SEROPREVALENCE OF *CHLAMYDIA TRACHOMATIS* CO-INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS AMONG WOMEN ATTENDING CLINICS IN ZARIA METROPOLIS, KADUNA STATE, NIGERIA.

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JUNE, 2014.

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMEMTS FOR THE AWARD OF MASTERS DEGREE IN MICROBIOLOGY, DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA, KADUNA STATE, NIGERIA.

JUNE, 2014.

DECLARATION

I hereby declare that the work in this thesis titled "Seroprevalence of *Chlamydia trachomatis* co-infection with Human Immunodeficiency Virus among pregnant and non-pregnant women attending clinics in Zaria metropolis, Kaduna State, Nigeria" was performed by me in the Department of Microbiology, under the supervision of Prof. E.D. Jatau and Dr. I.O. Abdullahi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

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CERTIFICATION

This thesis titled "Seroprevalence of *Chlamydia trachomatis* co-infection with Human Immunodeficiency Virus among pregnant and non-pregnant women attending clinics in Zaria metropolis, Kaduna State, Nigeria" by Anzaku, Shango Joshua (M.Sc./SCIE/1628/2010-2011) meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the "All Sufficient One", the giver of life, the source of my inspiration, wisdom, knowledge and understanding without reproach. Also to my dearly beloved and wonderful parents, late Mr. Joshua A. Anzaku and Mrs. Jessie C.J. Anzaku whose undying courage and belief in me spured me to carry out this thesis.

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ABSTRACT

Chlamydia trachomatis also known as the "Silent Epidemic" is a major threat to the reproductive health of women and a possible silent cofactor in the heterosexual spread of HIV in Africa. This study was aimed at determining the seroprevalence of *Chlamydia trachomatis* co-infection with Human Immunodeficiency Virus among women attending clinics in Zaria metropolis, Kaduna State. Each participant completed a researcher-devised questionnaire and quasi design was used in the selection of hospitals. Subsequently about 5mls of peripheral blood for serological analysis was obtained after informed consent. Presence of antibodies to *Chlamydia trachomatis* was determined using Enzyme Linked Immunosorbent Assay (ELISA) to detect IgG and screening for HIV was also done using Determine® HIV 1/2 as well as Uni-Gold™ HIV Test Kits. Out of the two hundred and seventy (270) samples collected, 32(11.9%) were positive for *Chlamydia trachomatis* IgG, 7(2.6%) for HIV and co-infection of *Chlamydia trachomatis* and HIV occurred in 1(0.4%) of the total population. *Chlamydia trachomatis* prevalence was found to increase with decrease in age with 11.5% in age groups 15-20 among pregnant women while in non-pregnant women, it was highest in <15 years 100.0%. Chlamydial infection was found to be significantly associated with level of education, marital status, number of years in marriage with those who have stayed in marriage for 26–30 years having the highest prevalence 50.0% among the women. Women in their third trimester of pregnancy had the highest prevalence 15.7% of chlamydial infection and it was found to be statistically significantly. There was no significant association between chlamydial infection and occupation, subjects' husbands' occupation, family type, number of sexual partners, age of women at first childbirth and also based on clinical symptoms. Similarly with HIV infection, significant association was found to exist with marital status and years spent in marriage among the women. Age, occupation, level of education, family type was not found to be significantly associated with HIV infection. No significant association also exist

between HIV infection and number of sexual partners as well as age of women at first childbirth. So also, history of stillbirth, history of premature birth, history of miscarriage and history of infertility showed no significant association with HIV infection among the women. Hence, there is an urgent need for a national policy on routine screening for *Chlamydia trachomatis* as treatment is cheap and effective, while the attendant morbidity resulting from delayed diagnosis is more difficult to manage and associated with severe sequelae.

CHAPTER ONE

INTRODUCTION

The genus *Chlamydia* belongs to the taxonomic family *Chlamydiaceae*, which also contains the genus *Chlamydophila* (Bush and Everett, 2001). The genus *Chlamydia* includes the human *Chlamydia trachomatis*, the mouse pathogenic *Chlamydia muridarum*, the avian *Chlamydia psittacis,* the swine *Chlamydia suis* and *Chlamydia pneumoniae*. *Chlamydia trachomatis* (CT) is a small Gram-negative bacterium that is an obligate intracellular parasite (McAdam *et al.,* 2005).

