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Seroprevalence of *Chlamydia trachomatis* Infection among Infertile Women in Khartoum State

الانتشار المصلي لبكتريا الكلاميديا تراكوماتس لدى النساء اللاتي يعانين من العقم في ولاية الخرطوم

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الآية

اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۚ الْمِصْبَاحُ فِي زُجَاجَةٍ ۚ الزُّجَاجَةُ
كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ
تَمْسَسْهُ نَارٌ ۚ نُورٌ عَلَى نُورٍ ۚ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۚ وَاللَّهُ بِكُلِّ
شَيْءٍ عَلِيمٌ

صدق الله العظيم

سورة النور ، رقم الآية (35)

DEDICATION

To my mother, father, brothers and friends

Acknowledgment

First and last great thanks the ALMIGHTY ALLAH; I would like to express my deep gratitude to my supervisor Prof. Humodi Ahmed Saeed Nasser for his priceless inspiration and encouragement.

I am grateful to my teachers and colleagues for their help and encouragement.

I would like to express my thanks to everyone who help me directly or indirectly in completing this study.

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Abstract

Chlamydia trachomatis is the common cause of bacterial sexually transmitted disease in the world. More than 85% of women with urogenital Chlamydial infections do not manifest obvious symptoms; the consequences of such untreated infections include pelvic inflammatory disease, infertility and ectopic pregnancy. This cross sectional study aimed to detect of Chlamydia IgG antibodies among infertile women in Khartoum State.

A total of ninety (90) infertile women with a history of primary and secondary infertility were enrolled in this study. 5 ml blood was collected from each woman. Serum was obtained by centrifugations at 3000 rpm for 5 min, Detection of *C. trachomatis* IgG antibodies were detected using Enzyme linked immunosorbant assay, and data analysis using spss.

The result revealed that out of 90 women investigated, only one (1/90) (1.1%) was positive for *C. trachomatis* IgG antibodies. the majority (89/90)(98.9%) were negative.

The study concluded that *C. trachomatis* infection is not common among infertile women in Khartoum state. Further studies are required with larger sample size to confirm this result.

مستخلص الدراسة

تعتبر بكتريا الكلاميديا تراكوماتس من البكتريات الشائعة المسببة للعدوى المنتقلة جنسيا. حوالي اكثر من 85% من النساء المصابات بهذه البكتريا لا تظهر عليهم اي اعراض. الاثار المترتبة على عدم معالجة هذه العدوى مرض التهاب الحوض ,والعقم والحمل خارج الرحم. هدفت هذه الدراسة للكشف عن وجود بكتريا الكلاميديا تراكوماتس في النساء اللاتي يعانين من العقم في ولايه الخرطوم. سجلت ما مجموعه تسعين (90) امرأه من النساء المصابات بالعقم مع تاريخ من العقم الابتدائي. والثانوي في هذه الدراسة. جمعت 5 مل من الدم من كل امرأة. وحصلت على المصل عن طريق الطرد المركزي عند 3000 دورة في الدقيقة لمدة 5 دقائق.كشفت عن الأجسام المضادة جي لبكتريا الكلاميديا تراكوماتس باستخدام فحص الانزيم المناعي المرتبط

كشفت النتيجة أنه من بين 90 امرأة خضعن للفحص ، كانت واحدة فقط (/901) (1.1 %) إيجابية للأجسام المضادة الايمونوجلوبولين جي لبكتريا الكلاميديا تراكوماتس. الغالبية (90/89) (98.9 %) كانت سلبية.

ونخلص إلى أن عدوى بكتريا الكلاميديا تراكوماتس ليست شائعة في ولاية الخرطوم ,يرجى عمل دراسات اخرى مستقبلية بحجم عينة اكبر للتحقق من نتائج هذه الدراسة.

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List of Abbreviations

ATP	Adenosine triphosphate
COPN	Chlamydia outer protein N
DFA	Direct fluorescent assay
EB	Elementary body
ELISA	Enzyme linked immunosorbent assay
LPs	Lipopolysaccharides
LVG	Lymphogranuloma venereum
MHC	Major histocompatibility complex
MIF	Microimmunofluorescence
MOMP	Major outer membrane protein
OMPs	Outer membrane protein
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
RB	Reticulate body
RFLP	Restriction fragment length polymorphism
STI	Sexually transmitted infection
TARP	Translocated actin recruiting phosphoprotein

