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Sudan University of Science and Technology

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Sero-detection of Anti-*Chlamydia trachomatis* IgG among Infertile Women with Blocked Fallopian Tube and Pregnant Women at Fertility Centers in Khartoum State

الكشف المصلي للجسم المضاد IgG للمتدثرة الحثرية لدى النساء العقيمات بانسداد في قناة فالوب والنساء الحوامل بمراكز الخصوبة في ولاية الخرطوم

A dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree. Medical Laboratory Sciences (Microbiology)

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2018

الآيـــــــــــــة

قال تعالى:

﴿لاَ يُكَلِّفُ اللَّهُ نَفْسًا إِلاَّ وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لاَ تُؤَاخِذْنَا إِن نَّسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلاَ تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِن قَبْلِنَا رَبَّنَا وَلاَ تُحَمِّلْنَا مَا لاَ طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنتَ مَوْلانَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ﴾

البقرة الآية (286)

DEDICATION

To my beloved parents who made me what I'm today

To my respectful brothers

To my lovely friends

ACKNOWLEDGEMENT

Piously my gratitude and prayers to ALMIGHTY ALLAH for the mercy that followed me during the long way of this research. I owe so much to my supervisor Dr. Omer Khalil for his immense effort to accomplish this work. Special thanks to my collegeaues: Saba Salah and Hind Yosuf for their efforts and boundless support. I am thankful for Dr/ Mohammed awad for his boundless support and helpful advices. Special thanks For Alzaytouna Specialist Hospital and especially to Mr. Ahmed Babiker for their greatest efforts in the accomplishment of this thesis. Thanks extended to Osman Abdelwahab Center, all the fertility centers and all the patients who agreed to participate in this research. Eventually I would like to thank anyone who contributed by any means to this research from commence, during the collection, processing of specimens or the final touches.

ABSTRACT

Chlamydia trachomatis is recognized as one of the most common sexually transmitted pathogen in the world, most infected female are asymptomatic. These untreated women are at risk of developing chronic sequelae leading to tubal pathology causing infertility. The aim of this study to detect the anti-*Chlamydia trachomatis* IgG among infertile women with blocked Fallopian tube and pregnant women and to determine the associatation between *Chlamydia trachomatis* and tubal blockage.

Sixty infertile women with blocked Fallopian tube diagnosed by Hysterosalpingography (HSG) attending fertility centers in Khartoum state and 30 pregnant women were included in this case control study during the period from July to December 2018.

Anti-*Chlamydia trachomatis* IgG was investigated by Enzyme Linked Immunosorbent Assay (ELISA). Infertile women with blocked Fallopian tube and pregnant women were compared and the data were statistically analyzed by Statistical package for the Social Sciences (SPSS) version 16.

The study showed that there was greater prevalence of *chlamydia trachomatis* infection in infertile women with blocked Fallopian tube (18.9%) compared pregnant women (1.1%), the different was found statistically highly significant (p value= 0.005). The prescence of Chlamydia is associated with 11.46 fold risk of tubal blockage (OR: 11.46, 95%CI:1.44-90.95, P=0.002). Also showed the significant high prevalence of *chlamydia trachomatis* IgG in women with age of 32 - 45 years (14.4%), women without history of pelvic inflammatory disease (11.1%) compared to women with pelvic inflammatory disease (8.9%) and infertile women with distal blocked tube (28.3%). The study concluded there was high prevalence

of anti-*Chlamydia trachomatis* IgG among women with blocked Fallopian tube compared to pregnant women and strong association between anti *Chlamydia trachomatis* IgG and tubal blockage.

المستخلص

تعتبر المتدثرة الحثرية واحدة من أكثر مسببات الأمراض المنقولة جنسيا في العالم ومعظم الاناث اللائي يصبن بها لا توجد لديهن اعراض. وهؤلاء النساء غير المعالجات معرضات لخطر تسلسلي مزمن للاصابة يؤدي الى أمراض البوق (الأنابيب) التي تسبب العقم.

تهدف هذه الدراسة للكشف عن الجسم المضاد IgG للمتدثرة الحثرية بين النساء العقيمات اللاتي لديهن انسداد في قناة فالوب والنساء الحوامل وتحديد العلاقة بين المتدثرة الحثرية والانسداد البوقي.

شملت الدراسة 60 مشاركة من النساء العقيمات بانسداد في قناة فالوب مشخصة بالتصوير الملون للرحم وقناتي فالوب (HSG)اللاتي يترددن لمراكز الخصوبة في ولاية الخرطوم و30 مشاركة من النساء الحوامل في الفترة من شهر يوليو الى شهر ديسمبر 2018.

فحص الجسم الضاد للمتدثرة الحثرية بواسطة الانزيم المناعي المرتبط (ELISA) وقورنت النساء العقيمات مع النساء الحوامل كما حللت البيانات إحصائيا باستخدام الحزمة الاحصائية للعلوم الاجتماعية (SPSS) النسخة 16.

أظهرت الدراسة ان انتشار المتدثرة الحثرية أكبر لدى النساء العقيمات بإنسداد الانابيب (18.9%) مقارنة بالنساء الحوامل(1.1%) كما أن الاختلاف كان ذا دلالة احصائية (P=0.005).

كما وجدت الدراسة ان الاصابة بالمتدثرة الحثرية لها ارتباط كبير بمعدل 11.46 مرة بخطورة الانسداد البوقي (OR: 11.46, 95%CI:1.44-90.95, P=0.002)

كما أظهرت الدراسة أيضا ارتفاع معدل انتشار المتدثرة الحثرية في النساء اللاتي تتراوح اعمارهن بين 32-45 سنة (14.4%) و النساء اللاتي ليس لديهن تاريخ اصابة بإلتهابات الحوض (11.1%) مقارنة مع اللاتي لديهن تاريخ اصابة بإلتهابات الحوض(8.9%) والنساء المصابات بالعقم بسسبب انسداد الجزء البعيد من قناة فالوب (28.3%).

وخلصت الدراسة الى ارتفاع معدل انتشارالجسم المضاد IgG للمتدثرة الحثرية بين النساء العقيمات بإنسداد في قناة فالوب مقارنة مع النساء الحوامل ووجود علاقة قوية بين الجسم المضاد للمتدثرة الحثرية والانسداد البوقي.

Table of Contents

CHAPTER ONE: INTRODUCTION

CHAPTER TWO: LITERATURE REVIEW

CHAPTER THREE: MATERIALS AND METHODS

CHAPTER FOUR: RESULTS

CHAPTER FIVE: DISCUSSION

List of Tables

List of abbreviations:

µm	Micromiter
ATP	Adenosine Tri-phosphate
CAT	Chlamydia Antibody Titer
CDC	Centers for Disease Control and Prevention
Chsp	Chlamydia-Heat Shock Protein
DFA	Direct Fluorescent Assy
DIF	Direct Immunefluorescence
DNA	Deoxyribonucleic Acid
EB	Elementary Body
EIA	Enzyme Immuno-assay
ELISA	Enzyme Linked Immuno-sorbent Assay
EMEM	Eagle's Minimal Essential Medium
G	Gram
GLXA	Glycolipid exoantigen
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
HSG	Hysterosalpingography
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunglobulin M
IL	Interleukein
IVF	In Vetro Fertilization
KDa	Killo Dalton
LGV	Lymphogranuloma Venereum

LPS	Lipopolysaccharaide
Mg	Milligram
MIF	Micro Immune-Fluorescence
MOMP	Major Outer Membrane Protein
NAA	Nucleic Acid Amplification
Nm	Nanometer
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Disease
RB	Reticulate body
RNA	Ribonucleic Acid
SPSS	Statistical Package for Social Science
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
TFI	Tubal Factor Infertility
Th	T helper cell
TNF	Tumor Necrosis Factor
VB	Variable Sequences
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

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INTRODUCTION

1.1 Introduction:

The Fallopian tubes are two thin tubes, one on each side of the uterus, which lead the mature egg from the ovaries to the uterus. When an obstruction prevents the egg from travelling down the tube, the woman has a blocked fallopian tubes, it can occur in one or both sides. This is also known as Tubal Factor Infertility (TFI) (Rajeshwari, 2012), which is a common cause of infertility among women and is estimated to be an aetiological factor in 30 % to 40% of infertile women (Tjiam *et* al., 1985). Pelvic inflammatory disease (PID) and upper genital tract infection causing endometriosis salpingitis, tubo-overian abscess and peritonitis in the small pelvis may contribute in tubal infertility, in most cases the infection is ascending, *Chlamydia trachomatis* and *Neisseria gonorrhea* are common with increased incidence (Tjiam *et* al., 1985). *Chlamydia trachomatis* is an obligate intracellular bacterium, infecting cervical epithelial cells in women. Generally, the infection resolves without sequelae, but occasionally it spreads from the lower to the upper genital tract and pelvic inflammatory disease may develop. This may lead to scarring of the fallopian tube, causing occlusion, possibly resulting in ectopic pregnancy or tubal factor infertility (TFI) (Astrid *et* al., 2011).