Genital infections with *Chlamydia trachomatis* are the most common sexually transmitted bacterial infections in European countries (Fenton and Lowndes, 2004; Manavi, 2006), in the United States (Miller *et al.*, 2004; Schillinger *et al.*, 2005) and worldwide, with more than 90 million cases annually (Coonrod, 2002). The Centres for Disease Control and Prevention (CDC, 2010) estimates that there are approximately 2.8 million new cases of *Chlamydia* in the United States each year and that about one million individuals in the United States are infected with *Chlamydia* (CDC, 2010). It has also been reported as an established cause of pelvic inflammatory disease (PID), ectopic pregnancy and infertility among women (Oakeshott and Hay, 1995). Although the major impact of the disease is on the female genital tract (Fenton and Lowndes, 2004; Manavi, 2006), men may suffer from urethritis, prostatitis, infertility and reiter's syndrome (Manavi, 2006). *Chlamydia trachomatis* is also the causative agent of trachoma, a chronic infection of the conjunctiva characterized by extensive scarring and blindness.

Sexually transmitted infections (STIs) contribute to a variety of obstetric and gynaecologic complications in women, including increased risk of tubal infertility and have been associated with chronic pelvic pain (Grodstein *et al*., 1993). They are also significantly associated with

adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, ectopic pregnancy, premature rupture of membranes, intrauterine infection of the foetus, and low birth weight in infants (Mardh, 2002).

Human Immunodeficiency virus (HIV) is a lentivirus, a member of the *Retroviridae* family that causes Acquired Immune Deficiency Syndrome (AIDS) (Douek *et al.,* 2009). An estimation of 33.2 million people globally are HIV-positive and 22.5 million of these people live in sub-Saharan Africa, with 61% (13.75 million) of these being women (Vuyst *et al.,* 2008). HIVprevalence in Nigeria has dropped from 4.6% in 2008 to 4.1% in 2011 with the number of infected people estimated at 3.1 million (NACA, 2011).

Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors (Smith and Daniel, 2006). Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle anew.

HIV acquisition, transmission and infectivity can be facilitated by other STIs as cofactors through multiple immunological and biological pathways (Cohen, 2004; Sangai *et al.,* 2004). The World Health Organization estimated in 2001 that per year 92 million new cases of *Chlamydia trachomatis* infection occur worldwide (WHO, 2001). This includes 5.2 million new cases of genital infections yearly in Western Europe out of which around 2.9 million are

in women. About 15.9 million cases of *Chlamydia trachomatis* occur in Sub-Saharan Africa of which 8.2 million occur in women.

1.1 STATEMENT OF RESEARCH PROBLEM

Chlamydia is known as the "Silent Epidemic" because in women, it may not cause any symptoms in 75% of cases (CDC, 2008), and can linger for months or years before being discovered. Symptoms that may occur include unusual vaginal bleeding or discharge, pain in the abdomen, painful sexual intercourse (dyspareunia), fever, painful urination or the urge to urinate more frequently than usual (urinary urgency).

Chlamydia trachomatis is a major threat to the reproductive health of women (Brunham *et al.*, 2005). It is the leading cause of tubal infertility and ectopic pregnancy in women and also the most common sexually transmissible bacterial pathogen worldwide. Since most *C. trachomatis* infections remain asymptomatic, up to 80% in women and 50% in men, a pool of individuals with subclinical infection continues to be responsible for the risk of transmission within the community (Fenton *et al.,* 2001).

Chlamydia trachomatis is responsible for a significant proportion of genitourinary tract infections in adult males and females, but like STIs in general, it is primarily a woman's health care issue since the manifestations and consequences are more damaging to the reproductive health of women than of men (Paavonen and Eggert-Kruse, 1999). Acute *C. trachomatis* infection is easy to treat, but the majority of chlamydial infection remains asymptomatic and untreated, and may lead to serious complications such as pelvic inflammatory disease (PID). Ectopic pregnancy, infertility and chronic pelvic pain in women (Malik *et al.,* 2006), as well as conjunctivitis and respiratory tract infection in infants (Darville, 2005) are also sequelae of infection with *C. trachomatis*. Hence is a major personal, social and health service burden.