CHAPTER ONE
INTRODUCTION

CHAPTER ONE

Introduction

1.1 introduction

C. trachomatis is one of the most prevalent sexually transmitted pathogens. This is because more of the sexual transmitted infection (STI) cases are caused by *C. trachomatis* than any other bacterial pathogen (Workowski and Bolan, 2015). *C. trachomatis* a coccoid bacillus closely related to Gram negative bacteria, presents a major public health concern both in industrialized and developing countries and is of high economic importance (Gomes, Bruno *et al.* 2007). Belongs to the family *Chlamydiaceae*, consisting of two genera; *Chlamydia* and *Chlamydiophila*. They in turn are divided into numerous species, of which three are responsible for human disease; *C. pneumoniae*, *C. psittaci* and *C. trachomatis*. The distinguishing characteristics between the three species concern the host range, clinical expression, and antibiotic susceptibility (due to folate biosynthesis), the staining characteristics (due to glycogen inclusions), inclusion morphology, shape of the elementary body, and limited DNA sequence homology (Rours, *et al.*, 2011) . *C. trachomatis* was the first Chlamydia species to be discovered and has been divided into subgroups based on antigenic variation in the major outer membrane proteins (MOMP) (serovars) and on clinical expression (biovars) (Rours *et al.*, 2011) .The serovars include A, B, C, D, E, F, G, H, I, J, K and L. Seventy percent of the non-lymphogranuloma venereum (LGV) STIs are due to serovars D, E and F, which are also responsible for neonatal disease. Serovars D through K deal with urogenital and neonatal chlamydial infections (Rours *et al.*, 2011)

In females, *C. trachomatis* causes cervicitis, urethritis, ectopic pregnancy, pelvic inflammatory disease (PID), tubal factor infertility and chronic pelvic pain (Morre *et al.*, 2000). Studies have also associated chlamydial infection with cervical and ovarian cancer as well as increase in HIV infectivity (Luostarinen *et al.*, 2004).

Antibiotics play a major role in treating chlamydial infection. Azithromycin and doxycycline are considered as first line drugs (Workowski and Berman, 2010). Though the efficacy of these drugs is high, many researchers have reported the problem of recurrent infections and treatment failures (Wang *et al.*, 2005). Recent studies have also indicated the emergence of antibiotic resistance in chlamydia which is feared to create severe problems in the treatment of the disease (Bhengraj *et al.*, 2012).

1.2 Justification

Data on the prevalence of Chlamydial infection in patients with tubal infertility are scarce especially in the developing countries due to unavailability and high cost of facilities necessary for diagnosing the infection (Tukur *et al.*, 2006). In the same vein, there is lack of sufficient surveillance data on the prevalence of chlamydial infection in ectopic pregnancy patients which has made it difficult to assess the contribution of the infection to the PID and ectopic pregnancy epidemics (Moss, 2001). The surveillance data and records on prevalence of chlamydial infection in khartoum state not enough so reliable epidemiological data is needed to determine the prevalence rate of the disease in the populations which will help in devising an effective Chlamydia infection control program.

1.3 OBJECTIVES

1.3.1 General objective

To investigate the prevalence of *Chlamydia trachomatis* among infertile women.

1.3.2 Specific objectives

1. To detect Chlamydia IgG among infertile women in Khartoum state.

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 *Chlamydia trachomatis*

2.1.1 Taxonomy and Classification

The chlamydia organisms belong to the family Chlamydiaceae in order Chlamydiales (Everett *et al.*, 1999). The genus *Chlamydia* was characterised by its unique developmental cycle and the two species were separated based on characteristics such as host range, accumulation of glycogen within the chlamydial inclusion and sensitivity to sulfadiazine (Beagley and Timms, 2000).

C. trachomatis and *C. pneumoniae* are the two chlamydial species pathogenic to humans, whereas the other species occur mainly in animals and birds (Schachter *et al.*, 2001). According to the recent review of the Bergey's Manual by Garrity *et al.* (2004) and Krieg *et al.* (2010), the family Chlamydiaceae contained two genera *Chlamydia* and *Chlamydophila*. The genus *Chlamydia* has six species namely; *Chlamydia trachomatis*, *C. muridarum*, *C. pecorum*, *C. pneumoniae*, *C. suis* and *Chlamydia psittaci*. *C. trachomatis* was initially classified into 15 different serovars. These serovars include A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2, and L3.

2.1.2 Reproductive Cycle

Key for understanding the pathophysiology of chlamydial disease is an appreciation of the chlamydial developmental cycle (Fredlund *et al.*, 2004). Members of the genus *Chlamydia* are obligate intracellular energy parasites of eukaryotic cells, which supply them with ATP, GTP and UTP. Chlamydiae do encode functional glucose-catabolizing enzymes, which can be used for generation of ATP (Hammerschlag, 2002). In addition, proteomic analysis of *C. trachomatis* showed that all of the glycolytic enzymes were readily detectable. However, chlamydiae with mammalian hosts appear to have lost the genes for the F1 ATPase (ATPase that uses energy released by transport of protons across the bacterial cell membrane to synthesise ATP) during evolution. It is therefore likely that these ATPase components are not involved in energy generation (Skipp *et al.*, 2005). Although chlamydiae are classified as bacteria, they have a unique biphasic developmental cycle (Figure 2), involving an extracellular form, the elementary body (EB), which is infectious but metabolically inactive and an intracellular form, the reticulate body (RB) which is non infectious but metabolically active (Mardh, 2005). The EBs multiply by binary fission in a cytoplasmic vacuole (termed the inclusion) surrounded by a membrane with similarities to the host cell's cytoplasmic membrane (Cevenini *et al.*, 2002). The EB adheres to the host cell with the aid of heparin-sulphate-like glucosaminoglycan molecules which are required for chlamydiae to enter a host cell. A number of substances are known to enhance contact between EBs and host cells, i.e., diethylaminoethyl dextran and idoxyuridine, which have been utilized in in-vitro cultures. After eclipse phase of approximately

