بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of graduate studies

Seroprevalence of *Chlamydia trachomatis* Infection among Sudanese Women Attending Infertility Clinics in Khartoum

الكشف المصلي عن وجود بكتيريا المتدثرة الحثرية في النساء المترددات على عيادة العقم في الخرطوم

"A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. in Medical Laboratories Sciences (Microbiology)

By:

Mrwa Sidahmed Mahmoud Sidahmed

B.Sc., Medical Laboratory Sciences

Sudan University of Science and Technology, 2011

(Microbiology)

Supervisor:

Dr. Elfadil Mustafa Abass

B.Sc., M.Sc. & Ph.D. Medical Microbiology

2016

الآية

(فَبَدَأَ بِأَوْعِيَتِهِمْ قَبْلَ وِعَاءِ أَخِيهِ ثُمَّ اسْتَخْرَجَهَا مِن وِعَاءِ أَخِيهِ ۚ كَذَٰلِكَ كِدْنَا لِيُوسُفَ ۖ مَا كَانَ لِيَأْخُذَ أَخَاهُ فِي دِينِ الْمَلِكِ إِلَّا أَن يَشَاءَ اللَّهُ ۚ نَرْفَعُ دَرَجَاتٍ مَّن نَّشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ (76)

سورة يوسف الآية 76

Dedication

To every woman who had a dream of having a baby,

I dedicate this humble effort to you, wishing that it lights the sky of science and reflects its light over you.

Acknowledgment

My great thanks to Almighty Allah who facilitates this work and gave me the power to finish it.

My dear parents who supported me in all steps of my life.

My man, who stood with me in my good and bad times.

My amazing family that I love.

A lot of thanks for my supervisor Dr.Elfadil Abass for his efforts and time and kindness.

More thanks for everyone who helped me even by a smile and gave me a motive to move on.

Abstract

The aim of the study was to determine seroprevalence of *Chlamydia trachomatis* infection among infertile women in three clinics for infertility in Khartoum during the period from September 2015 to December 2015. The study population age range was 18 – 47 years with mean 32.86 years.

Serum samples were obtained and preserved at -20˚C and were tested by ELISA for detection of anti-Chlamydia trachomatis IgG. Specimens included 60 sera of infertile women and 30 sera of pregnant women served as controls. Among the infertile women group, 10 (16.67%) showed positive IgG ELISA for *C. trachomatis*; while none of the fertile group showed positive results.

C. trachomatis infection should be suspected as a cause for infertility. However, infertile women should be considered for possibility of being infected by other sexually transmitted infections.

ملخص البحث

كان الهدف من هذه الدراسة هو تقييم بكتيريا المتدثرة الحثرية في النساء العقيمات في عيادات الخصوبة والانجاب في الخرطوم. أجريت هذه الدراسة في الفترة من سبتمبر 2015 حتى ديسيمبر 2015.

شملت الدراسة الفئة العمرية (18- 47) مع متوسط عمر 32.68.

تم جمع العينات المصلية وحفظها في درجة حرارة 20- درجة مئوية ثم فحصت للكشف عن الانتشار المصلي لمستضد المتدثرة الحثرية بواسطة تقنية الاليزا في مصل 60 من الاشخاص تحت الدراسة و 30 من الأشخاص لضبط الداراسة. اظهرت النتائج ان هنالك عشرة اشخاص بنسبة 16.67% لديهم نتائج ايجابية لبكتيريا المتدثرة الحثرية في وسط الاشخاص تحت الدراسة، ولم يكن هنالك أي شخص لديه نتائ ايجابية في وسط الاشخاص لضبط الدراسة.

لذلك يجب توقع بكتيريا المتدثرة الحثرية كواحدة من أسباب العقم لدى النساء.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER TWO

LITERATURE REVIEW

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

Results

CHAPTER FIVE

List of abbreviations

PID	Pelvic Inflammatory Disease
TFI	Tubal factor Infertility
GU	Gonococcal Urethritis
NGU	Non Gonococcal Urethritis
LGV	Lympho Granuloma Venereum

Chapter one

Introduction

1. Introduction

1.1. Introduction

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 (Nada *et al*., 2015).

Chlamydia trachomatis is a ubiquitous pathogen worldwide and causes ocular, urogenital, and respiratory infections in humans (Qayum and Bin-Saleem, 2013). It is the most prevalent bacterial sexually transmitted infections (STD) recognized throughout the world (Paavonen and Kruse, 1999). C. trachomatis infection is a widespread public health concern because of its prevalence and potentially devastating reproductive consequences, including pelvic inflammatory disease (PID), infertility, and ectopic pregnancy (Darville and Hiltke, 2010). *C. trachomatis* infection have been reported to cause silent infections (asymptomatic) in communities which becomes endemic and could remain unnoticed for very long time. The sharp worldwide increase in the incidence of PID during the last two to three decades has led to the secondary epidemics of primary and secondary tubal factor infertility (Malik *et al., 2015)*.

The WHO estimates that annually almost 100 million new cases occur worldwide (WHO, 2006), but the majority of women with lower genital tract infections remains asymptomatic and therefore undiagnosed (Land *et al*., 2010). More than two-thirds of these cases occur in the developing world where diagnostic and treatment services are scanty. Asymptomatic in nearly 80.0% of women and 40.0% of men, and untreated genital infections have serious ramifications for reproductive health of women as it may evolve into complications such as ectopic pregnancy, pelvic inflammatory disease, salpingitis with tubal scarring and infertility in

women. Undiagnosed and untreated chlamydial infections are thus not only creating major health problems and consequences for individuals but also result in major epidemiological, social and economical problems. It is also recently hypothesized that chronic infections with for example *C. trachomatis* might promote ovarian tumor development (Idahl, 2009). *C. trachomatis* whether symptomatic or asymptomatic can spread to upper genital tract (Keltz *et al*., 2013). More than 900.000 infections with *C. trachomatis* were reported and twice as many were unreported in the United States during 2004. Vaginal discharges, dysuria, postcoital bleeding, inter menstrual bleeding and abdominal pains are some of the symptoms that are associated with genital infection with *C. trachomatis* in women (Rashidi *et al*, 2013). Chlamydial infection produces less severe symptoms than other sexually transmitted diseases. These deceptively mild symptoms allow the infection to go unnoticed with minimal patient awareness until secondary or tertiary symptoms develop. The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease lead not only to significant morbidity but far more importantly to infertility. The association between *C. trachomatis* infection and infertility has been the subject of several researches. A significant association between *C. trachomatis* infection and female infertility has been suggested in many studies conducted in different countries. In addition, other studies suggested an association between positive serology screening result for *C. trachomatis* and prediction for both tubal damage, and a reduced pregnancy rate. A prevalence rate of 9.6% was found in female patients with infertility in Nigeria. *C.trachomatis* infection was detected in other countries including USA (5-15%), UK (16%), Jordan (3.9%), Iran (22%), and Brazil (10.9%) (Nada *et al*., 2015).

1.2. Rationale

Chlamydia trachomatis is most common bacterial sexually transmitted infections (STIs) throughout the world (Muvunyi et al., 2011). It is estimated that over 90 million cases occur annually worldwide which reflects its public health importance. More than two-thirds of these cases occur in the developing world where diagnostic and treatment facilities are almost absent. The developing countries have a high incidence of chlamydial infection, however, with the exception of sporadic testing; screening for *Chlamydia* is rare (Qayum and bin-Saleem, 2013). In most parts of Sudan these organisms are not screened for and relative information about frequencies of the organisms is sparse (Mohammed and Omar, 2012). *C.trachomatis* of the lower genital tract is predominantly asymptomatic in men and women. Between one half and two thirds of such infections in women remain undetected and hence untreated, resulting in serious longterm sequelae, such as ectopic pregnancy and tubal infertility. Consequently, screening is necessary to identify and treat this infection to help reduce duration of infectivity, transmissibility and long term sequelae (Abdella *et al.*, 2015). Infertility due to *C. trachomatis* represents a preventable type of infertility, if detected early (Malik *et al., 2015)*. Especially in poor countries in Africa, infertility is considered as an enormous social problem because siblings are still important to retain a families' economic standard in great parts of the population (Siemer *et al*., 2008).