The risk of vertical transmission is directly related to the prevalence of *C. trachomatis* infection in pregnant women and has been reported to be as high as 75% (Darville, 2005). *Chlamydia trachomatis* causes more than 250,000 cases of epididymitis and 250,000 to 500,000 cases of PID every year in the United States. Women infected with Chlamydia are up to five times more likely to become infected with human immunodeficiency virus (HIV), if exposed (CDC, 2007).

In many African settings, HIV is now the most prevalent STI in pregnant women. Overall, 15–30% of women attending prenatal care clinics are infected with HIV (Fawzi *et al.,* 2002). The most significant sequela of maternal HIV infection in pregnancy is mother to child transmission (MTCT) of HIV. Without intervention, rates of MTCT range from 15–30% without breastfeeding and rise to 30–45% with prolonged breastfeeding (De Cock *et al.,* 2000). MTCT is responsible for 90% of HIV infection in children worldwide (Ogundele and Coulter, 2003).

Available data from studies conducted so far on different subsets of the population in a number of African countries suggest that, incidences of *Chlamydia trachomatis* infection are on the rise and there are indications that the prevalence figures may exceed those reported in developed countries (Omo-Aghoja *et al*., 2007). This lends credence to the fear that *Chlamydia trachomatis*, like the ulcerative STIs, may have become an important, but silent, cofactor in the heterosexual spread of Human Immunodeficiency Virus (HIV) in these African countries.

The global HIV epidemic continues to expand, with an estimated five million people becoming infected each year. Over the decades, the epidemic, once dominated by infected males, has become progressively feminized. In sub-Saharan Africa, where about two-thirds of the global disease burden resides, 57% of adults living with HIV are women (UNAIDS,

2004). As more women contract the virus, the number of children infected from their mothers has been growing. Worldwide, an estimated 640,000 children acquired HIV in 2004 alone (UNAIDS, 2004), with more than 90% of the infections occurring through mother-to-child transmission.

1.2 JUSTIFICATION

Chlamydia trachomatis have been reported to cause silent infections in communities which become endemic and could remain unnoticed for a very long time. In most parts of Nigeria these organisms are not screened for, and hence relative information about frequencies of the organisms are sparse (Okoror *et al.,* 2007).

Despite recent advances in the prevention of transmission of HIV infection from mother to child during pregnancy, infants continue to be born and infected with HIV, particularly in Africa (Chris *et al.,* 2010).

Between half and three quarters of all women who have chlamydial infection of the cervix (cervicitis) are asymptomatic. Occasionally, the condition spreads to the upper genital tract in women (causing pelvic inflammatory disease, PID) or epididymis in men (causing epididymitis). If untreated, chlamydial infections can cause serious reproductive and other health problems with both short-term and long-term consequences (CDC, 2008).

Early detection followed by correct treatment of *Chlamydia trachomatis* may reduce the risk of complications resulting from untreated infections and the community-wide prevalence of sexually transmitted infections (STIs). The present findings are important to facilitate such intervention (Blake *et al*., 2008).

The importance of *Chlamydia trachomatis* as a possible silent cofactor in the heterosexual spread of HIV in Africa is further enhanced by the fact that many cases of *Chlamydia*

trachomatis infection remain asymptomatic in both sexes (van Dyek *et al.*, 1992) and therefore, remains undiagnosed and untreated for extended periods of time. The situation could be more critical in many African countries where knowledge of the disease is very low and the facilities for its routine diagnosis are not readily available.

The health-economic impact of *Chlamydia trachomatis* infections may therefore be enormous. Vaccines are not yet available, hence health gain and financial benefits can only be achieved by active case finding and treatment of *Chlamydia trachomatis* infection which can be done on an individual level or nationwide in a screening program.

There is an overall increasing trend in the chlamydia positivity rate in recent years, which may partly be due to the development of more sensitive laboratory tests, but also reflects a true increase in infections (Fine *et al.,* 2008).

1.3 AIM

The aim of this research is to investigate the seroprevalence of *Chlamydia trachomatis* co-infection with Human Immunodeficiency Virus among women attending clinics in Zaria metropolis, Kaduna State, Nigeria.