12 hours, the EB differentiates into the larger reticulate body (RB) which replicates inside the vesicle 200–500-fold by binary fission. The EB has a diameter of 200-300 nm, whilst that of the RB is 1000-1500 nm. During the synthesis of substances by the chlamydiae cells, the metabolism of the host cell is depressed through influence of the chlamydiae parasites. Eighteen to 24 hours after the EB has attached, the RBs undergo reorganization into EBs. Within another 6 hours, the EBs is released and the host cell dies. In vivo, the developmental cycle is longer than in in vitro cultures. Its exact time may differ by prevailing circumstances but may be up to a week (Beagley and Timms, 2000; Mardh, 2005).

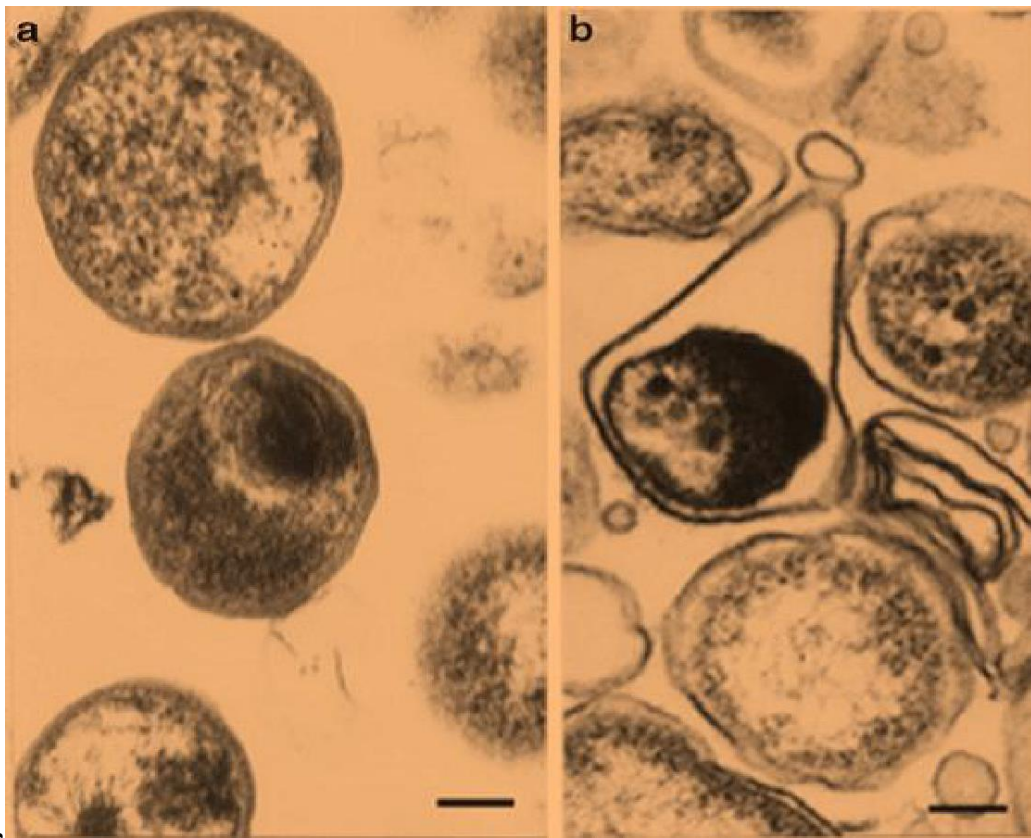


Figure 1: Elementary Body Form of Chlamydiae

The elementary body form of chlamydiae is round, except for *Chlamydia pneumoniae* which is pear-shaped. (a) *Chlamydia trachomatis* serovar B, strain TW-5, (b). *Chlamydia pneumoniae* strain TW-183. The reticulate body is round for all species. Bars = 0.1 mm. (Source: Grayston *et al.*, 1989