Association between *chlamydia trachomatis* antibody titers and tubal factor infertility has been known since 1979 and numerous studies have reported on value of chlamydia antibody titer (CAT) testing to predict tubal pathology. Pathogenic process of chlamydial infection is thought to be partly immunological (Singh *et* al., 2016).

Sequel of this infection namely PID is an important cause of tubal factor infertility. It has been observed that sequel is associated with persistent infection rather than single acute episode (Singh *et* al., 2016).

According to the World Health Organization, there is an estimated 448 million new cases of sexually transmitted infections (STI) which are acquired worldwide annually (Alfarraj *et* al., 2015). World Health Organization (WHO) estimates that, globally 98 million adults were infected by *Chlamydia trachomatis* at any point of time in the year 2005 (WHO, 2011). According to U.S. Centre for Disease Control and Prevention (CDC) STD surveillance report 2007: 1,030,911 *Chlamydia trachomatis* infection were reported in 2006 (CDC, 2008), which increased to about 1.24 millions in the year 2009 (Mallika *et* al., 2015). In Sudan the prevalence of *Chlamydia trachomatis* range from 6.2% in some studies to 47.2% in other (lamya *et* al., 2014). According to my knowledge relative information about prevalence of *chlamydia trachomatis* in infertile women in Sudan are spare and this study consider the first study to determine the prevalence of *Chlamydia trachomatis* among infertile women with blocked Fallopian tube.

1.2 Rationale:

Chlamydia trachomatis is the most common sexually transmitted pathogen. It can lead to pelvic inflammatory disease (PID), tubal factor infertility or ectopic pregnancy. Most of the infected patients are asymptomatic and are usually recognized when they are routinely screened as part of infertility investigations or before performing a gynecology procedure (Al-Ramahi *et* al., 2008). Although infection with *Chlamydia trachomatis* has been suggested to be a cause of infertility due to the sequelae on the genital tract according to several studies, many gynecology clinics do not perform routine screening for *Chlamydia trachomatis.*

This makes the magnitude of the problem unclear. Many patients are diagnosed to have unexplained infertility without screening for possible genital tract infection, which leads to inaccurate diagnosis. The infertility which was caused by *Chlamydia trachomatis* represented a preventable type of infertility, if it was detected early (Al-Ramahi *et* al., 2008). Therefor this study was conducted to determine the prevalence of *Chlamydia trachomatis* infection among infertile women with blocked fallopian tube attending fertility centers and to determine if screening for *Chlamydia trachomatis* is necessary as part of routine investigations of infertility.

1.3. Objectives:

1.3.1. General objective:-

To detect the anti-*Chlamydia trachomatis* IgG among infertile women with blocked fallopian tube and pregnant control at fertility centers.

1.3.2. Specific objectives:-

- To determine the seroprevalence of *Chlamydia trachomatis* among infertile women with blocked fallopian tube and pregnant control.
- To associated the risk of *Chlamydia trachomatis* for Tubal blockage.
- To determine the prevalence of *Chlamydia trachomatis* according to age, type of infertility, history of pelvic inflammatory disease, hydrosalpinx, number of blocked tube and type of blockage.

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2.1 Background:

Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium that belongs to the order Chlamydiales and the family Chlamydiaceae (Jeremiah *et* al., 2011). The word Chlamys is Greek for "cloack draped around the shoulder" this describe how the intracytoplasmic inclusions caused by the bacterium are draped around the infected cell nucleus (Hoque *et* al., 2012). It was discovered in 1907 by Helberstaed and Vonprowazek who observed in conjunctival scrapings from an experimentally infected orangutan (Budai, 2007). 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) (Alfarraj *et* al., 2015).

Chlamydia trachomatis is a major cause of genital and eye disease, currently it is recognized as the most common sexually transmitted pathogen (Jeremiah *et* al., 2011).

2.2 Morphology:

Chlamydia is an obligate intracellular bacterial pathogen that requires a eukaryotic host cell to subsequently grow and develop, they possess both Deoxy Nucleic Acid (DNA) and Ribo Nucleic Acid (RNA), have cell wall and ribosomes, replicate by binary fission. They are therefore accepted as bacteria. Unlike other bacteria, they don't have peptidoglycan cell walls. They lack enzymes of electron transport chain and so require Adenosin Tri Phosphate (ATP) and nutrient resources from host cells (Patrik *et* al., 2015).

Chlamydia alternates between two morphological forms during developmental cycle, the elementary body (EB) and the reticulate body (RB) (Patrik *et* al., 2015).

2.3 Developmental cycle:

During infection, Chlamydia undergo a characteristic unique biphasic life cycle within a period of 48 to 72 hours. The infection process is initiated by the attachment of elementary bodies (EBs) to epithelial cells (Kosma, 1999).

Elementary bodies are infectious particles measuring 200 to 300 nm in diameter, which display a three-layered outer membrane. EBs are quite resistant to environmental conditions outside the host cell, making them adapted to cell-cell and host-host transits. The cell walls of EBs are rigidified by major outer membrane protein units being highly cross-linked via disulfide bridges. In addition, the chlamydial major outer membrane protein omp-1 is a glycoprotein of the high-mannose type, and manno-oligosaccharides have been found to mediate attachment and infectivity of *Chlamydia trachomatis* to HeLa cells. Meanwhile it has been demonstrated that human mannose-binding protein is capable to bind high-mannose structures on the surface of chlamydiae and thus may be involved in host defense mechanisms against chlamydial infections (Kosma, 1999).

Heparan sulfate-like glycans being present on the surface of the host cell have been proposed to be involved in the adhesion process, but did not show protective properties when used as inhibitors of genital chlamydial infections in a mouse model. Entry of elementary bodies proceeds by host-driven endocytosis.Within a vacuole, elementary bodies undergo a significant morphological change into the larger (0.8-1 μm in size), metabolically active reticulate bodies (RBs), which are not capable to survive outside the host (Kosma, 1999).

The cell wall of RBs is much less rigid probably due to reductive opening of the covalent disulfide cross-linkings allowing for enhanced metabolic activities and transport of nutrients. By unknown mechanisms, phagosome-lysosome fusion is inhibited. Reticulate bodies multiply by binary fission forming microcolonies as so-called inclusion bodies. Finally, reticulate bodies condense, enhance the rate of biosynthesis of outer membrane proteins and reorganize into infectious elementary bodies, which are released by exocytosis or lysis of the host cells (Kosma, 1999).

2.4 Genome:

Chlamydia trachomatis has a genome that consists of 1,042,519 nucleotide base pairs and has approximately 894 likely protein coding sequences that lack many metabolic enzymes which makes these bacteria reliant on the host for many of their metabolic requirements. *Chlamydia trachomatis* strains have an extrachromosomal plasmid, which was sequenced to be a 7,493 base pair plasmid (Resinkoff *et* al., 2004). The plasmid of *Chlamydia trachomatis* is favored target DNA-based diagnosis of *Chlamydia trachomatis* because there are approximately 7-10 copies of the plasmid present per chlamydia particle. Some *chlamydia trachomatis* strains lack these plasmid, and the consequence aid in detection of the plasmid free variant *Chlamydia trachomatis* strains (Hoque *et* al., 2012).

Plaque purified *Chlamydia trachomatis* that do not contain plasmid have usually inclusion morphology, have no glycogen, and show no alteration in antibiotic sensitivity. However, the fact that existence of such strains show that the plasmid is not essential for *Chlamydia trachomatis* survival (Hoque *et* al., 2012).

2.5 Antigenicity:

Chlamydia antigens consist of four groups: group specific, species specific, Type specific and subspecies specific (Choroszy *et* al., 2012).

2.5.1 The group- specific antigen (genus) is a multi-saccharide -lipid complex which consists of two components:

2.5.1.1-Lipopolysaccharide (LPS), it is present on the surface of EB and RB during the developmental cycle of Chlamydia (Choroszy *et* al., 2012).

2.5.1.2-Glycolipid exoantigen (GLXA), It is located on the surface of EB and RB within the cell membrane of cytoplasmic inclusions, the weight of the molecules and genus-specific antigen features are similar to the lipopolysaccharide antigen of Gram-negative bacteria (Choro szy *et* al., 2012).

2.5.2 Species-specific antigens consist of Major outer membrane protein (MOMP) and heat shock proteins (Magdalena *et* al., 2015).

2.5.2.1- Major Outer Membrane Protein:

It is known to be an immune dominant surface antigen (Walf *et* al., 2001). Major outer membrane protein (MOMP) is a protein whose molecular weight is approximately 40 kDa; it comprises 60% of the weight of the outer membrane proteins (Jinaxin *et* al., 2014).