Because of increase of infertility and lack of studies in causative agent in Sudan that determine infertility to *C.trachomatis* infection, this study was carried out to show sero prevalence of *C.trachomatis* among Sudanese infertile women.

1.3. Objectives

1.3.1. General objective

To determine seroprevalence of *C. trachomatis* infection among infertile women who attending outpatient clinics of infertility.

1.3.2. Specific objectives

- To detect *Chlamydia trachomatis* IgG levels in infertile and fertile pregnant women.

- To detect risk factors associated with *C.trachomatis* infection in infertile women.

Chapter Two

Literature Review

Chapter two

2. Literature review

2.1. History

The effects of infection with *C. trachomatis* were first described in ancient Chinese writing in the Ebers papyrus (1500 B.C.) as trachoma of the eye. The name “trachoma” was first introduced in A.D. 60 and referred to the “roughness of the conjunctiva” that characterizes the ocular disease. The disease eventually became endemic but during the last century, the disease has disappeared from many parts of the world. The disappearance has been attributed to improvements in the standard of living and of hygienic practices. In hot, dry climates it still persists, and is a major cause of blindness in developing countries.

T’ang and colleagues (1957) in China were first to isolate the trachoma agent, which was at that time considered to be a virus. This finding boosted research on *Chlamydia* and by 1975 *C. trachomatis* was suggested to be the most common sexually transmitted bacterial pathogen worldwide.

C. trachomatis was recognized as a very common cause of urethritis in men and cervical infection in women. In 1977 Mårdh and colleagues stated that *C. trachomatis* was a major cause of pelvic inflammatory disease (PID), and subsequent studies found that this organism could be associated with tubal factor infertility and ectopic pregnancy. In 1980´s C. *trachomatis* was divided into two groups causing primarily either ocular disease or genital disease. Since then there has been a rapid progress in the knowledge of *C. trachomatis* and its effects and diseases in human. *C. trachomatis* is considered the world's leading preventable cause of blindness, with about 6 million people blinded as a result of this

disease (Idahl, 2009). The chlamydiaceae was once considered viruses because they are small enough to pass through 0,45 micro meter filters and are obligate intracellular parasites. However, the organisms have the following properties of bacteria, including:

1. Possess inner and outer membranes similar to those of Gram- negative bacteria.

2. Contain both DNA and RNA.

3. Possess prokaryotic ribosomes.

4. Synthesize their own proteins, nucleic acids, and lipids.

5. Are susceptible to numerous antibacterial antibiotics.

Unlike other bacteria, however, the Chlamydiaceae lack a peptidoglycan layer (Levinson, 2008)**.**

2.2. General features of Chlamydiae

They have been placed in their own order, *Chlamydiales*, with one family, *Chlamydiaceae*, and a single genus, *Chlamydia*. There has been some disagreement in the scientific community whether *Chlamydia* should be divided into two genera, *Chlamydia* and *Chlamydophila*, based on apparent differential clustering of the 16S rRNA gene; however this separation has not been commonly accepted. The genus *Chlamydia* consists of four major species, *Chlamydia trachomatis, Chlamydia psittaci, Chlamydia pneumoniae* and *Chlamydia pecorum* .

C. trachomatis has been divided into three biovariants (biovar): trachoma,lymphogranuloma venerum (LGV) and murine (mouse pneumonitis [MoPn] agent). The trachoma and LGV biovars are distinguished by different clinical features. LGV readily cause systemic infections and proliferate in lymph nodes, whereas growth of the

trachoma biovar has been believed to be limited to columnar epithelial cells at mucosal surfaces. However, chlamydial antigen and nucleic acid is also found in macrophages and smooth muscle cells deep within the lamina propria, and electron microscopic investigation has revealed *C. trachomatis* elementary bodies within spermatozoa (Idahl, 2009). The trachoma biovar consists of prototypical serovariants (serovars), determined by the serological immune response, and designated by the letters A through K. The serovariants A through C give rise to trachoma of the eye, whereas serovariant D through K in adults give rise to genital manifestations and in newborns pneumonia and conjunctivitis (Idahl, 2009).

Members of the genus *Chlamydia* are obligate intracellular bacteria, which have all the elements of bacteria except a rigid cell wall. Of the three species causing disease in humans, *Chlamydia trachomatis* is the most common as a major cause of genital infection and conjunctivitis. A chronic form of *C. trachomatis* conjunctivitis, called trachoma, is the leading preventable cause of blindness in the world.

Chlamydia pneumoniae and *Chlamydia psittaci* are respiratory pathogens. Knowledge of biology and pathogenesis of these bacteria is based primarily on the study of *C. trachomatis* (Ryan and Ray, 2003**)**.

2.3. Definition and Morphology of *Chlamydiae*

Chlamydiae are spherical or ovoid obligate intracellular bacteria that are ubiquitous. Intracellular parasitism of *Chlamydia* differentiates it from other bacteria. Unlike viruses, *Chlamydiae* possess both DNA and RNA, multiply by binary fission rather than self-assembly, contain their own ribosome, have a peptidoglycan free cell wall and are susceptibile to various antimicrobial agents (Malhotra *et al*., 2013).

The chlamydial cell envelope consists of two lipid bilayers resembling a gram-negative envelope. Although the presence of peptidoglycan has never been directly demonstrated in isolated organisms, genes for the biosynthesis of peptidoglycan are universally present in the genomes of the family. Cell wall active antimicrobials have negative impacts on the life cycle of the *chlamydiae*, inducing a persistent state that may contribute to the chronicity of infection.

The chlamydial DNA genome is small. For example, the genome of *C. pneumoniae* comprises 1,230 kilobase pairs (kbs), making it among the smallest found in prokaryotic cells. *Chlamydiae* possess ribosomes and synthesize their own proteins and, therefore, are sensitive to antibiotics that inhibit this process, such as tetracyclines and macrolides (Harvey *et al.*, 2013).

2.4. Physiology*:*

Chlamydiae are energy parasites, requiring living cells for growth. They are unable to synthesize their own pools of ATP or regenerate NAD+ by oxidation. With these high-energy molecules exogenously supplied, *Chlamydiae* produce CO_2 from compounds such as glucose, pyruvate, and glutamate and carry out the usual bacterial metabolic activities (Harvey *et al*., 2013). **.**

2.5. Replication cycle:

The developmental cycle, which is the unifying property that defines the genus, was first clearly described for the psittacosis agents after they had been isolated in the early 1930s (Idahl, 2009).

Chlamydiae have a unique life cycle, with morphologically distinct infectious and reproductive forms. The extracellular infectious form, the elementary body, is a tiny, condensed, apparently inert structure that can

survive extracellular cell-to-cell passage and initiate an infection. The elementary body is taken up by phagocytosis into susceptible host cells, a process facilitated by proteins in the chlamydial cell envelope that function as adhesins, directing attachment to glycolipid or glycopolysaccharide receptors on the host cell membrane. Once inside the cell, the elementary body prevents fusion of the phagosome and lysosome, protecting itself from enzymatic destruction. The particle reorganizes over the next 8 hours into a larger, noninfectious reticulate body, which becomes metabolically active and divides repeatedly by binary fission within an inclusion in the cytoplasm of the host cell. As the reticulate body divides, it fills the endosome with its progeny, forming an inclusion body. After 48 hours, multiplication ceases, and reticulate bodies condense to become new infectious elementary bodies. The elementary bodies are then released from the cell by cytolysis, ending in host cell death (Harvey *et al*., 2013).