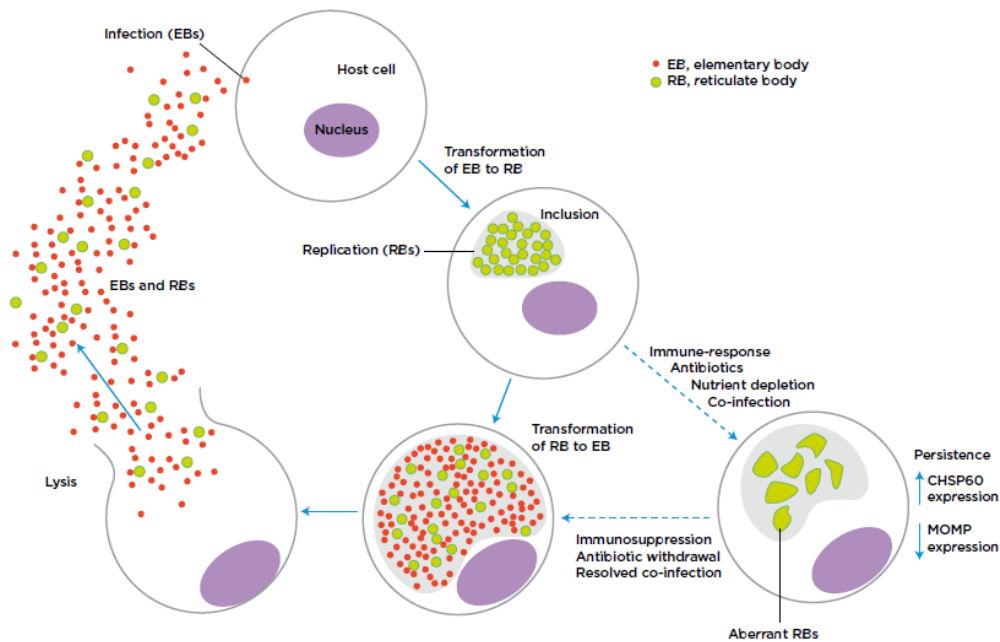


Figure 2: The Intracellular Developmental Cycle of Chlamydiae

At the end of the reproductive phase, the chlamydial inclusion may be so large that it displaces the nucleus of the host cell. This phenomenon may give the impression that the inclusion more or less surrounds the nucleus, like an overcoat. This once gave the organisms their name —chlamydia, which in Greek means —overcoat (Mardh, 2005). Deviations from the typical developmental cycle have been experimentally induced by a variety of stimuli, including IFN- γ , antibiotics, and nutrient deprivation. These stimuli, particularly IFN- γ can alter chlamydial growth and facilitate persistent or chronic infection (Fredlund *et al.*, 2004)

2.1.3 Clinical Spectrum

Typically, *C. trachomatis* serovars A through C of biovar trachoma infect the conjunctival epithelium and lead to ocular infections that can progress to trachoma, the leading cause of preventable blindness. In developing countries, these serovars remain endemic. It is estimated that there are about 162 million infected people worldwide, and 6 million of them are blind (Mabey and Fraser-Hurt, 2001; Mabey *et al.*, 2003). Serovars D–K, Da, and Ia infect the genital epithelium and cause urogenital tract infections. These serovars remain the major causes of STDs in developed as well as in developing countries, with about 92 million new infections each year (Bjartling *et al.*, 2000; Toth *et al.*, 2000). The LGV biovar has four invasive serovars (L1, L2, L2a and L3) that are able to infect not only the genital epithelium, but also monocytes and lead to a systemic disease known as Lymphogranuloma Venerum (LGV) (Mabey and Peeling, 2002). The different serovars display well-documented and unique tissue tropisms. The trachoma serovars (A–C) are rarely isolated from the genital tract with the exception of serovar B variants, which have been associated with a very low incidence of urogenital disease. In contrast, genital serovars D–K are not associated with blinding trachoma; however, they can cause ocular infection when newborn infants acquire the organism during passage through the infected birth canal or when adults accidentally inoculate the eye with infected genital secretions (Caldwell *et al.*, 2003). Despite this diversity in tissue tropism and disease manifestations, complete genomic sequencing of several Chlamydiaceae has shown that the gene order and content among the different species is remarkably conserved, with the exception of a region termed the plasticity zone (PZ). This observation led to the suggestion that

host organ and cellular tropism may be attributed to the few genes localized in the PZ (Caldwell *et al.*, 2003).

2.1.4 Genital chlamydial infection:

Genital chlamydial infection is the most common among STIs worldwide (Kučinskienė *et al.*, 2006). In the US in 2006, more than one million cases of chlamydial infection were reported to the Centers for Disease Control and prevention (CDC), corresponding to a rate of 347.8 cases/100000, with an increase of 5.6% as compared with the rate in 2005 (Be'be'ar and de Barbeyrac, 2009). Most of these infections are asymptomatic and, if not treated, can lead to severe complications, mainly in young women. Risk factors for infection include young age, high frequency of partner change, multiple partners, unprotected sex and being unmarried (Manavi, 2006). The majority of researchers think that higher prevalence of chlamydial infection among young-aged female adolescents may be due to insufficiently developed cervix, which is especially susceptible to STIs (Sedlecki *et al.*, 2001). *Chlmydia trachomatis* is a cause of cervicitis, non gonococcal urethritis (NGU) and pelvic inflammatory disease (PID) in women and NGU, epididymitis and proctitis in men. Infection of the urethra and lower genital tract may cause dysuria, whitish or clear urethral or mucopurulent vaginal discharge and post-coital bleeding. The bulk of infections is asymptomatic and therefore remains undetected (CDC, 2005). If untreated, ascending infection may result. In women, ascending infection can cause endometritis, salpingitis, PID and perihepatitis. This happens in up to 40 % of women with untreated chlamydial infection. Manifestations of upper genital tract infection in women are irregular uterine bleeding, pelvic discomfort or abdominal pain; however some infections may still remain silent (Molano *et al.*, 2003). Women infected with chlamydiae are up to 5