It is built of four variable hydrophilic domains exposed outside the cell membrane and surrounded by 5 hydrophobic domains occurring on the inner surface (Jinaxin *et* al., 2014).

Antibody recognition was restricted to the antigenically variable major outer membrane protein and a few antigenically conserved antigens. The MOMPs encoded by different *Chlamydia trachomatis* serovars share five well-conserved regions and four variable sequence (VS) domains. *Chlamydia trachomatis* VS domains, and homologous regions in MOMPs from other species (Findley *et* al., 2005).

2.5.2.2- Chlamydia Heat Shock Proteins:

The Chlamydia heat shock protein (c-HSP60) antigen, or heat shock protein, whose molecular weight is 60 kDa is moderately immunogenic in comparison to the poorly immunogenic smaller protein of 12 kDa molecular weight (Magdalena *et* al., 2015), they are invisibly present on the surface of the membrane, and antibodies specific to these proteins do not bind to live EB (Choroszy *et* al., 2012).

2.5.3 Type-specific antigens:

Are probably polypeptides, whose molecular weight is 30 kDa, placed on the surface of EB and RB They are used to differentiate serotypes using the Micro Immune-Fluorescence (MIF) test (Choroszy *et* al., 2012).

2.5.4 Subspecies-specific:

Polypeptides are subspecies-specific antigens. Based on the reactivity measured using the MIF test. *Chlamydia trachomatis* serovars were classified into three subspecies: B, Ba, D, Da, E, L1, L2 and L2A serotypes belong to the so-called group B, serotypes A, C, I, Ia, J and H belong to group C, and K and L3 serotypes that undergo cross-reaction. Group C, F and G serotypes and B subspecies belong to the so-called intermediate group (Choroszy *et* al., 2012).

2.6 Infections and complications:

Chlamydia trachomatis infect mainly mucosal membrane (epithelium) such as the cervix, rectum, urethra, throat and conjunctiva. And it is transmitted through infected secretion therefore, it is primarily spread via sexual contact and manifests as Sexually Transmitted Disease (STD). Most infections of *Chlamydia trachomatis* in men and women are asymptomatic (Gaydose *et* al., 2003).

Chlamydia trachomatis infections in women include acute urethral syndrome, urethritis, bartholinitis, cervicitis, upper genital tract infection (endometritis, salpingo-oophoritis, or pelvic inflammatory disease), perihepatitis (Fitz-Hugh–Curtis syndrome), and reactive arthritis (Jeffrey *et* al., 2003).

Symptoms depend on the site of infection. Infection of the urethra and lower genital tract may cause dysuria, abnormal vaginal discharge, or postcoital bleeding, whereas infection of the upper genital tract (e.g., endometritis or salpingitis) may be manifested as irregular uterine bleeding and abdominal or pelvic discomfort. In women, untreated chlamydial infection can lead to severe reproductive complications. *Chlamydia trachomatis* is an important causal agent in pelvic inflammatory disease (PID), with sequelae including infertility, ectopic pregnancy, and chronic pelvic pain (Jeffrey *et* al., 2003).Up to two thirds of cases of tubal-factor infertility (TFI) and one third of cases of ectopic pregnancy may be attributable to *Chlamydia trachomatis* infection.

Chlamydial infection during pregnancy is associated with a number of adverse outcomes of pregnancy including preterm labor, premature rupture of the membranes, low birth weight, neonatal death, and postpartum endometritis. Chlamydial infection during pregnancy may be transmitted to the infant during de-livery (Jeffrey *et* al., 2003).

An infant born to a mother with active infection has a risk of acquiring infection at any anatomical site of 50 to 75 percent. Approximately 30 to 50 percent of infants born to chlamydia-positive mothers will have conjunctivitis, and at least 50 percent of infants with chlamydial conjunctivitis will also have nasopharyngeal infection. Chlamydial pneumonia develops in about 30 percent of infants with nasopharyngeal infection (Jeffrey *et* al., 2003).

In men, the most common clinical manifestation of *Chlamydia trachomatis* infection is nongonococcal urethritis. In fact, *Chlamydia trachomatis* causes approximately 35 to 50 percent of all cases of nongonococcal urethritis in heterosexual men. Symptoms of nongonococcal urethritis may develop after an incubation period of 7 to 21 days and include dysuria and mild to-moderate whitish or clear urethral discharge. In most cases, physical examination reveals no abnormalities other than the discharge. Other clinical syndromes in men include acute epididymitis, acute proctitis, acute proctocolitis, conjunctivitis, and Reiter's syndrome. Male infertility, chronic prostatitis, and urethral strictures are possible results of infection (Jeffrey *et* al., 2003).

Both Reiter's syndrome (urethritis, conjunctivitis, arthritis, and mucocutaneous lesions) and reactive tenosynovitis or arthritis (without the other components of Reiter's syndrome) have been associated with genital *Chlamydia trachomatis* infection (Jeffrey *et* al., 2003).

Some strains of *Chlamydia trachomatis* cause lymphogranuloma venereum, a venereal disease with genital lesions and regional lymph node involvement (buboes) (Weinstock *et* al., 1994).

Infection with *Chlamydia trachomatis* is also believed to be a cofactor for the transmission of human immunodeficiency virus in both men and women (Jeffrey *et* al., 2003).

Chlamydia trachomatis also cause trachoma, which characterized by the development of follicles and inflamed conjunctivae. The cornea may become cloudy and vascularized; repeated infections are a common cause of blindness (Weinstock *et* al., 1994).

2.7 Pathophysiology:

Chlamydia trachomatis infection may be primary or a chronic recurrence/ re-infection (Malhorta *et* al., 2013).

2.7.1 Primary infection: A serial infection of the mucosal cells is seen during the primary infection. The damaging and infected epithelial cells secrete numerous pro-inflammatory chemokines and cytokines, including IL-1, IL-6, IL-8, granulocyte - macrophage colony stimulating factor (GM-CSF), growth regulated oncogene, and tumor necrosis factor alpha (TNFα). The released cytokines cause vasodilatation, increased endothelial permeability, activation and influx of neutrophils, monocytes and T-lymphocytes, and elevated expression of adhesion molecules. In addition, it stimulates other cells to secrete cytokines (Malhorta *et* al., 2013).

Neutrophils appear to play a role in reducing the initial amplification of *Chlamydia trachomatis* and possibly in limiting the spread within the female genital tract. IL-1 is secreted initially by the undamaged cells and stimulates the secretion of other cytokines from other non-infected cells, like TNF-α20. During the same period, Chlamydia passes via lymphatic vessels to local lymph nodes. The decaying

epithelial cells release a few elementary bodies which are phagocytosed by neutrophills through phagolysosomes (Malhorta *et* al., 2013).

T lymphocytes mainly T helper cells (Th1) play an important role during early phase of infection. Which, due to Chlamydia antigen-induced activation, secrete IFN-γ, necessary for infection regression (Malhorta *et* al., 2013).

It increases the potential of various phagocytes to destroy Chlamydia and stimulates the secretion of other cytokines, including IL-1. IL-1, in turn, by stimulating the secretion of IL-2 by Th1 cells causes increased replication of cytotoxic lymphocytes and natural killer cells. The role of secretory IgA has also been established in the neutralization of primary infection (Malhorta *et* al., 2013)

2.7.2 Chronic infection - recurrence/reinfection: Chronic infection, associated with persistence of Chlamydia in the host cells in the lymph node and spleen, recurrent infection or reinfection are more dangerous. A delayed hypersensitivity reaction or rarely type 3 hypersensitivity reactions (Arthus reaction) is observed in long term or recurrent stimulatory action of chlamydial antigens. Antibodies are not involved in the delayed type of reaction developing within 24-48 hours due to antigen interaction with specifically sensitized Th1 lymphocytes. Processes which occur during these reactions lead to tissue damage, fibrosis and cicatrization within the affected organs. Irreversible consequences like PID leading to mechanical infertility, ectopic pregnancy, chronic pelvic pains and chronic urethritis may occur. After a single episode of salpingitis about one in 10 patients become infertile because of tubal occlusion. After 2-3 episodes, infertility ensues in about 35-70 per cent cases (Malhorta *et* al., 2013).

2.8 Laboratory diagnosis:

Chlamydia trachomtis infection of women's urogenital systems can be diagnosed by urinanalysis or cytopathology of the urethra, cervix uteri or vagaina (Magdalena *et* al., 2015).

Chlamydia trachomatis in men's urethra can be detected by examination of urethra or urinanalysis. A nucleic Acid Amplification (NAA) test, cell culture, direct immune-fluorescence (DIF), and enzyme immunoassay (EIA) are used to detect *Chlamydia trachomatis* in cytopathology of men and women (Magdalena *et* al., 2015).