2.6. Pathogenesis

Chlamydiae infect primarily epithelial cells of the mucous membranes or the lungs. They rarely cause invasive, disseminated infections (Levinson, 2008)**.**

Chlamydiae have a tropism for epithelial cells of the endocervix and upper genital tract of women, and the urethra, rectum and conjunctiva of both sexes. The LGV biovar can also enter through breaks in the skin or mucosa. Once infection is established, there is a release of pro inflammatory cytokines such as interleukin-8 by infected epithelial cells. Chlamydial lipopolysaccharides probably also play an important role in initiation of the inflammatory process. This results in early tissue infiltration by polymorphonuclear leukocytes, later followed by lymphocytes, macrophages, plasma cells and eosinophils. If the infection

progresses further (because of lack of treatment and/or failure of immune control), aggregates of lymphocytes and macrophages (lymphoid follicles) may form in the submucosa; these can progress to necrosis, followed by fibrosis and scarring (Ryan and Ray, 2003).

Chlamydia trachomatis exists in more than 15 immunotypes (A-L). Types A,b and C cause trachoma, a chronic conjunctivitis endemic in Africa and Asia (Levinson, 2008)**.**

It has 3 human serovars; serovar Ab, B, Ba or C, which causes trachoma (an eye infection), serovar D to K which causes pelvic inflammatory diseases (PID), ectopic pregnancy and urethritis, and serovars La to L3 which causes lymphogranuloma venereum (LGV). Trachoma may recur over many years and may lead to blindness but causes no systemic illness (Levinson, 2008)**.**

Chlamydia trachomatis (serotypes D–K) are obligatory intracellular Gram-negative bacteria that primarily infect the female cervix, urethra, and fallopian tubes. The majority of infection is asymptomatic and goes undetected, with an increased risk of pelvic inflammatory disease, and is the leading cause of ectopic pregnancy, tubal factor infertility and chronic pelvic pains (Kamel, 2013).

2.7. Signs and symptoms

Up to as many as 85 to 90 percent of *C. trachomatis* infections in men and women are asymptomatic, and can persist for several months and years. The complications encountered by the female are many different such as urethritis, bartholinitis, cervicitis, endometritis, pelvic inflammatory disease (PID) sometimes with intraabdominal spread causing periappendicitis and perihepatitis, and in rare cases proctitis (Idahl, 2009). Consequences of *Chlamydia trachomatis* infection are

more damaging to the reproductive health of women than to men (Mania-Pramanik *et al*., 2012).

Symptoms range from pain when urinating to lower abdominal pain, modest fever and adnexal and uterine tenderness on pelvic examination, but often there is only a midcycle bleeding or no symptoms at all. Late sequels are infertility, ectopic pregnancy, chronic pelvic pain and probably also uterine cervix squamous cell carcinoma. In men the spectrum of disease covers urethritis, prostatitis, orchitis and epididymitis (Idahl, 2009).

2.8. Chlamydial Heat Shock Proteins

Heat Shock Proteins (HSPs) are a group of highly conserved cellular proteins that acts as chaperones, with a key role in intracellular folding and refolding, assembly, and translocation of proteins. The expression of HSPs was initially found to be elevated in reaction to heat stress but is also expressed as well in reaction to proteolytic, mechanical or chemical stress. There are four main groups of HSPs based on their molecular weights: HSP90, HSP70, HSP60 and the small HSPs. During persistent infection the HSP60 production is up-regulated while the production of other proteins is down-regulated, Chlamydial HSP60 is suggested to inhibit the apoptotic pathway of the host-cell supporting persistence of chlamydial infection. Human and chlamydial HSP60 share an approximately 50% amino acid homology and despite this homology, chlamydial HSP60s are highly immunogenic and are the HSPs most extensively studied in relation to infertility (Idahl, 2009).

2.9. Definition and prevalence of Subfertility/infertility

Infertility is defined as the inability of sexually active couples taking no contraceptives to achieve pregnancy within 1 year (Lavorato *et al*., 2015).

Primary infertility is defined as inability to get pregnant, while secondary infertility is the inability to get pregnant after an earlier pregnancy (Okonofua, 2005).

An often used definition is that the couple is regarded sub fertile after one year of unprotected sexual intercourse without conception. According to the definition by the World Health Organization (WHO), 2 years of unprotected sexual intercourse is required. Infertility is the most common used term for this condition, however in fact refers to a couple that cannot at all achieve pregnancy. Infertility affects approximately 5-26% of couples in the reproductive age-group, a figure that is fairly similar between less and more developed countries. An estimated 70 million couples are at the present experiencing infertility (Idahl, 2009).

The causes of infertility are in slightly more than 1/3 due to female factors, in 1/3 male factors and in 1/3 there is a combination of male and female factors. In 5-10% a cause cannot be established despite thorough investigation. Psychosocial aspect psychosocial aspects for many couples, the inability to bear children are a tragedy. There is both a biological and social loss that to most people is shocking and often leads to a psychological crisis following the well-known traumatic crisis, however often more prolonge (Idahl, 2009).

In a society where infertility represents a flawed social identity an infection with the propensity to damage reproductive capability represents a threat to their identity as mother and therefore woman (Piercy and Hilary, 2009).

Even though infertility is a common problem to the couple, the man and the woman might have different feelings and reactions, affecting their marital and social lives in different ways. A feeling of despair, anxiety,

grief's lack of self-esteem, and sexual inadequacy are common in the infertility situation and does not seem to be related to socio-economic class, race or culture. It is essentially "human" to respond to childlessness even though the nature of concern may differ (Idahl, 2009).

2.10. *Chlamydia trachomatis* and infertility

C. trachomatis is known to cause damage to the female reproductive tract, primarily due to adhesions or obstructions of the fallopian tubes secondary to the inflammatory response. Reduced chances to achieve an intrauterine pregnancy are the result. TFI (Tubal Factor Infertility) is the main cause of infertility in 10-30% of cases in developed countries and *C. trachomatis* is the most common causative agent to Pelvic Inflammatory Disease and TFI in the developed world, while the rate of *Neisseria gonorrhoeae* is low (Idahl, 2009).

2.11. Clinical significance

C. trachomatis causes a range of gonococcal urethritis and eye infections.

1. Nongonococcal urethritis: Annually, more than 4 million urogenital *C. trachomatis* infections occur in the United States in young, sexually active individuals of all socioeconomic groups. In men, the urethra is the initial site of infection. Women may present with cervicitis and/or urethritis. Infections are often asymptomatic, although communicable. Among women, the asymptomatic rate is higher than 50 percent. Whether locally symptomatic or not, the infection may ascend into the upper reproductive tract to involve the epididymis in men and fallopian tubes and adjacent tissues in women (pelvic inflammatory disease). Chlamydial NGU (Non Gonococcal Urethritis) is symptomatically similar to infections caused by *Neisseria gonorrhoeae*, although the average incubation time is longer (2 to 3 weeks), and the discharge tends to be

more mucoid and contains fewer pus cells. In addition, the two infections often occur simultaneously. Therefore, patients suspected of chlamydial infection should be treated for gonococcal infection. Non gonococcal urethritis is caused by serotypes D–K of *C. trachomatis*. These serotypes also cause eye infections, for example, in infants born to genitally infected women Infection with *C. trachomatis* confers little protection against reinfection, which commonly occurs. Repeated or chronic episodes may lead to infertility in both sexes and to ectopic pregnancies.