times more likely to become infected with HIV, if exposed (Joyee *et al.*, 2005). In pregnant women, there is little evidence and this is conflicting, to implicate *C. trachomatis* in chorioamnionitis and adverse pregnancy outcome. Babies who are born to infected mothers can get chlamydial infection during passage in the infected birth canal with the risk of developing pneumonia and conjunctivitis (Be´be´ar and de Barbeyrac, 2009). Complications among men are rare. Infection sometimes spreads to the epididymis, causing pain, fever, and rarely, sterility (CDC, 2005).

Serious sequelae (blindness, tubal infertility, ectopic pregnancy, etc.) due to chlamydial diseases are observed only if they remain chronic (Mpiga and Ravaoarinoro, 2006). Re-exposure or persistent infection is thought to drive an immunopathological inflammatory response resulting in tissue fibrosis and scarring that characterize all chlamydial diseases (Caldwell *et al.*, 2003).

2.1.5 Immune Response

Chlamydia trachomatis is a strong immunogen, which stimulates both humoral and cell mediated immune responses. In addition to the immunogenic antigens, the outcome of chlamydial infection depends on interaction and balance of cytokines secreted by the activated lymphocytes. Interferon gamma (IFN- γ) has been described as a single most important factor in host defense against *Chlamydia*, while disease susceptibility has been linked with enhanced expression of Interleukin- 10 (IL-10) (Rank *et al.*, 1992). Immune system changes or disturbances induced by *C. trachomatis* may favour its own survival in the infected host, and induce persistent infections (Malhotra *et al.*, 2013). Various studies have disclosed that the CD4+Th1 response is absolutely required to resolve primary infection, even when CD4+Th2 response is ineffective (Mpiga and Ravaoarinoro, 2006). The antichlamydial action of Th1 effectors is mediated

principally via cytokines, specially the chlamydistatic IFN- γ . The antimicrobial mechanisms of these cytokines include depletion of intracellular tryptophan by activation of indoleamine 2,3-dioxygenase (IDO), induction of elevated nitric oxide (NO) through inducible NO synthase, deprivation of iron, via down-regulation of transferrin receptors and possibly the stimulation of phagolysosomal fusion or disruption of selective vesicular nutrient transport (Hefty *et al.*, 2007).

Cytokines play an important role in induced immune response polarization. IFN- γ and IL-12 favour Th1 responses, but inhibit Th2 responses. In contrast, IL-4 and IL-10 stimulate Th2 responses, but suppress Th1 responses. For this reason, initial steps during infection are crucial in the induction of an appropriate response. Several authors have noticed that IFN- γ and IL-12 required polarizing the immune response towards the Th1 profile could come from natural killer cells and dendritic cells, respectively (Tseng and Rank, 1998; Matyszak *et al.*, 2002). However, the early source of IL-4 and IL-10 allowing the induction of Th2 responses is not known (Mpiga and Ravaoarinoro, 2006).

2.2 Diagnosis of Genital Chlamydial Infections

2.2.1 Clinical Diagnosis

Clinical picture of the patients suffering from chlamydial infection could be misleading as up to 70-80 per cent of the infected women and 50 per cent of the infected men are asymptomatic. Typically, a female with uncomplicated chlamydial infection will present with odourless, mucoid vaginal discharge without pruritis. Dysuria without frequency or urgency will be complained of if urethra is involved. Further, in PID, history of severe abdominal pain with high fever, dyspareunia, prolonged menstrual cycles and intermenstrual

bleeding can be elicited. On examination, cervicitis with a yellow, cloudy, mucoid discharge can be seen. The cervix tends to bleed easily when scraped with spatula or brush. Urinalysis will reveal the presence of >5 WBC/HPF (high power field), which is suggestive of urethritis (CDC, 2010).

2.2.3 Laboratory Diagnosis

a. Specimens

The type of specimen depends on the clinical picture, the diagnosis conditions and the laboratory technique used for detection, with the conditions of transport and storage being adapted to the particular technique (Essig, 2007). Invasive specimens include urethral swabs in men, and endocervical or urethral swabs and specimens taken from the upper genital tract, in women (liquid from Douglas's pouch, endometrium and tubal specimens) (Be´be´ar and de Barbeyrac, 2009). Non-invasive self-collected specimens include first-void urine (FVU), vulvovaginal swabs, anal swabs and penile swabs (Forbes *et al.*, 2007)

b. Microscopy

In earlier days, Giemsa was used to stain EBs, which are eosinophilic in contrast to the basophilic RBs. Also dark-field microscopy can be used to detect chlamydiae organisms where EBs appears as yellow bodies due to their natural auto fluorescence. However, this method is insensitive compared to culture or other methods of diagnosis (Singh *et al.*, 2002; Forbes *et al.*, 2007)

