

***Chlamydia trachomatis* IgM antibodies among women suffering infertility in Gezira State, Sudan**

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ABSTRACT

Background: Although the symptoms of genital chlamydia infection vary from symptomatic to asymptomatic, it has a profound impact on the female reproductive system causing ascending infections which may lead to infertility. We aimed to detect the genital chlamydia infection and common associated symptoms in both primary and secondary infertility cases.

Methods: One hundred and sixty two Sudanese women suffering infertility were recruited to this study. Blood samples were collected from each woman, and then the separated serum was tested for the presence of anti-*chlamydia trachomatis* IgM using ELISA technique. A designed questionnaire was used to collect demographic and clinical presentation.

Results: The percentage of chlamydia infection was 48.6% among women suffering primary infertility and 81.8% among those suffering secondary infertility with significant differences between the two groups (p value 0.00). Women with secondary infertility exhibited significantly more common chlamydia symptoms than those with primary infertility. Chlamydia infection was significantly associated with the age, burning in urination and urethritis (p value <0.05).

Conclusions: Secondary infertile women affected by chlamydia infection and symptoms more than women those have primary infertility. Screening for chlamydia infection is mandatory.

Keywords: *Chlamydia trachomatis*, Primary Infertility, Secondary Infertility, Symptoms

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Introduction

Genital chlamydia infection is a sexually transmitted disease (STD) of public health importance (1-3). Although asymptomatic *Chlamydia trachomatis* infection are quite common occurring in up to 70% of infected women, the symptomatic infection often cause

less severe symptoms than other sexually transmitted diseases. These may allow the infection to go unnoticed with minimal patient awareness until secondary or tertiary symptoms develop. The sequelae of undetected and untreated chlamydia infection such as urethritis, cervicitis, proctitis or epididymitis may lead not

only to significant morbidity but far more, importantly ectopic pregnancy, tubal problems and then infertility(1, 4).

Infertility is a medical problem affects more than 80 million people worldwide. Large proportion of the developed countries has no access to medical diagnosis and treatment for infertility, and even in Sudan (5). The term primary infertility used for a couple who have never achieved pregnancy, the secondary infertility used for a couple who have previous successful pregnancy even if it ended in abortion. Reports represent primary infertility as a common than secondary infertility in Gezira state and the most common etiology was high prolactin level and tubal factor respectively(5, 6).

Infertility due to *chlamydia trachomatis* represents a preventable type of infertility, if detected early. However, *C.trachomatis* infection is not a notifiable disease in Sudan and data pertaining to infertility attributed to *chlamydia trachomatis* infection is very limited particularly in Wad Madani City, thus preventing the policies from being formulated regarding screening of patients.(2)

Diagnosis, treatment and prevention of sexually transmitted Chlamydia infection has become an important public health priority (7), especially when strong evidence linked this infection with clinical signs and symptoms . Few reports are available on the prevalence of current *C.trachomatis* infection in women with different clinical complications like urethritis and vaginal discharge; however, there is less reports in Sudan

on its prevalence and association with infertility or related clinical symptoms. Evaluation of present infection with the aforesaid types of manifestations could help in treatment.

The aim of this study was to detect the rate of chlamydia infection in both primary and secondary infertile women, also accordingly the most common clinical symptoms as an attempt for more clinical identification of the infection.

Methods

Study design and setting: From January to June 2021, 162 women suffering infertility attending Wad Medani Maternity Hospital were selected to detect anti-*chlamydia trachomatis* IgM in their serum samples using ELISA. Permission to carry out this study was granted from the Ministry of Health, Gezira State. The written consent was obtained from study participants after they had been informed about the study objectives.

Participants and selection criteria: The study included married women, suffering infertility selected during the routine clinic of the Hospital. The subjects were grouped according to infertility type into: primary infertile group (n= 74) those failed to get pregnant for at least one year of marriage, and the secondary infertile group (n= 88) those failed to get pregnant for at least five years after a history of abortion and/or successful pregnancy before. Pregnant women were excluded from this study.

Data collection and analysis: A designed questionnaire was used to collect data including demographic characteristics and relevant clinical symptoms. Collected data was analyzed using

Statistical Package for the Social Science (SPSS) software version 20.0 (IBM, USA). Descriptive statistics such as frequency tables, cross-tabulation, and chi test were used. The mean and standard deviation was also used for age. The level of confidence was at 95% and P value <0.05 was considered significant.

Clinical data was collected according to standard guidelines, from each participant using a sterile syringe five ml of venous blood were collected into a plain tube. Then the serum was separated in another tube after centrifugation of the blood sample. The serum samples were analyzed using ELISA technique to detect the presence of anti-*chlamydia trachomatis* IgM antibodies.