2. Lymphogranuloma venereum:

C. trachomatis serotypes L1, L2 and L3 cause lymphogranuloma venereum (LGV), a more invasive sexually transmitted disease. It is uncommon in the United States but endemic in Asia, Africa, and South America. LGV is characterized by transient papules on the external genitalia, followed in 1 to 2 months by painful swelling of inguinal and perirectal lymph nodes. Adenopathy (swelling of the lymph nodes) is often accompanied by mild constitutional symptoms. The inguinal ligament often forms a cleft known as the "groove sign" between masses of inguinal lymph nodes. The affected lymph nodes suppurate (to form or discharge pus), and chronic inflammation and fibrosis lead to extensive ulceration and blockage of regional lymphatic drainage.

3. Trachoma:

C. trachomatis serotypes A, B, Ba, and C cause a chronic keratoconjunctivitis that often results in blindness. Trachoma is transmitted by personal contact, for example, from eye to eye via droplets, by contaminated surfaces touched by hands and conveyed to the eye, or by flies. Because of persistent or repeated infection over several

years, the inflammatory response with attendant scarring leads to permanent opacities of the cornea and distortion of eyelids.

4. Neonatal conjunctivitis and other infections:

Over 50 percent of infants born to women infected with *C. trachomatis*, serotypes D–K will contract symptomatic infection on passage through the birth canal. The most common presentation is inclusion conjunctivitis of the newborn. This acute, purulent conjunctivitis (named for the inclusion bodies seen in infected conjunctival epithelial cells) usually heals after appropriate antimicrobial therapy, without permanent damage to the eye. If untreated, the infection can lead to permanent scarring of the cornea or conjunctiva. Approximately 1 of 10 infected infants will present with or develop pneumonia, which can be treated with erythromycin.

5. Inclusion conjunctivitis in adults:

Individuals of any age may develop transient purulent conjunctivitis caused by *C. trachomatis* serotypes D–K. Such individuals are often found to be genitally infected as well (Harvey *et al.*, 2013).

2.12. Transmission:

Chlamydia trachomatis has a very limited host range with infections restricted humans (Levinson, 2008)**.** Spread is by contact with infective human secretions, directly via hands to the eye, or via fomites transmitted on the feet of flies (Ryan and Ray, 2004).

When spread by sexual intercourse, the infection can ascend to the upper genital tract where it can cause pelvic inflammatory disease (PID), leading to subsequent infertility and ectopic pregnancy (Wilson *et al*., 2002).

Infects only humans and is usually transmitted by close personal contact, e.g., sexually or by passage through the birth canal. Individuals with asymptomatic genital tract infections are an important reservoir of infection for others (Levinson, 2008).

Patients diagnosed with Chlamydia must receive a partner notification interview (Scottish Intercollegiate Guidelines Network, 2009).

2.13. Epidemology:

C. trachomatis is most common agent leading to congenital infection in both men and women. Worldwide estimated annual incidence goes up to 50 million cases. *C. trachomatis* in women has a clinical course varying from asymptomatic infections to ascending infections leading to pelvic inflammatory disease (PID) associated with late ectopic pregnancy and tubal infertility (Land, 2010; Mohammed and Omar,2012).

As with most STIs, incidence and prevalence are highest among young people, and decline steeply with age. Infection is more common among the more sexually active individuals, with the greatest number of sexual partners (Price *et al*., 2016).

Chlamydia trachomatis are mainly spread through sexual contact and most time neonatal which have been reported to be the commonest sexually transmitted diseases in European countries and the USA with paucity of reports from sub-saharan Africa (Okoror and Agbonlahor, 2012).

It is spread by secretions and is the most common sexually transmitted disease. In the United States over 700,000 cases are reported each year, which is twice the number for gonorrhea. Humans are the sole reservoir. Each of the major disease syndromes caused by *chlamydiae* are associated with several different strains. Inclusion conjunctivitis is seen

among population groups in which the strains causing *C. trachomatis* genital infections are common. This disease is the most common form of neonatal conjunctivitis in the United States, occurring in 2 to 6% of newborn infants. The infection results from direct contact with infective cervical secretions of the mother at delivery.

Trachoma, a chronic follicular conjunctivitis, afflicts an estimated 500 million persons worldwide and has blinded millions, particularly in Africa. The disease is usually contracted in infancy or early childhood from the mother or other close contacts.

The prevalence of chlamydial urethral infection in US men and women ranges from 5% in the general population to 20% in those attending sexually transmitted disease clinics.

Approximately one third of male sexual contacts of women with *C.trachomatis* cervicitis develop urethritis after an incubation period of 2 to 6 weeks. The proportion of men with mild to absent symptoms is higher than in gonorrhea. Nongonococcal urethritis is most commonly caused by *C. trachomatis* and less frequently by *Ureaplasma urealyticum*. Reinfection is common (Ryan and Ray, 2004).

2.14. Laboratory identification

C. trachomatis can be demonstrated in clinical material by several direct procedures and by culturing in human cell lines. Samples, particularly from the urethra and cervix in GU infection and conjunctivae in ocular disease, should be obtained by cleaning away overlying exudate and gently scraping to collect infected epithelial cells (Harvey *et al*., 2013).

1. Direct tests

Microscopic examination using direct fluorescent antibody staining reveals characteristic cellular cytoplasmic inclusions.

This permits cost-effective screening of large numbers of individuals without the need for access to a medical clinic and a pelvic examination (Harvey *et al*., 2013).

2. Culturing methods

For diagnosis of *chlamydia* infection, cell culture of urogenital specimens has been considered the ideal method, although few laboratories could offer this, due to its cost and lack of experience in the cell culture technique.

Accurate results depend on the proper sample taking, carrying, storage, and interpretation *(*Kamel, 2013*)*.

C. trachomatis can be cultivated by tissue culture in several human cell lines. In the standard procedure using McCoy cells, addition to the culture medium of a eukaryotic metabolic inhibitor, such as cycloheximide, enhances growth of the parasite. The presence of chlamydial inclusions can be demonstrated after 2 to 7 days of incubation (Harvey et al., 2013).

Culture was for many years the only method available for the diagnosis of *C. trachomatis*, but has in the light of newer methods such as nucleic acid amplification tests (NAAT) been abandoned for routine diagnosis in large-scale laboratories (Idahl, 2009).

The method of choice for the isolation of *C. trachomatis* is cell culture but other techniques like DIF and ELISA are more feasible, faster, less expensive and adequately specific and sensitive(Kajbaf and Gholamnezhad, 1998).

3. A presumptive diagnosis of acute chlamydial infection in male patients is often made when a Gram stained urethral smear contains more than 5 pus cells/high power field (100/objective) and no intracellular Gram negative diplococcic or more than 20 pus cells in a first voided urine specimen (Cheesbrough, 2006).

4. Serology

Use of rapid serological methods that rely on immunology of the infection as proxy determinants of either an on‑going or previous infections were developed. It involves use of immunoglobulin G (IgG), A and M detections in either the serum or urine samples. Several studies have confirmed the reliability of using this simple serological test as a predictor of tubal blockage in different settings (Morhason-Bello *et al*., 2015).

These methods are widely used for research purposes to compare sample populations for prior or current exposure to an infection (Idahl, 2009).