c. Culture

The gold standard for diagnosing chlamydial infection is culture performed as described by Mardh *et al.* (1977). Chlamydial infection diagnosed by 2 non-culture tests, is now known as the expanded gold standard (Stary *et al.*, 1996; Watson *et al.*, 2002). The most widely applied culture method is application of cycloheximide-treated McCoy cells (Ripa and Mardh, 1977). The use of both urethraly and cervical cultures has been recommended for detection of genital chlamydial infection, increasing the positive rate by approximately 5%. The current sampling recommendations are to obtain the specimen for *C. trachomatis* culture after all other specimens (i.e. those for Gram-stained smear, *N. gonorrhoeae* culture or Pap smear) (Mardh, 2005; Forbes *et al.*, 2007). Use of urine for culture is inadequate, as the positive rate is only 30% as compared to cervical swabs from the same individuals (Mardh, 2005). Several types of media can be used to transport specimens and include 2-sucrose phosphate, sucrose– glutamate phosphate, or other commercial media (Hammerschlag, 2003). Specimens should be refrigerated upon receipt, and if they cannot be processed for culture within 24 hours, they should be frozen at -70°C (Forbes *et al.*, 2007). Other drawbacks of chlamydial culture are that it requires a cold chain during transport and that results are not available for 3 to 7 days. For these reasons, culture should not be used routinely to screen patients for *C. trachomatis*.

d. Non-amplification, Non-culture Tests

The first generation of nonculture tests to diagnose chlamydial infections includes direct fluorescent antibody staining (DFA), ELISA, and DNA probe tests. These tests use direct visualization techniques to detect *C. trachomatis*. Although these tests largely have been replaced by NAATs, they are still used in some clinical settings (Olshen and Shrier, 2005).

e. Serological Diagnosis

Serology is useful only in some cases of *C. trachomatis* infection and in sero-epidemiological studies. It suffers from several drawbacks, including the serological cross-reactivity between *C. trachomatis* and *C. pneumoniae* species and the persistence of antibodies, which prevents a distinction being made between past and present infection. Although it is not recommended for the diagnosis of lower genital tract infections, or for screening in asymptomatic patients, serological testing may be useful for diagnosing LGV, neonatal pneumonia and upper genital tract infections, and for the evaluation of tubal-factor of infertility (Persson, 2002; Be'be'ar and de Barbeyrac, 2009). The serological methods available are complement fixation, MIF and ELISA. The latter two allow the distinction among IgG, IgA and IgM. The MIF method, which is species and serovar-specific and which is considered to be the reference method, is laborious, and reading of the assay is subjective and therefore it is not suitable for a daily routine. ELISAs provide objective reading and allow the handling of more samples at the same time (Bax *et al.*, 2003).

f. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA tests were widely used to diagnose genital chlamydial infections. Such tests are still used in economically less developed countries, mainly because of the price being lower than that for DNA-based tests. ELISA uses an enzyme-linked monoclonal or polyclonal antibody directed at the *C. trachomatis* LPS which is more soluble than MOMP. These tests are not species-specific for *C. trachomatis* and may cross-react with *Staphylococcus aureus*, or LPS of other Gram-negative bacterial species e.g., *E. coli*. This problem is accentuated when rectal; urine and pharyngeal samples are to be tested. Because of the low specificity, it has been recommended to use

confirmatory tests in case of a positive ELISA (Essig, 2007; Forbes *et al.*, 2007). ELISA tests can be automated. They are more reproducible than DFA, and the sensitivity of the best ELISA is comparable to that of culture and lower than that of NAATs (Be'be'ar and de Barbeyrac, 2009).

2.3 Chlamydai and some condition

2.3.1 Chlamydia and Pregnancy

The prevalence of *C. trachomatis* infection in pregnant women ranges from 2-35 per cent (Black, 1997). Pregnant women with chlamydial infection are at increased risk for adverse outcomes of pregnancy and post-partum PID. Sequelae like still birth, low birth weight, neonatal death, decrease gestational periods, preterm delivery and premature rupture of membranes (PROM) have been reported (Ward, 1999). Nine per cent of the women with chlamydial infection who develop PID have tubal pregnancy (Johnson *et al.*, 2002). Early pregnancy loss or recurrent pregnancy loss may be induced by asymptomatic chlamydial infection through the operation of immune mechanism.

2.3.2 Chlamydia and Pelvic Inflammatory Disease

Pelvic inflammatory disease is a general term that refers to infection and inflammation of the upper genital tract in women. It can affect the uterus (womb), fallopian tubes, ovaries and other organs related to reproduction (Moss, 2001). Twenty per cent of the women with chlamydial lower genital tract infection will develop PID (Price *et al.*, 2013) and 4 per cent will develop chronic pelvic pain (Paavonen and Eggert-Kruse, 1999). The clinical spectrum of chlamydial PID ranges from subclinical endometritis to frank salpingitis, tubo-ovarian masses, pelvic peritonitis, periappendicitis

and perihepatitis. However, symptomatic chlamydial infections represent only the tip of the iceberg of all chlamydial infections as majority of genital chlamydial infections are asymptomatic.