2-8.1 Direct cytological examination:

Infection of the conjunctiva can be diagnosed by the detection of typical intracytoplasmic inclusions by Giemsa stain. The inclusion are basophilic and stain pinkish-blue. Cytological testing to detect inclusions is particularly useful in diagnosing acute inclusion conjunctivitis of the newborn; the sensitivity of this method exceeds 90% (Cherneskey, 2005).

2.8.2 Isolation in cell culture:

Until recently, culture was considered the gold standard for detection of *Chlamydia trachomatis* in urogenital specimens because it has aspecificity that approachs 100%. Since culture detects only viable infectious Chlamydial elementary bodies and has minimal potential for contamination (Carolyn, 1997).

Culture is performed by inoculating specimens onto cell culture monolayers and infection appear in the cell as intracytoplasmic inclusion. The inclusions are visualized following 48-72 hours of incubation by staining with fluorescently labeled antibodies that bind chlamydial LPS to recognize all chlamydial species or

MOMP for Chlamydia trachomatis specific recognition. Cells monolayers for culture of *Chlamydia trachomatis* are grown in dram or shell vial on glass coverslips or in the wells of multi-well cell culture dishes. Traditionally, McCoy (195, 200, 242), HeLa 229(108, 170), and more recently, BGMK (89, 106) cells have been used to support the growth of *Chlamydia trachomatis*. The most commonly used growth medium is Eagle's Minimal Essential Medium (EMEM) (Carolyn, 1997).

2.8.3 Enzyme immune assay (EIA):

A number of commercial EIAs are available for the detection of Chlamydial antigens in clinical specimens (Cherneskey, 2005).

These procedure use either monoclonal or polyclonal antibodies to detect chlamydial lipopolysaccharide (LPS), which is more soluble than the major outer membrane protein (MOMP). Most EIAs take several hours to perform and are suitable for batch processing. The sensitivity profiles of the commercially available *Chlamydia trachomatis* EIAs range from 65% to 75% compared with NAA assay. Without confirmation the test have a specificity of 97% (Cherneskey, 2005).

2.8.4 Direct Fluorescent Assay (DFA):

Several DFA assays are commercially available and use monoclonal antibodies directed against MOMP or LPS. Monoclonal antibodies to the LPS will stain all Chlamydiae, but the specimen may be more difficult to read because of the uneven distribution of LPS on the chlamydial particle. The anti-MOMP monoclonal antibodies are prepared against *Chlamydia trachomatis*; therefor, they are species-specific (Carolyn, 1997).

The quality of fluorescence is better because MOMP is evenly distributed on the chlamydial particle. This procedure offers rapid diagnosis. A variation on the DFA

procedure involves centrifugation of the transport medium being used for other tests (cell culture, NAAT or EIA), preparation of a slide from the sediment, and staining with the fluorescent antibody reagents (Cherneskey, 2005).

This is often used as a confirmatory test for positive in other tests. DFA requires the expertise of an experienced microscopist, and the procedure is presently not amenable to automation (Cherneskey, 2005).

2.8.5 Nucleic Acid Hybridization assay (NAH):

Commercially available NAH tests for *Chlamydia trachomatis* in some parts of the world have been used. One commercially probe test (PACE 2, Gen-Probe Inc, USA) uses DNA-RNA hybridization in an effort to increase sensitivity by detecting chlamydial RNA (Cherneskey, 2005).

2.8.6 Nucleic Acid Amplification assay (NAA):

Polymerase Chain Reaction (PCR) Ligase Chain Reaction and strand displacement amplification assays amplify nucleotide sequences of cryptic plasmid, which is present in multiple copies in each *Chlamydia trachomatis* EB. The transcription mediated amplification reaction is directed against ribosomal RNA, which is also present in multiple copies. The NAA assays have become the tests of choice for the diagnosis of *Chlamydia trachomatis* infection in routine clinical laboratories (Cherneskey, 2005).

2.8.7 Serology:

Serology is not recommended for diagnosing chlamydial infections, with the exception of infection neonates (high immunoglobulin”Ig” M), patients with tubal factor infertility (TFI) (high IgG), and occasionally for LGV infection. The microimmunofluorescence test is the current method of choice for the

serodiagnosis of chlamydial infection. Other assays include the whole inclusion immunofluorescence test, EIA using RBs, RBs or infected cells and recombinant enzyme-linked assay (Cherneskey, 2005).

2.9 Prevention:

There is no vaccine against any chlamydial disease (Warren, 2008). The chlamydial infection is highly prevalent, usually asymptomatic are associate with significant morbidity, can be reliably diagnosed and treatable (Egget, 1999).

Screening programs for Chlamydia trachomatis will be of paramount importance in the prevention of long-term sequelae. Current strategies to control Chlamydia trachomatis still largely depend on clinic-based screening of symptomatic patient (Egget, 1999).

There are a number of ways to prevent, or at least significantly reduce, the incidence of genitourinary chlamydia infection. The most definitive methods of prevention are practicing abstinence and being in a long-term, mutually monogamous relationship. Patients should be encouraged to avoid high-risk behaviors such as having unprotected sex or multiple sex partners. In addition, the correct and consistent use of condoms has been shown to reduce the risk of transmission of sexually transmitted diseases. For adolescents who are considered high risk, specific education about the transmission of disease through unprotected vaginal, anal, or oral sex is warranted (Mishori *et* al., 2012).

2.10 Treatment:

Azithromycin and doxycycline are very efficient in the treatment of Chlamydia infection (Magdalena *et* al., 2015).

Uncomplicated genitourinary chlamydia infection should be treated with azithromycin (Zithromax; 1 g, single dose) or doxycycline (100 mg twice daily for seven days). Studies indicate that both treatments are equally effective. Alternative regimens for uncomplicated chlamydia infection include erythromycin (500 mg four times daily for seven days), erythromycin ethylsuccinate (800 mg four times daily for seven days), levofloxacin (Levaquin; 500 mg once daily for seven days), or ofloxacin (Floxin; 300 mg twice daily or 600 mg once daily for seven days). Erythromycin is reported to have higher occurrences of gastrointestinal adverse effects. Pregnant women may be treated with azithromycin (1 g, single dose) or amoxicillin (500 mg three times daily for seven days). Alternative regimens include erythromycin (500 mg four times daily for seven days or 250 mg four times daily for 14 days) and erythromycin ethylsuccinate (800 mg four times daily for seven days or 400 mg four times daily for 14 days) (Mishori *et* al., 2012).

In complicated genitourinary Studies suggest that prolonged antimicrobial therapy, up to six months of combination antibiotics, may be effective.

Trachoma treated with Azithromycin, 1 g (single dose) or Doxycycline, 100 mg twice daily for 21 days (Mishori *et* al., 2012).

2.11 Tubal Blockage:

Fallopian tubes are very delicate structures that are responsible for picking up the egg and providing the site of for fertilization of the egg as well as early embryo development and transport to the uterine cavity. The cells lining the tube produce secretion that nourish the egg and embryo. The tubes may be damaged by infections or other pelvic condition. A prior history of pelvic inflammatory disease (PID), tubal surgery, ectopic pregnancy, rupture appendix, ovarian surgery or septic abortion strongly suggests the possibility of tubal disease. PID is clearly the

major cause of tubal factor infertility and ectopic pregnancies. The number and severity of pelvic infections increase the risk of tubal damage. However, some women who are found to have pelvic adhesions and/or tubal disease have no known prior history of pelvic infection. These (salient) infection are most often caused by Chlamydia (Laurence and Jacobe, 2015).

Tubal Factor Infertility (TFI) is due to any anatomic abnormality that prevents the union of sperm and egg. Proximal tubal occlusion prevents sperm from reaching the distal portion of the Fallopian tube where fertilization normally occurs. Distal tubal adhesions or occlusion prevent egg from pickup from adjacent ovary. Distal tubal disease can range from mild (fimbrial adhesion) to sever (complete occlusion). If blockage occurs only at the distal end, then the secretions of the fallopian tube will not be able to drain out of the end of the tube. The resulting accumulation of fluid in the tube (Hydrosalpinx) has a very negative affect on fertility even when couples utilize in vitro fertilization (IVF) for treatment (Laurence and Jacobe, 2015).

2.11.1 Diagnostic evaluation:

The diagnostic evaluation of tubal patency in infertile women can be accomplished by performing: laparoscopy, a Hysterosalpingogram (HSG) or saline sonohystogram. Each procedure has advantages and dis advantages (Laurence and Jacobe, 2015).

2.11.2 Treatment:

Essentially, with (TFI), treatment options are reconstructive surgery or IVF. Over the last decade, for most conditions IVF success rates have steadily increased to now exceed those that can be achieved with surgery. In addition, ectopic pregnancy rates are considerably higher after tubal surgery compared to IVF.

Consequently, IVF has become the treatment of choice for much or most TFI. However, surgery remains a valid option under certain circumstances (Laurence and Jacobe, 2015).