Enzyme Linked Immune-sorbent Assay: The ELISA kit from SERION ELISA classic *Chlamydia trachomatis* IgM Best-Nr: ESR 1372M company was used. When a participant's serum added to the microtitration plate, the fixed antibodies were bind to make an immune complex. After the incubation time the conjugate solution containing the enzyme and the secondary antibody was added. After that, the colourless substrate (p-nitrophenylphosphate) was added, and then the enzyme interacted with it to form a yellow colour compound. Finally, the signal intensity was measured photometrically using 405 nm filter. Steps and the cut off value were followed according to instructions by the kit sheet.

Results

The study included 162 participants, their age ranged between 17 and 46 years, with mean ages

of 33.9 ± 7.05 and 34 ± 7.08 for primary infertile women and secondary infertile women respectively. Almost half (54.1%) of the primary infertility women and quarter (27.3%) of the secondary infertility women were between 21-40 years of age. The majority of both are housewives residing in Wad Madani City with varying educational levels and socioeconomic status as shown in table 1.

Out of 162 participants 66.7% of them were suffering Chlamydia infection as the anti-*chlamydia trachomatis* IgM antibodies were detected in their blood

Using ELISA technology, the anti-chlamydia IgM antibodies were detected in 66.7% of study participants. According to study groups, 48.6% of women with primary infertility and 81.8% of women with secondary infertility with significant differences between the two groups (p value 0.00) as shown in table 2. The associated symptoms studied among these women were lower abdominal pain, urethritis, burning during urination, and vaginal discharge. Almost (90%) of the secondary infertile women suffer from these Chlamydia symptoms more than the primary infertile women. The risk factors associated with chlamydia infection were the age (p value 0.041), burning in urination (p value 0.036) and urethritis (p value 0.004) as shown in table3.

Statistical analysis showed significant differences between primary and secondary infertility groups.

Table 1. Demographic characteristics and common symptoms of study participants

Characteristics		Primary Infertility (n= 74) N (%)	Secondary Infertility (n= 88) N (%)
Age Group/yrs	0-20	4 (5.4)	0 (0)
	21-30	20 (27)	32 (36.4)
	31-40	40 (54.1)	24 (27.3)
	41-50	10 (13.5)	32 (35.4)
Occupation	Employees	36 (48.6)	32 (36.4)
	House Women	38 (51.4)	56 (63.6)
Residence	Wad Medani City	62 (83.8)	56 (63.6)
	Out Side Wad Medani City	12 (16.2)	32 (36.4)
Educational Level	High	26 (35.1)	16 (18.2)
	Medium	26 (35.1)	40 (45.5)
	Low	22 (29.7)	32 (36.4)
Socioeconomic Status	High	2 (2.7)	0 (0)
	Moderate	52 (70.3)	64 (72.7)
	Low	20 (27)	24 (27.3)
Common Symptoms	Lower abdominal pain	30 (31.9)	64 (68.1)
	Burning in urination	24 (27.3)	64 (72.7)
	Vaginal discharge	24 (27.3)	64 (68.1)
	Urethritis	26 (28.9)	64 (71.1)

Table 2. Frequencies and percentages of ELISA positive and negative samples case groups.

Test Cases		Primary infertility group N (%)	Secondary infertility Group N (%)	Total	P-value
ELISA (IgM)	Positive	36 (48.6)	72 (81.8)	108 (66.7)	0.00
	Negative	38 (51.4)	16 (18.2)	54 (33.3)	
Total		74 (100)	88 (100)	162 (100)	

Table 3. Risk factors associated with Chlamydia infection in study participants

Parameters		ELISA		OR (95% CI)	P-value
		Positive (n= 108)	Negative (n= 54)		
Age Group/ years	0-20	2 (1.9)	2 (3.7)	0.24 (0.03-1.90)	0.041
	21-30	42 (38.9)	10 (18.5)	3.27 (1.40-7.63)	
	31-40	36 (33.3)	28 (51.9)	0.65 (0.29-1.44)	
	41-50	28 (25.9)	14 (25.9)	0.64 (0.29-1.44)	
Lower Abdominal Pain	Yes	94 (87.0)	44 (81.5)	1.5 (0.63-3.70)	0.348
	No	14 (13.0)	10 (18.5)		
Burning in Urination	Yes	88 (81.5)	36 (66.7)	2.20 (1.04-4.64)	0.036
	No	20 (18.5)	18 (33.3)		
Vaginal Discharge	Yes	88 (81.5)	38 (70.4)	1.90 (0.87-3.96)	0.109
	No	20 (18.5)	16 (29.6)		
Urethritis	Yes	90 (83.3)	34 (63.0)	2.94 (1.39-6.22)	0.004
	No	18 (16.7)	20 (37.0)		

OR; odd ratio, CI; confidence interval

Discussion

Infertility is becoming an emerging health problem in many countries of the world (8), including Sudan. A WHO study reported the current chlamydia infection in infertile women 18-20 per cent is high (2). When compared with other published studies (2-4, 9), the prevalence of *C.trachomatis* infection in our study is also high. This alarming finding should alert doctors and point out the importance to include the screening of chlamydia infections in the diagnostic workup for infertility. Screening for *C.trachomatis* in high risk populations is recommended by the Center for Disease Control and Prevention (CDC) (4). Early detection and treatment can prevent serious long-term sequelae of *C.trachomatis* infection including infertility.