2.3.3 Chlamydia and Infertility

Tubal infertility occurs when the infection spreads from the cervix, its point of initiation to the fallopian tubes keeping the eggs from being fertilized (Gerbese *et al.*, 1998). A major cause of infertility in sub-Saharan Africa is Pelvic Inflammatory disease (PID) (Bello, 2004). In PID cases due to *Chlamydia trachomatis*, high level of persistent circulating IgG antibody is produced (Joyner *et al.*, 2002). *Chlamydia trachomatis* antibody testing could provide a clinically useful screening test for predicting or confirming tubal factor infertility in women and is therefore a desirable way to avoid laparoscopy (Thomas *et al.*, 2000; Akande *et al.*, 2003).

Chlamydial PID is the single most important preventable cause of infertility. Approximately, 3 per cent of women with chlamydial genital tract infection develop infertility. After a single episode of PID, the risk of tubal factor infertility is approximately 10 per cent, each repeat episode doubles the risk (Ray, 2006). Although the majority of patients are asymptomatic but re-infection/persistent infection with *C. trachomatis* leads to more severe tubal damage than other agents. The role of *C. trachomatis* in the development of urethritis, epididymitis and orchitis in men is widely accepted. Though the role of this organism in prostatitis is controversial, but up to 35- 50 per cent incidence has been reported in patients with prostatitis (Cunningham and Beagly, 2008).

2.4.4 Chlamydia and Ectopic Pregnancy

Ectopic pregnancy is the condition which results when chlamydial infection ascends to the fallopian tubes causing scarring of the tubes which may interfere with the passage of fertilized egg to uterus and when this happens, the egg may attach itself to the fallopian tube and start to develop there. Ectopic pregnancy is an important cause of maternal deaths in Nigeria and in other developing countries. In Lagos, Nigeria, it was responsible for 8.6% of maternal deaths, and had a case fatality rate of 3.7%. The incidence was 23.1/1000 (1:43) deliveries and was responsible for 48.5% of gynecologic emergencies (Anorlu *et al.*, 2005). Studies have also shown a strong correlation between ectopic pregnancy rates and chlamydial infection (Johnson *et al.*, 1994).

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

3.1.1 Type of study

This was a descriptive cross-sectional study.

3.2 Study setting

The study was carried out in Almoalim Medical City Khartoum, Sudan

3.3 Study period

The study was carried out during the period from March to September 2017.

3.4 Study population

Infertile women attending to gynecologic clinic in Almoalim Medical City.

3.5 Ethical consideration

Each individual invited to participate in the study was verbally informed about the purpose of study before data collection. The study was approved by College of Medical Laboratory Science, Sudan University of Science and Technology.

3.6 Sample size

A total of 90 women whom agreed to participate were enrolled in this study

3.7 Data collection

Data were collected using questionnaire covered general information which include Age and education, residence, sign and symptoms (Appendix 1).

3.8 Collection of blood specimens

Under sterile condition, 5ml of venous blood was withdrawn from each one using vacutainer tubes. Serum was separated by centrifugation at 5000rpm for five minutes. The Serum was store at -20°C until used.

3.9 Detection of *Chlamydia Trachomatis*

Sera were tested for the presence of *Chlamydia Trachomatis* using Enzyme Linked Immuno-Sorbent Assay ELISA (Euroimmun) (Anti *Chlamydia Trachomatis* IgG) (appendix 2)

The procedure was done according to the instructions of the manufacturer.

3.10 Principle of the test

The test kit contain microtiter strips each with 8 break-off reagent wells coated with purified *Chlamydia Trachomatis* antigens MOMP (major outer membrane protein) which is a trans-membrane protein and major part of the outer membrane of the elementary bodies . Protein purification start with BGM cells infected with *Chlamydia Trachomatis* of serotype K . In the first reaction step diluted patient samples are incubated in the wells. In case of positive sample specific IgG antibodies well bind to the antigens .to detect the bound antibodies a second incubation is carried-out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

3.11 Procedure

The reagent and sera were allowed to reach room temperature. Human serum are diluted 1:101 in sample buffer transfer 100 µL of the calibrators positive negative controls and diluted patient samples in to the individual microplate wells according to the pipetting protocol. Incubate 30 minutes at room temperture. Empty the wells and subsequently wash 3 times using 300

μL of working strength wash buffer for each wash. Leave the wash buffer in each well for 30 to 60 seconds per washing cycle then empty the wells. After washing thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer. Pipette 100 μL of enzyme conjugate into each of the micoplate wells. Incubate for 30 minutes at room temperature. Empty the wells and wash as described above. Pipette 10 μL of chromo gens substrate solution into each of the micro plate wells incubate for 15 minutes at room temperature protected from the sun light. Pipette 100 μL of stop solution in to each of the microplate wells in the same order and at the same speed as the chromogen substrate solution was introduced. Photometric measurement of the colour intensity should be made at wave lenegth of 450 nm and reference wavelenegth between 620nm and 650nm within minutes of adding the stop solution.