2.12 *Chlamydia trachomatis* and tubal blockage:

Chlamydia trachomatis is the leading cause of bacterial sexually transmitted disease worldwide resulting in 4-5 million new cases of chlamydia annually and an estimated 100 million cases per annum. Infection of the lower female genital tract frequently are asymptomatic; thus, they often remain undiagnosed or untreated. If infections are either not resolved or left untreated, chlamydia can ascend to the upper genital tract and infect the fallopian tubes causing salpingitis that may lead to functional damage of the fallopian tubes and TFI. Clinical observations and experimental data have indicated a role of antibodies against chlamydia trachomatis proteins such as 60-kDa heat shock protein 60 (cHSP60) in the immunopathogenesis of TFI. When released from infected cells, cHSP60 can induce proinflammatory immune responses that may functionally impair the fallopian tubes leading to fibrosis and luminal occlusion (Hafner, 2015).

Chlamydial pathogenesis of irreversible and permanent tubal damage is a consequence of innate and adaptive host immune responses to ongoing or repeat infections (Hafner, 2015).

2.13 Previous study:

The association between *Chlamydia trachomatis* infection and infertility has been the subject of several research. In developing countries, data about the prevalence of genital *chlamydia trachomatis* infection and their sequelae, especially tubal infertility, is scare (Siemer *et* al., 2008). A study in Iran suggested the significant association between *Chlamydia trachomatis* infection and female infertility with a

prevalence rate of 15.3% (Marashi *et* al., 2014). The same finding was suggested by a study conducted in India, which confirmed the significant association between infertility and the duration of *Chlamydia trachomatis* infection (Hajikhani *et* al., 2013). Furthermore, another study suggested that a positive serology screening result for *Chlamydia trachomatis* is predictive for both tubal damage, and a reduced pregnancy rate (Keltz *et* al., 2013). A prevalence rate of 9.6% was found in female patients attending the infertility clinic in a study carried out in Nigeria (Nwankwo *et* al., 2014).

Other prevalence rates include studies carried out in the USA (5-15% prevalence rate) (Alsharief, 2011), UK (16%) (Pimenta *et* al., 2000), Jordan (3.9%) (Al-ramahi *et* al., 2008), Iran (22%) (Afrakhtch *et* al., 2008) and Brazil (10.9%) (Fernandes *et* al., 2014).

Another study was carriedout in Ghanaian women with primary and secondary infertility and healthy pregnant women (as control group). Significantly higher prevalence of specific IgG (39% versus 19%) antibody was found among infertile women than control. Their data suggest that previous *Chlamydia trachomatis* infection may contribute to infertility in Ghanaian women (Siemer *et* al., 2008).

In other study was performed in India to find out the association of genital *Chlamydia trachomatis* with female infertility, IgG seropositivity was significantly higher (15%) in cases than (0%) controls (Mallika *et* al., 2015).

Other study was performed in Saudi Arabia to determine the prevalence of *Chlamydia trachomatis* among Saudi women attending the infertility clinic their result was found a statistically significant greater prevalence of *Chlamydia trachomatis* infection in infertile women (8.0%) compared with fertile group (1.0%) (Alfarraj *et* al., 2015).

Also Chlamydial serologic studies with blocked fallopian tube was performed in Indian women with primary infertility to investigate the association between antibodies to *Chlamydia trachomatis* and age, type and site of blockage, their result showed that a peak seropositivity was noticed in the 21-25 years, also showed the maximum number of subjects who had a positive Chlamydial serology with bilateral tubal blockage (59.94%), followed by those with a bilateral hydrosalpinx (19.98%) (Surana *et* al., 2012).

The pattern of the blockage at various sites within the fallopian tube and it is correlation with the anti-Chlamydia antibody suggested that the Chlamydial infection were more likely to be associated with peripheral occlusion (distal) (Surana *et* al., 2012)

Akande *et* al., (2003) in study of tubal damage in infertile women: prediction using Chlamydia serology showed that the antibody titers of *Chlamydia trachomatis* were found higher among infertile women with secondary infertility than women with primary infertility ($P<0.001$). Also titers was found higher among women without history of Pelvic inflammatory disease ($P<0.001$) (Akande *et* al., 2003).

Another study was performed in Saudi Arabia to detect *Chlamydia trachomatis* among infected women, they detected infection with *Chlamydia trachomatis* according to the age of patients, and found that infection with *Chlamydia trachomatis* was interversly related to age, and prevalent associations were recorded between *Chlamydia trachomatis* infection and age less than 25 years (Fadwa, 2011).

Theils *et* al., (1991) performed study in infertile Sweden women to detect *Chlamydia trachomatis* using tissue culture, direct antigen detection and serology and when analyzed Chlamydia serologically were found high prevalence of

Chlamydia was found in women with Tubal factor infertility had no signs of prior pelvic inflammatory disease in compared with women had signs of prior pelvic inflammatory disease also found the high prevalence of *Chlamydia trachomatis* was found in women with secondary infertility (Theils *et* al., 1991)

On study carried by Singh *et* al., (2016) that aimed to define the role of Chlamydia antibody test in predicting tubal pathology and its nature at India. The women on their study were evaluated according to age, and type of infertility and site and type of blocked (Singh *et* al., 2016).

They revealed only 5% (10/200) of women were seropositive for anti-Chlamydial IgG antibody, also their result showed that the high prevalence was found in women with secondary infertility and age group 24-30 years, according to tubal pathology high prevalence was found in women with distal tubal blocked and women unilateral blocked tube without hydrosalpinx (Singh *et* al., 2016).

According to my knowledge in most parts of Sudan these organism are not screened for, and hence relative information about frequencies of the organisms are sparse.

CHAPTRE THREE
MARERIAL AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design:

This was case control study.

3.2 Study area:

Fertility centers in Khartoum state (Banoon center for Assisted Reproduction and Obstetric Gynecology, Elsir Abu Elhasan fertility center, Khartoum university center for fertility, Nile fertility center, Military hospital" Fertility clinc", and Osman Abdelwahab center for Radiology) .

3.3 Study duration:

This study was conducted during the period from July to December 2018.

3.4 Study population:

Study was conducted in infertile women with blocked fallopian tube and healthy pregnant women as control.

3.5 Inclusion criteria:

Cases of primary and secondary female infertility with blocked Fallopian tube confirmed by hysterosalpinography (HSG). were included in the study

3.6 Exclusion criteria:

Diagnosed cases of female infertility due to other reasons such as endometriosis, polycystic ovarian disease….etc. were excluded from the present study.

3.7: Sample size:

Total of Ninety (n=90) blood samples were collected in plain containers, 60 infertile women with blocked fallopian tube and 30 pregnant women as controls. According to single proportion formula: $n = \frac{z^2pq}{d^2}$

n = sample size

z = the normal standard deviate (z = 1.96)

p = the frequency of occurrence of an event from the previous surveys

q = 1-p (the frequency of non-occurrence of an event)

d = degree of precision (0.04%)

3.8: Sampling technique:

Infertile women with blocked fallopian tube were randomly selected.

3.9: Data collection:

Data was collected using self-administrated questionnaire (Appendix 1).

3.10 Ethical consideration:

Approval had been taken from Sudan University of Science and Technology (SUST) College of Medical Laboratory Sciences, Khartoum State Ministry of Health "Research Department", Permission to carriedout the study had been taken from fertility centers and verbal consent had been taken from infertile women with blocked fallopian tube and pregnant control.

3.11 Specimens collection:

Three milliliters (ml) of venous blood sample was collected from infertile women with blocked fallopian tube and pregnant control under study after their consent into plain containers for serological procedure.

3.12 Laboratory work:

3.12.1 Preparation of specimens:

Blood samples were allowed to clot and then centrifuged at 3000 rpm for 5 minutes to obtain serum which was transferred into Eppendorf tubes and stored at -20 °C until the serological analysis.

3.12.2 Sample analysis:

The samples were analyzed for *Chlamydia trachomatis* immunoglobulin IgG by a commercially available Enzyme Linked Immuno-sorbent assay (ELIZA) kit (indirect ELISA) "EUROIMMUNE Midizinische Labordiagnostica AG (Germany)" The assay was performed following the instruction of the manufacturer. Positive and negative controls were included in the assay. According to information included in the kit, the immunoassay used has specificity of 97.0%.

3.12.3 Principle of the assay:

The ELISA test kit provides a semi quantitative or quantitative *in vitro* assay for human antibodies of the immunoglobulin class IgG against species specific *Chlamydia trachomatis* MOMP " major outer membrane protein" in serum or plasma for the diagnosis of trachoma, infection of urogenital tract and lymphogranuloma venereum.

Persisting or chronic *Chlamydia trachomatis* infection can be reliably diagnosed.

The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified *Chlamydia trachomatis* antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens.

To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction (instruction sheet).