5. Nucleic acid amplification assays –NAATs

NAATs are widely used for *C. trachomatis* diagnosis today and have proven to be more sensitive and specific than previous diagnostic tests (culture, IF and EIA) because they don't need viable *chlamydiae* (more tolerant to transports) and due to the amplification process. A further advantage is that they can be used in non invasive specimens such as a first-void urine or vaginal swabs with nearly identical sensitivity and specificity to those in cervical or urethral samples (Idahl, 2009).

6. Immunofluorescence (IF) and antigen detection enzyme immunoassays (EIA)

The key to this entire process is the ability to visualize an antibody attached to an antigen. The direct immunofluorescence method uses a fluorescent dye that is covalently attached to the antibody. When a light illuminates the fluorescent dye, it absorbs the light and emits a different color light which is visible to the investigator and can be photographed. This was the first method not dependent on viable *C. trachomatis*, but had sensitivities and specificities lower than culture (Idahl, 2009).

In enzyme immunoassays, the attached enzyme-tagged antibody is detected by adding a substrate indicator that produces a color reaction. The optical density of the enzyme is read by a spectrophotometer (Idahl, 2009).

Enzyme-linked immunosorbent assay (ELISA)

Is the first generation of non-cultural tests to diagnose chlamydial infection. ELISA uses an enzyme-linked monoclonal or polyclonal antibody directed at the *C. trachomatis* lipopolysaccharide. In the presence of *C. trachomatis*, the antibody binds to LPS, and the linked enzyme induces a change in color that can be detected by spectrophotometer (Rashidi *et al*, 2013).

2.15. Treatment

Doxycycline 100 mg twice daily for 7 days, or a single dose of azithromycin 1g, are the most rigorously investigated treatment regimens for uncomplicated chlamydial infections, but doxycycline 200 mg on the first day with 6-9 subsequent doses of 100 mg daily is also studied (Idahl, 2009).

The recommended treatment during pregnancy is erythromycin base or amoxicillin (Miller, 2006).

In the absence of treatment, women infected with *C. trachomatis* may develop pelvic inflammatory disease (PID) which can result in tubal factor infertility (TFI), ectopic pregnancy (EP) and chronic pelvic pain (Akande *et al*., 2011). Treatment of PID and TFI engender elevated financial and psychological costs. Screening programmes for chlamydial infection have been implemented in order to decrease these costs. The major aims of *C. trachomatis* screening are to reduce morbidity by early detection and treatment of lower genital tract infection as well as to decrease prevalence of this infection and consequently reduce their transmission (Lavorato *et al*., 2015).

Chapter Three

Materials and Methods

Chapter three

3. Materials and Methods

3.1. Study design

The study design was case control hospital based study.

3.1.1. Study area and duration

Clinical specimens were collected from different health setting at Khartoum State including Ashmeeg Centre, Sudan Centre for Fertility and Dream hospital. The study was performed during September to December 2015 at National Health Laboratory – Khartoum.

3.2. Study population and sample size

Study population included infertile women who were attending infertility clinics (n = 60) and pregnant women (n = 30) having follow up visits as controls.

3.2.1. Inclusion criteria

Study group: infertile women attended infertility clinics.

Control group: pregnant women.

3.2.2. Exclusion criteria

Fertile non pregnant women.

3.3. Sampling

3.3.1. Sample type

Non-probability sampling.

3.3.2. Sampling technique

The proposed data collection tool from proposed study population is convenience.

3.4. Study variables

Qualitative variables are PID, vaginal bleeding, urogenital tract infection, age, previous pregnancy, history of ectopic pregnancy, history of previous abortion and history of stillbirths.

3.5. Data collection

Data was collected from patients by direct interviewing by closed answer questionnaire contains all study variables (appendix).

3.6. Data analysis

The collected data were analyzed using SPSS (Statistical Package of Social Science) soft program version 22. Association between study variables was done using chi-square test.

3.7. Ethical consideration

Permission for carry out this study was obtained from College Ethical Board of Sudan University of Science and Technology. The objectives of the study clearly and simply explained to all participants in the study and verbal informed consent was obtained.

3.8. Experimental work

3.8.1. Method of specimens' collection

Blood specimens were collected using sterile syringes. After sterilization of puncture site with 70% alcohol using impregnated cotton, puncture was made with needle smoothly and 2ml whole blood specimens were

collected in sterile plain containers. After blood was clotted, specimens were centrifuged at 3000 RPM for 5 minutes to get serum.

3.8.2. *Chlamydia Trachomatis* IgG ELISA:

ELISA kit purchased from AccuDiag Company (United States of America). It contains the following: microwell strips 12X8, sample diluent, Calibrator, Negative control, Positive control, washing concentrate 10X, Enzyme conjugate,TMB chromogenic substrate and stop solution.

The kit uses microwell strips coated with purified *Chlamydia trachomatis* antigen. If patient sera contain *Chlamydia trachomatis* IgG specific antibodies, they bind to the antigen. Unbound materials are washed away during washing steps. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic Substrate is added. The enzyme conjugate catalytic reaction is stopped at specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a micro well reader compared in a parallel manner with calibrator and controls.

3.8.3. Preparation of samples and washing buffer:

Patients' samples were diluted 1:40 in sample buffer by adding 5microlitre of the test samples, negative control, positive control and calibrator to 200 microlitre of sample diluent and mixed well using vortex.

Concentrated wash buffer diluted from 10x to 1x by adding 100ml of washing buffer to 900 ml of distilled water.

3.8.4. Assay procedures

1-Desired number of coated strips was placed into the holder.

2-1:40 dilutions were prepared and 5 µL of test samples, negative controls, positive controls and calibrator was added to 200 µL of sample diluent and were mixed well.

3-100 µL of diluted sera, calibrator and controls were dispensed into the appropriate wells. For the reagent blank, 100 µL sample diluent was dispensed in 1A well position. The holder was tapped to remove air bubbles from the liquid and mixed well and then incubated for 30 minutes at room temperature.

4-Liquid was removed from all wells. Washing with washing buffer was repeated three times.

5-100 µL of enzyme conjugate was dispensed to each well and incubated for 30 minutes at room temperature.

6-Enzyme conjugate was removed from all wells. Washing with washing buffer was repeated three times.

7-100 µL of TMB Chromogenic substrate was dispensed to each well and incubated for 15 minutes at room temperature.

8-100 µL of stop solution (H_2SO_4) was added to stop the reaction.

9-O.D. were read at 450 nm with a micro well reader

3.8.5. Results

1- Cut off OD Value was obtained by multiply the OD of calibrator by factor (f) printed on label calibrator.

(factor f value = 0.4)

2-The Chlamydia IgG Index of each determination was calculated by dividing the OD value of each sample by obtained OD value of cut off.

3.8.6. Interpretation

IgG Index of 0.90 or less were considered seronegative for IgG antibody. Whereas samples of IgG Index of 0.91-0.99 considered equivocal and were retested.

IgG Index of 1.00 or greater were considered seropositive.

Chapter Four

Results

4. Results

A total of 90 consented women were enrolled in the study; comprising 60 infertile women and 30 pregnant women as control group. All study population was married; their age ranged between 18 – 48 years. The mean age of the control group was 29 and the mean of infertile cases was 34.

Serum specimens were collected from all women in the study and were tested for detection of *C. trachomatis* antibodies using IgG ELISA assay.

Ten samples of infertile women group were positive for *C. trachomatis* antibodies, demonstrating a seroprevalence rate of 16.67% (Table 4.1). Analysis showed that there is a significant association between *C. trachomatis* infection and infertility (p.value 0.03).