3.12 Dada analysis

Processing and analysis of data were carried out by means of the statistical package for the social sciences (SPSS). A descriptive statistic frequency was used to assess the risk; Cross tabulation (chi-squire) was used to compare the variable with positive result.

3.13 Quality control

Reagents standard were checked for storage stability and preparation before starting work.

CHAPTER FOUR
RESULTS

CHAPTER FOUR

RESULTS

4.1 Results

Total of 90 infertile women were participated in this study. The participants were divided to four age groups (Table4.1). Based on education levels the participants divided to four major groups (Table 4.2). All woman were participated in this study were already diagnosed as infertile. (16)(17.8%) of the recruits had abortion during life (Table4.3). In all infertile woman the results of *Chlamydia trachomatis* IgG Ab revealed that (1) (1.1%) were positive and (89) (98.9%) were negative (Table4.4). This study show no association between presence of *Chlamydia trachomatis* IgG Ab and abortion (p -value = 0.640) (Table 4.5).

Table (4.1) Distributions of participants (n=90) according to the age group

Age groups	Frequency	Percent
<20	7	7.8
21-30	50	55.6
31-40	23	25.6
>40	10	11.1
Total	90	100.0

Table (4.2) Distributions of participants (n=90) according to the education level

Education level	Frequency	Percent
Primary	10	11.1
Secondary	14	15.6
University	50	55.6
post graduate	16	17.8
Total	90	100.0

Table (4.3) Distributions of participants (n= 90) according to the abortion

Abortion	Frequency	Percent
Yes	16	17.8
No	74	82.2
Total	90	100.0

Table (4.4) Distributions of participants (n=90) according to the results of *Chlamydia trachomatis* IgG

Result	Frequency	Valid Percent
positive	1	1.1
negative	89	98.9
Total	90	100.0

Table (4.5) Results among aborted woman (n=90)

Abortion	RESULT		Total
	positive	negative	
Yes	0	16	16
No	1	73	74
Total	1	89	90

CHAPTER FIVE
DISCUSSION

CHAPTER FIVE

Discussion

5.1 Discussion

Chlamydia trachomatis is one of the most prevalent sexually transmitted pathogens. This is because more of the STI cases are caused by *C. trachomatis* than any other bacterial pathogen (Workowski and Bolan 2015). In females, *C. trachomatis* causes cervicitis, urethritis, ectopic pregnancy, pelvic inflammatory disease (PID), tubal factor infertility and chronic pelvic pain (Morre, Rozendaal *et al.* 2000). Studies have also associated chlamydial infection with cervical and ovarian cancer as well as increase in HIV infectivity (Luostarinen, Lehtinen *et al.* 2004).

This study conducted to determine the presence of *C. trachomatis* IgG among infertile women at Khartoum state. Out of 90 serum sample 1.1% was positive IgG to *C. trachomatis*.

This result is similar to those reported in algezira state the result was (0%) (Albashir 2016) as well as in Nigeria the result was 0.7% (Adesiji, Iyere *et al.* 2015) . other studies gave close result in United Arab Emirates their study showed a prevalence of chlamydial infection was (2.6%)(Ghazal-Aswad *et al.* 2004).

other study among infertile women in albasra the result was (5.5%)(Abdulrahman, Jassim *et al.* 2016) The difference in prevalence of Chlamydia Trachomatis of all this studies and our finding may be due to different method adopted that is seemed much less sensitive and the number of participant and type of samples were used.

5.2 Conclusion

It was concluded from the current study that:

The overall prevalence of *Chlamydia trachomatis* among women at Khartoum State was very low

5.3 Recommendations

1. There is need for more public awareness by Researchers and Clinicians of STIs including chlamydia due to its asymptomatic nature and therefore the need for its integration into routine health system for sexually active males and females to get tested.
2. More innovative ways must be found to test sexually active young people, since the disease remain subclinical. For example, integrating it in the form of routine checkups as a general hospital routine.
3. Clinicians should be encouraged to use NAATs as a diagnostic tool since they can be used without using invasive methods (urine) in acquiring samples.
4. In the absence of laboratory testing, syndromic approach (burning sensation during urination, pain and urethral discharge) could be used for diagnosis.
5. Increase the sample size in further research and use more specific investigations (PCR).

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APPENDICES

Appendices

Questionnaire

Sero prevalence of Chlamydia trachomatis infection among infertile woman in Khartoum State:

1. Number:.....

2. Age groups:

Less than 20 (), 21-30 (), 31-40 (), More than 40 ()

3. Place of residence?

.....

4. Education level?

Primary () Secondary () University () Post graduate ()

5. Did you have Any abortion? Yes () No ()

If YES how many times?

○Laboratory test results: Detection of (IgG) by ELISA for:

Chlamydia trachomatis (IgG): Positive () Negative ().