3.12.4 Procedure:

The technique was done according to the instruction sheet . All reagents and samples had been brought to room temperature (18-25 °C) at least 30 minutes before use and then mixed thoroughly by vortex.

The patient samples were diluted 1:101 in sample buffer for analysis and washing buffer was prepared with distilled water, then 100µl of the calibrator, positive and negative controls and diluted patient samples were added to their respective wells by using separate disposable tips for each sample, then covered the plate with plate cover and incubated for 30 minutes at room temperature.

At the end of incubation the plate cover was removed and discharged. Each well was Washed 3 times with diluted wash buffer, then the plate was tapped on clean towel to remove any reminders. A100µl of enzyme conjugate (peroxidase-labelled anti-human IgG) in each of the wells was added then the plate was incubated for 30 minutes at room temperature. After that the wells was empty and washed 3 times.

Finally a100µl of chromogen/substrate solution was dispensed into each well, then the plate was incubated for 15 minutes at room temperatures and light was avoided. The reaction was stopped by using the stop solution. The blue colour was turned to yellow colour after addition of stop solution.

The colour intensity measured by photometer device at wavelength of 450nm within 30 minutes of adding the stop solution. The intensity of colour directly proportional to the amount of antibodies in samples.

3.12.5 Quality control and calculation of results:

Reagent, standard and control were checked for storage, stability and preparation before starting work.

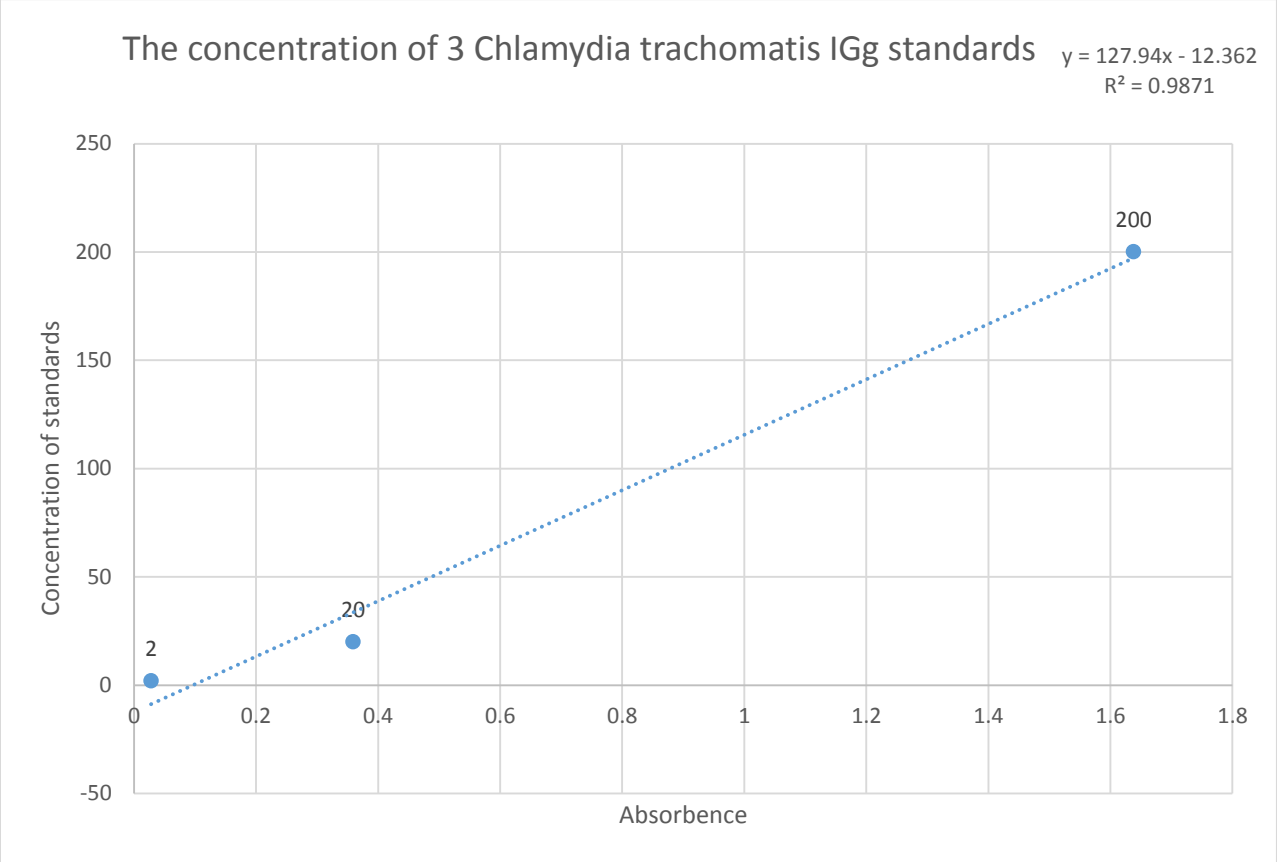
Stat fax 4200 Reading device was used to estimate absorbance in each well then, the absorbance was converted into concentrations by drawing an absorbance/concentrations curve from the given 3 standards. All the calculations were carried out using Excel program depending on the following Equation according to manufacturer's instructions.

Y= 127.94X-12.362

Y= Concentration.

X= Absorbance.

R2= Linearity



The concentration of three *Chlamydia trachomatis* IgG standards

3.12.6 Interpretation of results:

< 16 RU/ml:	Negative
≥ 16 to < 22 RU/ml:	Borderline
≥ 22 RU/ml:	Positive

3.13 Data management and statistical analysis:

Data was computed and analyzed by using Statistical Package for Social Science (SPSS) for computer software version 16 for interpretation of results. Significance of difference was determined using Chi-square test and logistic regression was used to determine the association *between Chlamydia trachomatis* and tubal blockage.

CHAPTER FOUR
RESULTS

CHATER FOUR

RESULTS

A total of 90 blood serum samples were screened for presence of *Chlamydia trachomatis* IgG antibody. Of these samples, 60 were from infertile women with blocked Fallopian tube and 30 from pregnant women as control.

Most of the infertile women with blocked fallopian tube (35.6%) were between the ages of 18-31 years, while (16.7%) of pregnant controls were between the same ages.

Fifteen infertile women with blocked fallopian tube had history of previous pelvic inflammatory disease and so was sex of pregnant control (Table 1).

Among the infertile women with blocked fallopian tube17 (18.9%) had IgG antibody to *Chlamydia trachomatis* in their serum, while 1 (1.1%) of pregnant women was positive for the antibody (Table 2). This differences was statistically significant (P =0.005).

When associated the risk of *Chlamydia trachomatis* IgG for tubal blockage, *Chlamydia trachomatis* was found 11.46 Fold risk for Tubal blockage like in table (5).

The subjects aged 32-45 had the highest positivity rate (14.4%) for *Chlamydia trachomatis* IgG antibody compared to (5.6%) of positivity in age group 18-31.The differences was statistically significant (P=0.020).

Ten (11.1%) of subjects who tested positive to *Chlamydia trachomatis* IgG had no history of pelvic inflammatory disease while 8(8.9%) of the subjects who tested positive had history of pelvic inflammatory disease. The differences was statistically significant (P=0.018) (Table 3).

In Comparing *Chlamydia trachomatis* IgG with type of infertility and histerosalpinography characteristics in infertile women with blocked fallopian tube as shown in table 4. A total of 8 (13.3%) of those with primary infertility and 9 (15.0%) of those with secondary infertility tested positive. The differences was of no statistical significance (p=0.437). Statistically significant highest prevalence of 17 (28.3%) was found in women had distal blocked tube in comparison with women had proximal blockage 0 (0%)

Also high prevalence was found in women without hydrosalpinx and women who had unilateral blocked tube (25.0%) and (21.7%), respectively and the difference was statistically insignificant (P> 0.05).

