common feature, even despite courses of antibiotic therapy. The current focus on TFI has been on the immunopathology of tubal chlamydial infections. Differences in host factors, such as genetic polymorphism in cytokine response and human leukocyte antigen type, may play a role in the outcome of pelvic inflammatory disease. Hysterosonography is a more convenient model for diagnosing tubal occlusion than hysterosalpingography. The use of new species-specific antibody tests for *C.trachomatis* has decreased previous specificity problems when used to detect tubal occlusion in women consulting work-up because of infertility[2]. In most women, chlamydia infections remain asymptomatic, but they may increase tubal factor sub-fertility risk. Pelvic inflammatory disease (PID) and its chronic sequelae are associated with chlamydial IgG antibody formation in serum. A correlation between the height of antibody titers and the presence of tubal factor sub-fertility has been established. However, the predictive value of chlamydia antibody testing (CAT) is limited. Several factors affecting the sensitivity and specificity of CAT have been identified. Because it is assumed that the presence of chlamydial heat shock proteins (HSPs) may indicate chronic inflammation, chlamydial HSP60 antibody testing has been evaluated in its prediction of tubal factor sub-fertility[3].

In the Czech Republic,the authors discussed the impact of the assembled results from impaired fertility of women[4], their study was performed in Tehran from April 2007 to April 2008 in 234 infertile and 223 pregnant

women, using ELISA and PCR, methods and PCR results, revealed that 29(12.4%) of the infertile and 19(8.5%) of the fertile women were positive for *C. trachomatis* infection[5].

In Idia,*C. trachomatis* was detected in 23% of patients attending gynecology OPD and among 19.9% of patients attending the STD clinic in a study from New Delhi[6]. In Mumbai, in a study conducted on female sex workers (FSW) and married contacts attending an STD clinic, 23.2% chlamydial positivity was found[7]. In a survey from Aligarh[8], *C. trachomatis* was detected in 28.1% of infertile women. The prevalence of *C. trachomatis* in asymptomatic and symptomatic women attending a gynecology clinic at Delhi was 4 and 30.4%, respectively[9.]Anti-chlamydial IgG antibodies were present in 68% of women with infertility, 50% with bad obstetric history (BOH), and 10% of healthy pregnant women in a study conducted in Amritsar district in Punjab, India[10]. Joyee*et[11]* found the prevalence of *Chlamydia* in STD patients to be 30.8%.

In contrast, in another study, the prevalence of *C. trachomatis* infection in male patients with urethritis was found to be 17.5%[12.] Research from the U.K.[13] has shown that health care settings had higher prevalence estimates than population-based studies. Among less than 20 years, prevalence estimates were 17.3% in genitourinary clinics, 12.6% in antenatal clinics, 12.3% in termination of pregnancy clinics, 10.7% in youth clinics, 1% in family planning clinics compared to 5% in population-based studies. Vuylstek*e et al*[14] reported a 7.3% prevalence of *C. trachomatis* in females attending the STD/genitourinary clinic in Belgium. In Europe[15], *C. trachomatis* infection prevalence was estimated to be 5 to 12% for women undergoing pregnancy termination. Several Latin American studiesshowed *C. trachomatis* prevalence rates of 1.9 to 4.5% in Chile, Peru, Brazil, Mexico[16,17] and 12.2%[18] in women attending family planning clinicsJamaica.No previous studiespublished in Sudan. This study aims to detect IgM and IgG antibodies of *Chlamydia Trachomatis* by using ELISA among infertile women and correlate the infection with age,duration of mirage without having kids.

MATERIALS AND METHODS

This was a descriptive cross-sectional study conducted at Khartoum State (targeting Infertile women in ALSIR Abu Elhassan Fertility Center) in Khartoum during the period from October 2018 to May 2019.

A total of 90infertile women were enrolled in this study(60women with recurrent assisted reproduction failure;and 30 as control from known infertile women with a history of successful assisted reproduction). Blood samples were collected, serum separated, investigated to detect *Chlamydia Trachomatis*Antibodies(IgM and IgG) by Enzyme-Linked Immunosorbent Assay (ELISA). A direct interviewing questionnaire was used to collect the data from all study participants and analyzed using SPSS version 21.

Method for detecting IgM and IgG(The same procedure for both): serum samples for analysis were diluted 1:101 in a green-colored sample buffer. (10ul sample to 1.0 ml sample buffer) and mixed well by vortex, and then the mixture was incubated for 10 minutes at room temperature. 100ul of calibrator was transferred, positive and negative controls or diluted patient samples into the individual microplate wells, incubated for 30 minutes at room temperature, then washed by wash the reagent wells 3times with 450 ul of working strength wash buffer, left the wash buffer in each well for 30 to60 seconds per washed cycle, then the wells were emptied,after washed, thoroughly disposed of all liquid from the microplate by tapped it on absorbent paper with the openings facing downwards to removeall residual wash buffer and then 100ul of enzyme conjugate (peroxidase-labeled anti-human IgM) was added into each of the microplate wells.

Incubated for 30 minutes at room temperature, then washed, and then added 100ul of chromogen/ substrate solution into each of the microplate wells. Set for 15 minutes at room temperature,and then added 100ul of stop solution into each of the microplate wells in thesame order. At the same speed, as the chromogen/ substrate solution was introduced, the last step measurement usesthe photometric measurement of the color intensity wasmade at a wavelength of 450 nm and a reference wavelength between 620 nm and 650nm within 30 minutes of adding the stop solution.