Although the increase of primary infertility cases than secondary infertility cases in last previous report by Osman in Sudan(5), at this study, the *C. trachomatis* infection was more common in women with secondary infertility than primary infertility with significant statistical differences between them. Women with secondary infertility were more likely to be screened positive for chlamydia has been reported earlier by others in Nigeria, India, china, Turkey and Sweden (2, 3, 10-13). This higher rate could be due to the presence of tubal factor as the most common etiology among them and also their longer period of active sexual life thus enhancing their exposure and also susceptibility to chlamydia infection.

There was a statistical significant correlation between age and chlamydia infection in women

included in this study. The age distributions of study participants with *C. trachomatis* infection revealed a high proportion of women were in the group older than 20 years of age. With the exception of Japan (14), India (15) and New Zealand (16), where the rates were higher in women younger than 20 years old, other reports from Denmark, Australia, Nigeria and Sweden is similar and in harmony with this study showed that increasing chlamydia infection rates were associated with increasing age (3, 16). Our result supports other previous studies in Sudan, a study conducted by Mohammed at 2012 and another study conducted by Omer at 2020 whose documented highest rates of chlamydia infection in older age (8, 17). The reasons of why old adults are more susceptible for genital chlamydia infection than young adult in Sudan may be associated with certain aspects of social factors including the later sexual intercourse experience with optimum age of marriage usually above 20 years old. In addition, this infection was mostly asymptomatic; women only came to this hospital usually after they had married and/or developed complications such as symptoms of lower genital tract infection, repeated pregnancy loss, and had infertility at later age; thus, present observation revealed an increasing rate of infection with age. Ninety percentages of women those with secondary infertility suffer from chlamydia symptoms more than those with primary infertility as they exhibit high rate of infection. The classical symptoms of chlamydia infection are dysuria (burning in urination), urethritis,

abnormal vaginal discharge and lower abdominal pain (13). Urethritis is a condition in which the urethra, or the tube that carries urine from the bladder to outside the body, becomes inflamed and irritated and usually accompanied with the burning sensation during urination, it typically causes pain while urinating and increased urge to urinate. Urethritis is generally defined as > 4 PMNL per high power field (HPF) (13). In the current study, in the context of the routine urine general analysis at this hospital, smears with > 10 PMNL per HPF were considered as urethritis(15), however, urethritis was observed in 83.3% (90/108) of positive cases with chlamydia suggesting a significant association between urethritis and chlamydia infection. Also this was in line with Falk findings in Sweden, when he recorded that the chlamydia infection is strongly statistically associated with urethritis, when he observed 71% of infected women with chlamydia suffering urethritis versus 40% of women free of chlamydia infection(13).

The vaginal discharge has no significant association with chlamydia infection among study women. This finding was inconsistent with most of the *C.trachomatis* related studies (2, 4, 10) and this is explained by that the vaginal discharge among study participants may be associated commonly with other conditions than chlamydia infection, as it may be associated with a normal physiologic occurrence or a pathological manifestation such as psychosocial factors or vaginal or cervical infections. It is often challenging to distinguish abnormal from

normal discharge, both for the patient's and the health care provider's perspective. (18). In the majority of settings here in Sudan, abnormal vaginal discharge is highly indicative of a vaginal infection, thus all women presenting with vaginal discharge receive treatment for *T.vaginalis*, *B.vaginosis* and *Candida spp.*

While the majority of women are asymptomatic, the lower abdominal pain considered as one of the classical symptoms of genital chlamydia infection and the leading cause to pelvic inflammatory disease(19). The latter is not easy to be diagnose, and the minimum criteria for diagnosis includes but not limited to the presence of the lower abdominal pain, thus, those women need better advice on when to seek early medical attention to avoid risk of reproductive damage. For this study, the lower abdominal pain was observed in 87% and 81% of infected women and non-infected women for chlamydia respectively. Several previous studies reported different rates of chlamydia among those present with lower abdominal pain (8, 20). Moreover, it may be associated with other infection such as *N.gonorrhoea* (20)

Prevalence of *C. trachomatis* varies considerable cross regions, subject population under study and the sensitivity of the laboratory method used. Our study suggests that all infertile women should be screened for *C. trachomatis* especially those suffering the secondary infertility. In the absence of requisite infrastructure and skills for culture, ELISA can play a significant role in screening for *C. trachomatis* high risk women. Studies with

larger sample size should further elucidate the extent of infertility caused by *C. trachomatis* in Sudan.

In conclusion, the chlamydia affects 66.7 % of infertile women, and the secondary infertile women suffer more than the primary infertile women in concerning the significant differences between them. Hence, we highly recommend active screening for *C.trachomatis* infection in women above 20 years of age irrespective of clinical presentation. Moreover, *C.trachomatis* infection should become one of the mandatory notifiable diseases in Wad Madani City.

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