Table 1: Socio-demographic and reproductive characteristics of cases and controls n (%)

Characteristics		Cases (60)	Controls (30)	Total
Age in year	18- 31	32 (35.6)	15 (16.7)	47 (52.2)
	32 -45	28 (31.1)	15 (16.7)	43 (47.8)
History of PID	Yes	15 (16.7)	6 (6.7)	21 (23.3)
	No	45 (50.0)	24 (26.7)	69 (76.7)

Table 2: Sero-detection of *Chlamydia trachomatis* IgG antibody among cases and controls

Chlamydia IgG antibody	Cases n(%)	Controls n(%)	P value
Positive	17(18.9)	1(1.1)	0.005
Negative	43(47.8)	29(32.2)	

Table 3: *Chlamydia trachomatis* IgG seropositivity accordindg to age and history of pelvic inflammatory disease in cases and controls

Variable		IgG positive n(%)	IgG negative n(%)	P value
Age	18-31	5 (5.6)	42 (46.7)	0.020
	32-45	13 (14.4)	30 (33.3)	
	Total	18 (20.0)	72 (80.0)	
History of PID	Yes	8 (8.9)	13 (14.4)	0.018
	No	10 (11.1)	59 (65.6)	
	Total	18 (20.0)	72 (80.0)	

Table 4: *Chlamydia trachomatis* IgG serpositivity according to type of infertility and HSG characteristics (N=60)

Characteristics		IgG positive n (%)	IgG negative n (%)	Total	P value
Type of infertility	Primary	8 (13.3)	25 (41.7)	33 (55.0)	0.437
	Secondary	9 (15.0)	18 (30.0)	27 (45.0)	
	Total	17 (28.3)	43 (71.7)	60 (100.0)	
Site of blockage	Distal	17 (28.3)	33 (55.0)	50 (83.3)	0.029
	Proximal	0 (0.0)	10 (16.7)	10 (16.7)	
	Total	17 (28.3)	43 (71.7)	60 (100.0)	
Number of blocked tube	Unilateral	13 (21.7)	34 (56.7)	47 (78.0)	0.826
	Bilateral	4 (6.7)	9 (15.0)	13 (21.7)	
	Total	17 (28.3)	43 (71.7)	60 (100.0)	
Hydrosalpinx	Yes	2 (3.3)	5 (8.3)	7 (11.7)	0.988
	No	15 (25.0)	38 (63.3)	53 (88.3)	
	Total	17 (28.3)	43 (71.7)	60 (100.0)	

Table 5: Risk of *Chlamydia trachomatis* IgG for Tubal blockage by logistic regression

Variable	OR	95% CI	P value
Chlamydia trachomatis IgG	11.465	1.445 – 90.954	0.002

CHATER FIVE
DISCUTION

CHAPTER FIVE
DISCUSSION

In this study the prevalence of *Chlamydia trachomatis* IgG antibody was significantly higher (18.9%) in the infertile women with blocked Fallopian tube than (1.1%) in the pregnant women (P=0.005). The significant difference in IgG seropositivity between cases and controls of present study is in agreement with studies of siemer *et* al., 2008 at Ghana and Mallika *et* al., 2015 in India. And Alfarraj *et* al., 2015 at Saudi Arabia who also found statistically significant differences between the IgG seroprevalence of infertile and pregnant women ($p<0.001$) (Alfarraj *et* al., 2015). This significant difference of IgG seropositivity was further strengthend by the finding in this study of 11.46 fold increase in the risk of tubal blockage in women with Chlamydia antibody (OR 11.46, 95%CI .1.44 – 90.95, P =0.002).

This study showed that subjects aged 32-45 years had the highest positivity rate (14.4%) for *Chlamydia trachomatis* antibody (p=0.020). This finding is inconsistent with those of most studies that shown a decline in the prevalence rate after 25 years of age. The reason for the high positivity of *Chlamydia trachomatis* IgG antibody in subject aged 32-45 years in this study, may be because of the frequent persistence of anti-Chlamydial IgG antibodies for prolonged period of time, even among women who have been treated with antibiotics or due to reinfection with *Chlamydia trachomatis*.

Eight (8.9%) of respondents with and 10 (11.1%) without history of pelvic inflammatory disease tested positive to IgG, and this was statistically significant (P=0.018) this similar to result obtained by Akedne *et* al (2003) and theils *et* al (1991) who found the high sero-prevalence of *Chlamydia trachomatis* IgG in

women without history of pelvic inflammatory disease (Theils *et* al., 1991) (Akedne *et* al., 2003). This lends credence to the fact that clinical history is not reliable in making a diagnosis of Chlamydial infection and World Health Organization (WHO) estimates that 70% - 80% of women infected with *Chlamydia trachomatis* are symptom-free (WHO, 2011).

Comparing *Chlamydia trachomatis* IgG with type of infertility, 9 (15.0%) and 8 (13.3%) of those with secondary and primary infertility, respectively tested positive. The difference was of no statistical significance (p=0.437) this finding was similar to that obtaind in Gavle/ Sweden (Thieils et al., 1991) and inconsistent in analysis with study by Akande *et* al (2003) which showed that antibody titers were found to be significantly higher among infertile women who had previously conceived compared to those with primary infertility (Akande *et* al., 2003). This finding may be related to vaginal delivery and abortion which considered a risk factor for acquiring ascending genital infection.

According to site of tube which blocked, seropositivity of *Chlamydia trachomatis* was found with highest rate (28.4%) in women with distal blocked in comparison to (0%) in women with proximal blocked (p=0.029), similar result obtained in India by Singh *et* al., (2016) and Surrana *et* al., (2012). This may be due to the coloumner epithelium cells which Chlamydia infect was found in the distal site of Fallopian tube. Prevalence of (25.0%) and (21.7%) was found in infertile women without hydrosalpinx and unilateral tubal blocked, respectively compared to prevalence of (3.3%) and (6.7%) in women with hydrosalpinx and bilateral tubal blocked, respectively (p>0.05) Similar result obtained in India (Singh *et* al., 2016). This finding may be due to the infection was diagnosed in early stage and not spread yet.

Conclusion:

The study concluded that the prevalence of *Chlamydia trachomatis* were higher in infertile women with tubal factor infertility (18.9%) compared to pregnant control (1.1%) and there was strong association between Chlamtdia IgG antibody positivity and tubal factor infertility (OR 11.46, 95%CI 1.44 – 90. 95).

Recommendations:

- Routine screening for *Chlamydia trachomatis* in gynecology clinics to avoid the complications of Chlamydial infection for infertility.
- Further studies (large longitudinal studies) with advanced technique are required to clarify the other risk factor for tubal factor infertility.

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Appendix (1)

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Sero-detection of anti-*Chlamydia trachomatis* IgG among infertile Women with Blocked Fallopian Tube and Pregnant Women at Fertility Centers in Khartoum State

Sample number ( )

Age:

18-31 ( ) 32-45 ( )

History of pelvic inflammatory disease:

Yes ( ) No ( )

Type of infertility:

Primary ( ) Secondary ( )

Number of blocked tube:

Bilateral ( ) Unilateral ( )

Site of Blockage:

Distal ( ) Proximal ( )

Hydrosalpinx:

Yes ( ) No ( )

APPENDIX (2)

Reagents of ELISA kit

Yellow colour of positive reaction

Stat fax reader

EUROIMMUN Medizinische Labordiagnostika AG

Anti-Chlamydia trachomatis ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2191-9601 G	Chlamydia trachomatis	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the immunoglobulin class IgG against Chlamydia trachomatis in serum or plasma for the diagnosis of trachoma, infections of the urogenital tract and lymphogranuloma venereum.

Application: By determination of antibodies against the species specific Chlamydia trachomatis MOMP antigen (major outer membrane protein), persisting or chronic Chlamydia trachomatis infections can be reliably diagnosed and clearly delimited by differential diagnosis from infections with other species of Chlamydia. In acute and peripherically localised Chlamydia trachomatis infections however, the direct detection of the pathogen (e.g. PCR) is the method of choice.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified Chlamydia trachomatis antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. **Microplate wells coated with antigens** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	—	12 x 8	STRIPS
2. **Calibrator 1** 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. **Calibrator 2** 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. **Calibrator 3** 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. **Positive control** (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. **Negative control** (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. **Enzyme conjugate** peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. **Sample buffer** ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. **Wash buffer** 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. **Chromogen/substrate solution** TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. **Stop solution** 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. **Test instruction**	—	1 booklet	
13. **Quality control certificate**	—	1 protocol	

LOT Lot description
IVD In vitro diagnostic medical device
CE 0197
Storage temperature
Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

EUROIMMUN Medizinische Labordiagnostika AG · 23560 Lübeck (Germany) · Seekamp 31 · Tel. +49 451 5855 0 · Fax +49 451 5855 591

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer.
For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantitative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), 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.

Note: Residual liquid (>10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.
Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 2	P 6	P 14	P 22			C 1	P 4	P 12	P 20		
B	pos.	P 7	P 15	P 23			C 2	P 5	P 13	P 21		
C	neg.	P 8	P 16	P 24			C 3	P 6	P 14	P 22		
D	P 1	P 9	P 17				pos.	P 7	P 15	P 23		
E	P 2	P 10	P 18				neg.	P 8	P 16	P 24		
F	P 3	P 11	P 19				P 1	P 9	P 17			
G	P 4	P 12	P 20				P 2	P 10	P 18			
H	P 5	P 13	P 21				P 3	P 11	P 19			

The pipetting protocol for microtiter strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7 to 10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

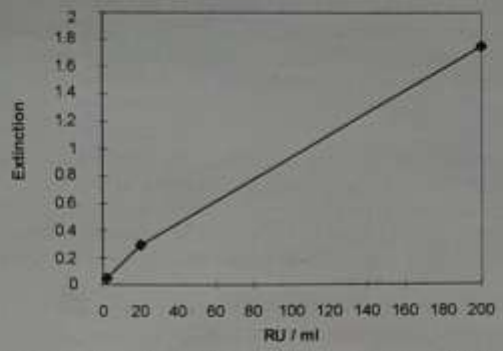
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as ">200 RU/ml". It is recommended that the sample be retested in a new test run at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against Chlamydia trachomatis, the calibration is performed in relative units (RU/ml). The calibration is performed with internal reference sera, which were used for evaluation of the test system.