Calculation by using (cut-off),

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of the calibrator}} = \text{Ratio}$$

RESULTS

A total of ninety infertile women were enrolled in this study to detect the anti-sperm antibodies in their serum. Sixty women with assisted reproductive technology failure (cases) and thirty women had successful assisted reproductive technology (control). Their age ranged from 20 to 50 years, with a mean of 31 years old. Among cases, 53 women had primary infertility, while the remaining (7 women) had secondary infertility. Out of the total of 60 infertile women with recurrent ART failure, 4(6.7%) were positive for *Chlamydiae* IgG, while the entirecontrol group was negative (Table1). Regarding IgM antibodies,all participants were negative. Out of total IgG positive, 75% belonged to (30-40) age group, and 50% were married forless than five years.75% of them committed ART one time. Statistical analysis results revealed no relation between IgG seropositivity between case and control (P =0.1), having no kids (P=0.3),age (P=0.4), duration of marriage(P=0.2), and recurrent failure of ART (P=0.8)(Table 2,3).

Table 1: Frequency of anti-Chlamydiae IgG among case and control groups

***Chlamydiae* IgG**	**Case**	**Control**	**Total**
Positive	4	0	4
Negative	56	30	86
Total	60	30	90

P value 0.148

Table 2: Frequency of anti-Chlamydiae IgG concerning age, infertility type, and number of assisted reproductive trials among case group

Variables		IgG(+ve)	IgG(-ve)	Total	*p* –value
Age	20-30	1	29	30(50%)	0.465
	31-40	3	20	23(38.3)	
	41-50	0	7	7(11.7%)	
Total		4(100%)	56(100%)	60(100%)	
Infertility	Primary	3	50	53(88.3%)	0.390
	Secondary	1	6	7(11.7%)	
Total		4(100%)	56(100%)	60(100%)	
No.trials	One	3	47	50(83.3%)	
	Two	1	5	6(10%)	0.864
	Three	0	2	2(3.3%)	
	Four	0	1	1(1.7%)	
	Five	0	1	1(1.7%)	
Total		4(100%)	56(100%)	60(100%)	

Table 3: Frequency of anti-Chlamydiae IgG concerning age, infertility type, and number of assisted reproductive trials among the control group

Variables		
Age	**IgG(+ve)**	**ASAs (-ve)**
20-30	1	23(76.7%)
31-40	3	6(20%)
45-50	0	1(3.3%)
Total	4	30(100%)
Number of trials		
One	3	22(73.3%)
Two	1	6(20%)
Three	0	1(3.3%)
Five	0	1(3.3%)
Total	4	30(100%)

DISCUSSION

In most women, chlamydia infections remain asymptomatic, but they may increase tubal factor subfertility risk. Pelvic inflammatory disease (PID) and its chronic sequelae are associated with chlamydial IgG antibody formation in serum. A correlation between the height of antibody titers and the presence of tubal factor subfertility has been established. However, the predictive value of chlamydia antibody testing (CAT) is limited[3]..

The present study results revealed that Out of the total of 60 infertile women with recurrent AST failure, 4(6.7%) were positive for *Chlamydia* IgG; this finding was slightly lower than (7.3%) of a study conducted in Nigeria that estimated *Chlamydia* infection among infertile women[19].

Also,it is lower than the result of the study conducted in Sudan that found C. trachomatis positive in 52 women (51.2%) of women, using the PCR test, while (10.7%) positive when used cytological method. This difference in the present study andtheir finding may be using different techniques.75% of positive results belonged to the (30-40) age group; this was similar to the Sudanese report, which showed that most positive cases were in the age range 26-40 years[(20)].U.S. survey showed younger age group correlated with the infection, and that might be due to differences in sexual activity culture between U.S. and Sudan.

In contrast, our findings disagreedwith CDC surveillance, reported that In 2017, 97.4% of all reported *chlamydia* cases in women were among those aged 15–44 years. The highest age-specific rates of reported cases of *chlamydia* in 2017 were among those old 15–19 years (3,265.7 cases per 100,000 females) and 20–24 years (3,985.8 cases per 100,000 females). Within these age groups, rates were highest among women aged 19 years (5,398.6 cases per 100,000 females) and 20 years (5,141.4 cases per 100,000 females[21].

The present study showed a lower seroprevalence of *chlamydiae*among ART failure cases than otherresearch on patients having *chlamydia trachomatis* and spontaneous abortion following in vitro fertilization. It concluded that a previous infection with *C.trachomatis* might increase susceptibility to subsequent spontaneous abortion. Even in the absence of detectable current disease, they found that incidence of antichlamydial IgG in aborting women (20 of 29; 69.0%) was greater (P <0.001) than the incidence in either woman with successful pregnancies (9 of 38; 23.7%), or women who did not become pregnant (20 of 78; 25.6%) after IVF[22].

CONCLUSION

Seroprevalence of *Chlamydia Trachomatis* infection among women older than 30 years is the highest; also, this study revealed there is no relation between Chlamydia infection and recurrent failure of ART.

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Authors' contributions: AAB designed and conducted the study and drafted the paper. NAA and MAR participated in practical work. WIE supervised all study starting from proposal writing, coordinated with the laboratory quality-control team, and revised and approved the manuscript, particularly the critical contents. All authors read and approved the final manuscript.

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