Seropositivity among different age groups is shown in Table 4.2. The highest seropositivity was shown in the age group 39 - 48 years (60%). There is insignificant association between *C. trachomatis* infection and age distribution of the population (p.vaues $\geq$0.16).

Association between *C. trachomatis* seropositivity and history of no pregnancy among infertile women is shown in Table 4.3. Results showed no significant association (p.value 1.0) between these variables.

Frequency and association of *C. trachomatis* seropositivity and urogenital tract infections were shown in Table 4.4. Majority of seropositive infertile women (80%) had urogenital infections. The association between *C. trachomatis* infection and urogenital infection was significant (p.value 0.03). A proportion of the infertile women that were seronegative for *chlamydia* showed history of urogenital infections; 20/50 (40%).

Relationship between *C.trachomatis* seropositivity with symptoms and signs of PID was studied and results are shown in Table 4.5. Majority of infertile women showed positive symptoms and signs of PID (80%). A significant association was shown between *C. trachomatis* and PID (p.value 0.01).

Vaginal bleeding is shown to be non-specific for infertility, as it prevalent in both infertile and fertile women (Table 4.6). In addition, *C. trachomatis* seronegative infertile women showed higher vaginal bleeding as compared to infertile seropositive women, demonstrating, no significant association between vaginal bleeding and *C. trachomatis* (P. value 1).

Frequency and association of stillbirths and *C.trachomatis* seropositivity were shown in Table 4.7. All seropositive infertile women showed no history of stillbirth. History of stillbirths was detected in 2 (6.7%) among pregnant fertile women, which is higher than seronegative infertile women (4%). No significant association between history of stillbirths and *C.trachomatis* seropositivity (P.value 1).

As shown in Table 4.8, both infertile and fertile groups showed history of ectopic pregnancy, demonstrating that ectopic pregnancy is not specific for infertility and thus there is no significant association with *C.trachomatis* infection (P.value 1).

Association between *C.trachomatis* seropositivity and history of abortion was shown in Table 4.9. Previous abortion was higher in seronegative group for *C.trachomatis* than seropositive women, 3(30%). There is no significant association between previous abortion and *C.trachomatis* (P.value 1).

Table 4.1

Seroprevalence of *C. trachomatis* infection among infertile and pregnant women control groups:

Clinical condition	*C.trachmatis* IgG ELISA	
	Seropositive	**Seronegative**
Infertile women (n=60)	10 (16.67%)	50 (83.33)
Pregnant women (n=30)	0 (0%)	30 (100%)
P.value	0.03	

Values are number tested positive or negative (percentage) for

C. *trachomatis.*

* There is significant association between *C.trachomatis* and infertile women.

Table 4.2

Seropositivity of *C. trachomatis* infection among different age groups of infertile women:

Age group	*C.trachomatis* IgG ELISA		P.value
	seropositive	seronegative	
18-28	2/10 (20%)	11/50 (22%)	0.16
29-38	2/10 (20%)	24/50 (48%)	0.16
39-48	6/10 (60%)	15/50 (30%)	0.22

Values are number tested positive or negative (percentage) for *C. trachomatis* in different study population among different age groups.

*There is no significant association between *C.trachomatis* and different age groups.

Table 4.3

Association between *C. trachomatis* seropositivity and history of no pregnancy among infertile women:

No previous pregnancy	***C.trachmatis* IgG ELISA**	
	Seropositive	**Seronegative**
Yes	4/10 (40%)	23/50 (46%)
NO	6/10 (60%)	27/50 (54%)
P.value	1.0	

*There is no significant association between *C.trachomatis* and history of no pregnancy.

Table 4.4

Frequency and association of *C. trachomatis* seropositivity and urogenital tract infections among infertile women:

Urogenital Tract Infection	***C.trachmatis* IgG ELISA**	
	Seropositive	**Seronegative**
Yes	8/10 (80%)	20/50 (40%)
No	2/10 (20%)	30/50 (60%)
P.value	0.03	

* There is significant association between *C.trachomatis* and urogenital tract infection.

Table 4.5.

Association between *C.trachomatis* seropositivity with symptoms and signs of PID among infertile women:

Symptoms and signs of PID	*C.trachmatis* IgG ELISA	
	Seropositive	**Seronegative**
Yes	8/10 (80%)	17/50 (34%)
No	2/10 (20%)	33/50 (66%)
P.value	0.01	

*There is significant association between Chlamydia trachomatis infection with symptoms and signs of PID.

Table 4.6.

Association between *C. trachomatis* seropositivity and bleeding per vaginum among infertile women:

Vaginal bleeding	*C. trachmatis* IgG ELISA			
	Infertile women		**Pregnant women**	
	Seropositive	Seronegative	Seropositive	Seronegative
Yes	0/10 (0%)	2/50 (4.0%)	0/30 (0%)	3/30 (10%)
No	10/10 (100%)	48/50 (96.0%)	0/30 0%%	27/30 (90%)
P.value	1.0			

Values are number tested positive or negative (percentage) for

C. trachomatis in different study populations.

*There is no significant association between Chlamydia trachomatis infection and vaginal bleeding.

Table 4.7.

Frequency and association of *C. trachomatis* seropositivity with history of stillbirths among infertile and pregnant control women:

History of stillbirth	*C. trachmatis* IgG ELISA			
	Infertile women		Pregnant women	
	Seropositive	Seronegative	Seropositive	Seronegative
Yes	0/10 (0%)	2/50 (4.0%)	0/30 (0%)	2/30 (6.7.0%)
No	10/10 (100%)	48/50 (96.0%)	0/30 (0%)	28/30 (93.3%)
P.value	1.0		-	

*There is no significant association between *C.trachomatis* and history of stillbirths.

Table 4.8.

Association between *C.trachomatis* seropositivity and history of previous ectopic pregnancy among infertile and pregnant control women:

History of ectopic pregnancy	*C. trachmatis* IgG ELISA			
	Infertile women		**Pregnant women**	
	Seropositive	Seronegative	Seropositive	Seronegative
Yes	0/10 (0%)	3/50 (6.0%)	0/30 (0%)	3/30 (10.0%)
No	10/10 (100%)	47/50 (94.0%)	0/30 (0%)	27/30 (90%)
P.value	1.0		-	

*There is no significant association between *C.trachomatis* and history of ectopic pregnancy.

Table 4.9.

Association between *C.trachomatis* seropositivity and history of previous abortions among infertile and pregnant control women:

History of previous abortion	*C. trachmatis* IgG ELISA			
	Infertile women		**Pregnant women**	
	Seropositive	Seronegative	Seropositive	Seronegative
Yes	3/10 (30%)	15/50 (30.0%)	0/30 (0%)	3/30 (10.0%)
No	7/10 (70%)	35/50 (70.3%)	0/30 (0%)	27/30 (90%)
P.value	1.0		-	

*There is no significant association between *C.trachomatis* and previous abortion. .

Chapter Five

Discussion, Conclusion, Recommendations

5.1. Discussion

C. trachomatis is most common agent leading to congenital infection in both men and women. Worldwide estimated annual incidence goes up to 50 million cases. *C. trachomatis* in women has a clinical course varying from asymptomatic infections to ascending infections leading to pelvic inflammatory disease (PID) associated with late ectopic pregnancy and tubal infertility (Land, 2010; Mohammed and Omar,2012). *C.trachomatis* infection is becoming a public health problem in Khartoum. Studies have shown high frequency among fertile and non-fertile Sudanese women (Mohammed and Omar, 2012*). C. trachomatis* infection can cause numerous serious disease states including urethritis, proctitis, trachoma, and infertility (Qayum and Bin-Saleem, 2013). Screening of *C. trachomatis* is needed to define measures of prevention, modes of transmission to newborn, and ways to reduce sexual spread. Such screening program is lacking in Sudan and might be resulted from the lack of awareness of the high rate of infection among young asymptomatic women and its serious late complications (Mohammed and Omar, 2012). The main problem of *C.trachomatis* infection is that it might be a symptomatic, and women only came to the clinics when they developed complications such as signs and symptoms of lower genital tract infection, experienced repeated pregnancy loss, and had infertility at later age; thus, present observation revealed an increasing rate of infection with age.