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio values determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The reagent wells are coated with MOMP antigen (major outer membrane protein) which is a transmembrane protein and the major part of the outer membrane of the elementary bodies. Protein purification starts with BGM cells infected with Chlamydia trachomatis of serotype K.

Linearity: The linearity of the Anti-Chlamydia-trachomatis ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was >0.95. The Anti-Chlamydia trachomatis ELISA (IgG) is linear at least in the tested concentration range (2 RU/ml to 177 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Chlamydia trachomatis ELISA (IgG) is 0.6 RU/ml.

Cross reactivity: The quality of the antigen used ensures high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the Anti-Chlamydia trachomatis ELISA (IgG). For this ELISA there is no known cross reactivity with other Chlamydia pneumoniae positive samples.

Antibodies against	n	Anti-Chlamydia trachomatis ELISA (IgG) positive
Adenovirus	12	0%
Chlamydia pneumoniae	10	0%
CMV	12	0%
EBV-CA	12	0%
Helicobacter pylori	12	0%
HSV-1	12	0%
Influenza A virus	12	0%
Influenza B virus	12	0%
Measles virus	12	0%
Mumps virus	12	0%
Mycoplasma pneumoniae	12	0%
Parainfluenza pool	12	0%
Rubella virus	12	0%
RSV	12	0%
Toxoplasma	12	0%
VZV	12	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	8	9.2
2	65	8.2
3	137	3.2

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	7	10.6
2	62	9.5
3	139	6.5

Sensitivity and specificity: 53 clinically pre-characterised patient samples (INSTAND) were investigated with the EUROIMMUN Anti-Chlamydia trachomatis ELISA (IgG). The sensitivity amounted to 100%, with a specificity of 97.0%. Borderline results were not included in the calculation.

n = 53		INSTAND		
		positive	borderline	negative
EUROIMMUN Anti-Chlamydia trachomatis ELISA (IgG)	positive	17	0	1
	borderline	0	0	2
	negative	0	1	32

Prevalences: For different collectives of samples the following prevalences could be determined:

Panel	**Number of samples**	**Prevalence (IgG)**
Patients with pos. direct determination for C. trachomatis	100	67.0%
Risk group (prostitutes)	134	42.5%
Patients with reactive arthritis	54	27.8%
Pregnant women	200	12.5%
Healthy blood donors I	200	8.0%
Healthy blood donors II	200	4.0%

Reference range: The levels of anti-Chlamydia trachomatis antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, 13.4% of the blood donors were anti-Chlamydia trachomatis positive (IgG).

Clinical significance

The infectious agent Chlamydia trachomatis belongs to the human pathogenic Chlamydia genus, together with Chlamydia pneumoniae and Chlamydia psittaci. It is one of the smallest intracellular, gram-negative bacteria. It subsists as an energy parasite on the ATP of infected cells. Around 700 million people are infected worldwide, with approximately 50 million new infections taking place each year. In the USA the prevalence of mainly asymptomatic C. trachomatis infections in 16- to 25-year-old women is 22%, and in Western Europe 2.7% (in Italy) to 8% (in Iceland) according to the WHO. The disease is transmitted by contact with infected humans.

C. trachomatis is the pathogenic agent of non-gonorrheal urethritis, lymphogranuloma venereum, trachoma, inclusion conjunctivitis, neonatal pneumonia and Reiter's syndrome.

Sexually transmitted non-gonorrheal urethritis caused predominantly by C. trachomatis serotypes D to K is nowadays the most frequent sexually transmitted disease. The bacteria live mostly in the cells of the urethra, in men also in the prostate and the seminal vesicles and in women in the cervix or oviducts (salpinx). Infections proceed asymptomatically in around 50% of men and 70 to 80% of women. If symptoms develop they are urethritis, epididymitis and prostatitis in men, and in women urethritis, cervicitis and salpingitis/adnexitis with itching, pain and discharge. Chronic infections of the inner female organs lead in many cases to sterility. In Germany, more than 100,000 women suffer from Chlamydia-caused infertility. Secondary infertility in men has also been shown. There is an evident connection between acute C. trachomatis infections during the first three months of pregnancy and early abortions, premature deliveries or stillbirths (32nd to 34th week of pregnancy).

Lymphogranuloma venereum (lymphogranuloma inguinale, lymphopathia venera, Durand Nicolas Favre disease) is caused by C. trachomatis serotypes L1, L2 and L3. It is a rare venereal disease which occurs worldwide but mainly in tropical areas. Approximately 40% of men and 70% of women become infected after sexual contact with an infected person.

In tropical regions, C. trachomatis leads to trachoma (serotypes A, B, Ba and C), an eye infection of varying severity which is also known as trachomatous conjunctivitis, granular conjunctivitis or Egyptian ophtalmia. It is caused by direct contact between the mucous membranes of the eye, nose and mouth or may be transmitted by the mutual use of towels or washcloths. The first symptoms of severe conjunctivitis occur after an incubation period of 5 to 12 days. Around 400 million people suffer from trachoma, which is the most frequent cause of blindness worldwide (trachoma blindness).

The disease must be differentiated from an infection with C. trachomatis serotypes D to K, which causes paratrachoma in adults, also known as acute suppurative inclusion conjunctivitis or swimming pool conjunctivitis. It is generally transmitted through bathing water.

In newborns, particularly premature infants, prenatally or perinatally transmitted C. trachomatis causes conjunctivitis (ophthalmia neonatorum) and pneumonia (serotypes D to K). The latter is noticeably often accompanied by pneumothorax and lifelong health problems.

In 1 to 3% of cases, an urogenital infection with C. trachomatis is followed by reactive arthritis (Reiter's disease with the triad urethritis, conjunctivitis and arthritis). This is an oligoarthritis which predominantly affects the lower extremities, particularly the knee and ankle joints, causing local swelling. The distal interphalangeal joints and the spine (inflammatory back pain) are also frequently involved.

In reactive arthritis C. trachomatis occurs as a metabolically active agent in the joints. Due to the persisting infection, Chlamydia antigens such as major outer membrane protein (MOMP) and lipopoly-saccharide (LPS) are continuously produced, stimulating and sustaining an inflammatory process and the production of antibodies. The immune response is an intra-articular production of anti-C. trachomatis IgG. Type-specific serological test methods using MOMP as the target antigen allow a reliable diagnosis.

Despite distinct clinical symptoms, C. trachomatis antibodies are not necessarily produced in the serum in localised processes. IgM antibodies are not formed in all cases of florid infection. An increase in IgG titer is also not always found. In problem cases, it is therefore useful to determine the presence of Chlamydia in infectious secretions using direct immunofluorescence, or to determine the specific genetic sequences using PCR, although this is often unsuccessful in cases where the infection has taken place some time ago. Nevertheless PCR is often used for the detection of first infections, in particular as part of the screening programmes that are offered to young women in various countries in order to prevent asymptomatic ascending Chlamydia infections from causing sterility. The test results show that on average 10.9% of the young women investigated (up to age 24) were infected but asymptomatic. The ethnic breakdown was: white 18.1%, black Caribbean 9.9%, black African 15.2%, black British/other 5.9%, Asian subcontinent 6.7%, Chinese/other Asian 6.4% and other ethnic groups 14.9%.

The fastest and most reliable investigation of specific antibodies in infections with C. trachomatis, even with low antibody titers, is obtained with MIF (microimmunofluorescence) as "gold standard", IIFT (indirect immunofluorescence test) and ELISA (enzyme linked immunosorbent assay) using MOMP as the target antigen and taking into consideration different serotypes. In the MIF assay purified elementary bodies of the species C. trachomatis are used and the lipopolysaccharide (LPS) antigen common to all three species (C. trachomatis, C. pneumoniae and C. psittaci) is inactivated. This minimises cross reactions.

Studies throughout Europe show that these methods are suited to confirming C. trachomatis-induced infertility in women and men by the determination of C. trachomatis specific serum IgA and IgG antibodies. C. trachomatis specific IgA and IgG antibodies are frequently found in women who have had a premature delivery or a stillbirth, mostly in connection with high IgM titers. Recognised medical centres therefore recommend C. trachomatis screening for both parents before pregnancy.

Diagnosed Chlamydia infections can generally be cured with various antibiotics within 7 days, even during pregnancy. In reactive arthritis, a long-term, differentiated treatment is required, which acts locally and systemically.

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