This study was conducted to estimate seroprevalence of *C.trachomatis* infection among Sudanese infertile women in ninety blood samples of infertile and fertile women at Khartoum.

The main finding of this study was the high seroprevalence of *C.trachomatis* inection among infertile women (16.67%). The pregnant

fertile women showed 0% seropostivity. Similar results have also been shown previously in similar population (Muvunyi *et al*., 2011; *Kamel*, 2013; Lavorato *et al*., 2015). Various studies from different countries have reported different infection levels of *C.trachomatis*; 2.6% in United Arab Emirates , 3.9% In Jordan , 5.3% in Qatar , and 15% in Saudi Arabia *(Kamel*, 2013). In this study, highest seropositivity was shown in the age group 39 – 48 (60%), which disagree with another study conducted in a similar population in Khartoum (Mohammed and Omar, 2012); whereas, the majority of seronegative cases were in the same age group (39-48) as shown by Mohammed and Omar (2012).

In the study conducted in Khartoum in a similar population, a higher prevalence rate of *C. trchomatis* infection has been shown (Mohammed and Omar, 2012). These authors have reported a prevalence rate of 22.3%, which is higher than what was reported here. In this study, prevalence of *C. trachomatis* infection was determined based on ELISA. Mohammed and Omar (2012) in their study used PCR for detection of *C. trachomatis* DNA in specimen taken from gynecological patients in Khartoum. Molecular technique is known to be more sensitive and specific than ELISA due to the amplification process.

In addition, the study revealed no significant association between *C.trachomatis* infection and vaginal bleeding (p.value =1), stillbirth (p.value =1), ectopic pregnancy (p.value =1) and previous abortion

(p.value =1). These findings are disagreed with those published earlier in India (Malik *et al*., 2006).

Complications such as stillbirth, ectopic pregnancy and previous abortion although detected in infertile women, were also detected in fertile

pregnant women, indicating non-specificity of these complications. Such findings have also been shown by others (Mohammed and Omar, 2012).

The study revealed that there is significant association of *C. trachomatis* infection with PID and urogenital infections, these findings have been shown earlier (Mohammed and Omar, 2012).

5.2. Conclusion

The findings of this study highlight the need for *chlamydia* screening programs. Since seroprevalence of infection among Sudanese infertile women is higher than those of fertile women, *C.trachomatis* should be suspected as a possible cause of infertility.

3.3. Recommendations

- Screening of *C.trachomatis* should take a place in routine investigations in infertility clinics.

- Beside testing for *C.trachomatis* infection, infertile women should be also tested for other sexually transmitted infections, since *C.trachomatis* infection is not proved in all infertile women.

- Further studies about *Chlamydia trachomatis* and its rule in infertility and other sexual transmitted diseases should take place in medical laboratories and science field.

References

1. **Abdella R. M. A., Abdelmoaty H. I., Rasha H. Elsherif R. H.,Sayed A. M.,Sherif N. A.,Gouda H. M.,El Lithy A.,Almohamady M., Abdelbar M., Hosni A. N., Magdy A. and MA Y.,**(2015). Screening for *Chlamydia trachomatis* in Egyptian women with unexplained infertility, comparingreal-time PCR techniques to standard serology tests: case control study, *BMC Women's Health,*15:45,pp 1-8.

2. **Akande V. , Turner C., Horner P., Horne A., and Pacey A.,(**2011). Impact of *Chlamydia trachomatis* in the reproductive setting: British Fertility Society Guidelines for Practice, , *Hum Fertil* (*Camb),*13(3), 115–125.

3. **Cheesbrough M**.,(2006). District Laboratory Practice in Tropical Countries Part 2,2nd Edition ,Cambridge University Press, Cambridge, UK.

4. **HARVEY R. A., CORNELISSEN C. N., FISHER B. D**.,(2013). Third Edition, Lippincott's Illustrated Reviews: Microbiology, Lippincott Williams & Wilkins, Philadelphia, USA.

5. **Darville T. and Hiltke T.J.**,(2010). Pathogenesis of Genital Tract Disease Due to *Chlamydia trachomatis*, *Fertility and Sterility*, JID,201 (Suppl 2) *14-25.*

6. **Idahl A.,**(2009). *Chlamydia trachomatis* as a risk factor for infertility in women and men, and ovarian tumor development, ISBN 978-91-7264-759-6.

7. **Kajbaf M. J. and Gholamnezhad A**.,(1998). Prevalence of *Chlamydia trachomatis* Antigen and Antibody in Infertile Women in Ahwaz, *Iran. Biomed. J*. 2(1): 45-48.

8. Kamel R. M.,(2013). Screening for *Chlamydia trachomatis* infection among infertile women in Saudi Arabia ,*International Journal of Women's Health* :5 ,277–284.

9. Keltz M. D.,Sauerbrun-Cutler M.,Durante M. S.,Moshier E., Stein D. E. and Gonzales E.,(2013). Positive *Chlamydia trachomatis* Serology Result in Women Seeking Care for Infertility Is a Negative Prognosticator for Intrauterine Pregnancy, *Sexually Transmitted Diseases*,Volume 40, Number 11,pp842-845.

10. Land J.A, J.E.A.M. Van Bergen J. E.A.M,A. Morre S., and J.Postma M.,(2010). Epidemiology of *Chlamydia trachomatis* infection in women and the cost effectiveness of screening. *Human Reproduction Update*, Vol.16, No.2,189–204.

11. Lavorato, H.L., Moço, N.P., Martin, L.F., Santos, A.G.P., Pontes, A., Duarte, M.T.C. and da Silva, M.G.,(2015). Screening of *Chlamydia trachomatis* Infection among Women Attending Outpatient Clinic of Infertility. *Open Journal of Obstetrics and Gynecology*, 5, 600-607.

12.Levinson W.,(2008). Review of Medical Microbiology and Immunology,1oth edition,McGraw-Hill Companies,United States of America.

13. **Malhotra M.,Sood S., Mukherjee A., Muralidhar S. and Manju Bala M**.,(2013). Genital *Chlamydia trachomatis*: An update, *Indian J Med Res* 138,303-316.

14. Malik A.,Jain S.,Hakim S.,Shukla I. &Rizvi M.,(2006). *Chlamydia trachomatis* infection & female infertility, *Indian J Med Res* 123, 770-775.

15. Malik A., Jain S., Rizvi M.,Shukla I.,andHakim S.,(2015). *Chlamydia trachomatis* infection in women with secondary infertility, *Fertil Steril*. Vol. 91, No. 1, *91-95.*

16. **Mania-Pramanik J. , Kerkar S. ,Sonawane S. , Mehta P. and Salvi V.,** (2012). Current Chlamydia trachomatis Infection, A major cause of infertility, J Infertil,13(4):204-210.

17. Miller K. E.,*(*2006). Diagnosis and Treatment of *Chlamydia trachomatis* Infection, *American Family Physician* ,Volume 73, Number 8,1411-1416.

18. Mohammed M. A. and Omer A.A.,(2012). Molecular detection of *Chlamydia trachomatis* among gynecological patients attending Khartoum Teaching Hospital, *J. Bacteriol. Res.*,Vol. 4(4). 42-45.

19. Morhason-Bello IO,Ojengbedo OA,Oladokun A,Adedokun BO,Ajayi A,Adeyanju AA,Ogundepo O,Kareem OI.,(2015). The Prevalence and Outcome of A symptomatic Infection Screening Among Infertile Women Attending Gynecological Clinic in Ibadan, South West Nigeria, *Annals of Medical Health Science Research*, vol (4), iss(2),253-257.

20. Muvunyi C.M.,Dhont N.,Verhelst R.,Temmerman M.,Claeys G.and Padalko E.(2011). Human Reproduction,Vol.26, No.12,3319–3326.

21. Nada Adel M., Hassan Fatma M., Al-Azhary Nermeen H.(2015). Detection of *Chlamydia Trachomatis* in patients with unexplained infertility: A case control study, *Egypt J Med Microbiol*, Volume 24 / No. 2, 35-38.

22. Okonofua E.F., 2005, Female and Male Infertility In Nigeria,Kongl Carolinska Medico Chirurgiska Institute,ISBN:91-7140-355-8.

23. Okoror LE1 and Agbonlahor DE2. *(*2012). High Prevalence of *Chlamydia Trachomatis* in the Sperm of Males with Low Sperm Count in Nigeria,J. Volume 1 • Issue 3.1-5.

24. Paavonen J. and Kruse W. Eggert.,*(1999). Chlamdia Trachomatis: Impact* on Human Reproduction,*Hum Reprod update*,vol(5),5, 433-447.

25. PIERCY and Hilary,(2009). An uncertain future: infertility and chlamydial infection, http://shura.shu.ac.uk/3371/,pp1-13.

26. Price M.J., Ades A.E., Soldan K., Kate S., Welton N.J., Macleod J., Simms L., DeAngelis D., Turner K.ME. and Horner P.J., (2016). The natural history of Chlamydia trachomatis infection in women: a multi-parameter evidence synthesis. Southampton (UK): NIHR Journals Library. (Health Technology Assessment, VOLUME 20, ISSUE 22, ISSN 1366-5278),12.

27. Qayum M, Bin-Saleem M. K.,(2013). Prevalence Of *Chlamydia trachomatis* among Asymptomatic Women, *J Ayub Med Coll Abbottabad*;25(1-2),28–30.

28. Rashidi B.H., Chamani-Tabriz L. Haghollahi F., Jeddi-Tehrani M., Naghizadeh M.M., Shariat M., Akhondi M.M., Bagheri R., Asgari S. and Wylie K.,(2013). Effects of *Chlamydia trachomatis* Infection on Fertility; A Case-Control Study, *J Reprod Infertil* ,Volume 14, Issue 2, Number 55 ,67-72.

29. Ryan K.J., **Ray C.G**.,(2003). Sherris Medical Microbiology,4th edition, McGraw Hill Professional, united states.

30. Scottish Intercollegiate Guidelines Network.,(2009). Management of genital *Chlamydia trachomatis* infection *A national clinical guideline, ISBN 978 1 905813 44 5.*

31. Siemer J.,Theile O., Larbi Y., Fasching P. A.,Danso K. A., Rolf Kreienberg R. and Essig A.,*(2008). Chlamydia trachomatis* Infection as a Risk Factor for Infertility among Women in Ghana, West Africa, *Am. J. Trop. Med. Hyg*., 78(2),323–327.

32. Wilson J.S.,Honey E.,Templeton A., Paavonen J., Mardh P.-A.,Stry A. and Stray-Pederson B.,(2002). A systemic Review of The Prevalence of *Chlamydia Trachomtis* Among European Women, *Hum Reprod Update*, vol.8,No.4,385-394.

Appendixes

Appendix

Sudan University of science and Technology

College of graduate Studies

Questionnaire on

Seroprevalence of *C. trachomatis* infection among Sudanese infertile Women attending infertility clinics in Khartoum

Patient No. ……

Date: …….

Age: ……..

1- Did you get pregnant before.....

2- Current status:

a- Pregnant b-Not pregnant

3 -Symptoms of urogenital tract infection:

-Vaginal discharge: Yes ….. No …..

-Burning during urination: Yes …. No ……

-Itching around the outside of the vagina: Yes… NO ….

4- History of stillbirth: ……..…..

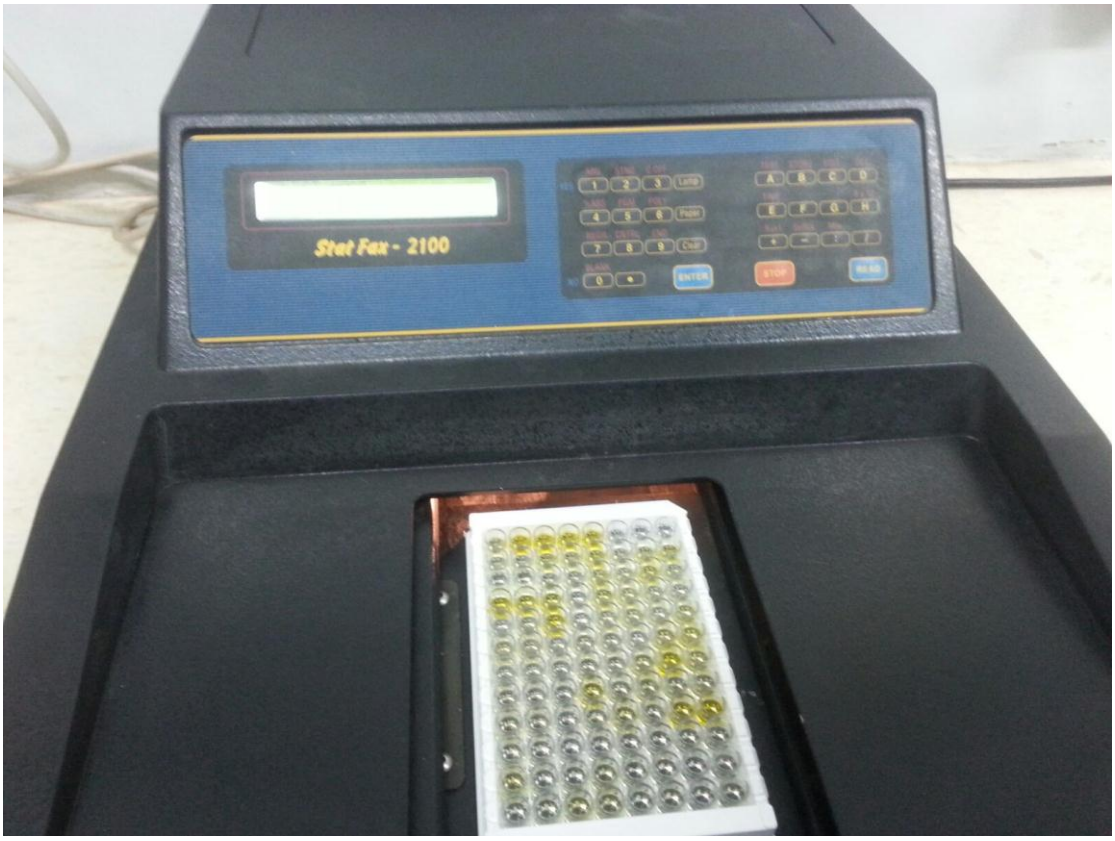
5- Bleeding per vaginum: ……..

6- Signs and symptoms of PID ……..

7- Previous abortions……..

8-History of ectopic pregnancy:…….

An ELISA KITT for detection of C.trachomatis IgG



Reading of ELISA microtiter plate