1.4 OBJECTIVES

1. To determine the seroprevalence of *Chlamydia trachomatis* and Human Immunodeficiency Virus among women (pregnant and non-pregnant).
2. To determine the seroprevalence of co-infection of *Chlamydia trachomatis* and Human Immunodeficiency Virus seropositive women in the study population.
3. To identify the risk factors associated with *Chlamydia trachomatis* and Human Immunodeficiency Virus infection in the study population.

CHAPTER TWO

LITERATURE REVIEW

2.1 *CHLAMYDIA TRACHOMATIS* (CT)

2.1.1 HISTORY AND BIOLOGICAL BACKGROUND OF CT

The Chlamydiae belong to the order *Chlamydiales* and family *Chlamydiaceae.*They were first recognized in the ancient times of Greece and Egypt. Three species were first recognized which includes; *Chlamydia trachomatis, Chlamydia pneumoniae* and *Chlamydia psittaci* (Graytson *et al*., 1990). A new addition to the family is called *Chlamydia pecorium.* They Chlamydiae were first detected with cytotoxic stains in the early 20th century in specimens from persons with trachoma and thereafter in infants with conjunctivitis and their parents (Schachter and Grossman, 1981). The wide spread importance and frequencies of genital and infant chlamydial infection were first appreciated in the 1960s.

The bacterium *C. trachomatis* is one of three species that belongs to the taxonomic genus *Chlamydia*. The word chlamys is Greek for "cloak draped around the shoulder." This describes how the intracytoplasmic inclusions caused by the bacterium are "draped" around the infected cell's nucleus. Because the symptoms of the disease resemble other pathologies, *Chlamydia* was not recognized as a sexually transmitted disease until recently. Isolation from embryonated eggs in 1957 and from cell culture in 1963 confirmed its existence as a bacterium (Gale, 2012). However, they were thought to be viruses and were referred to as large viruses because of their intracellular nature. However, they contain DNA, RNA and ribosomes and make their own proteins and nucleic acids and are now considered to be true bacteria. They possess an inner and outer membrane similar to gram-negative bacteria and a lipopolysaccharide but do not have a peptidoglycan layer. Although they synthesize most of their metabolic intermediates, they are unable to synthesize their own ATP and thus require

an exogenous source of this high energy compound. This is why it is called an 'energy parasite'. It undergoes a unique biphasic developmental cycle, forming distinctive intracellular inclusions that permit identification by light or fluorescent microscopy (Gene, 2010; Wariso *et al*., 2012). It can thus be found with the other well-known intracellular parasites, rickettsiae, in diagnostic manuals. Chlamydia has a genome size of approximately 500-1000 kilobases and contains both RNA and DNA. The organism is also extremely temperature sensitive and must be refrigerated at 4°C as soon as a sample is obtained.

2.1.2 Virulence Factors of CT

There are numerous factors that contribute to the pathogenicity of *Chlamydia trachomatis*. Colonization of Chlamydia begins with attachment to sialic acid receptors on the eye, throat, or genitalia. It persists at body sites that are inaccessible to phagocytes, T-cells, and B-cells. There are 19 human serovars and numerous variants of *C. trachomatis* that differ in their cell biology and also in their clinical presentation (Ngandjio *et al*., 2004). Serovars A, B, Ba, and C are largely restricted to causing infections of the eye and are associated with endemic trachoma, whereas serovars D, Da, E, F, G, Ga, H, I, Ia, J and K cause both ocular and genitourinary infections. Serovars LI, L2, L2a, and L3 are the cause of lymphogranuloma venereum (LGV) which causes genitourinary infections in addition to a more significant involvement of the lymphatic system. Its unique cell wall structure is another virulence factor. Studies reveal that Chlamydia, because of its cell wall, is able to inhibit phagolysosome fusion in phagocytes. The cell wall is proposed to be gram-negative in that it contains an outer lipopolysaccharide membrane, but it lacks peptidoglycan in its cell wall. This lack of peptidoglycan is shown by the inability to detect muramic acid and antibodies directed against it. It may, however, contain a carboxylated sugar other than muramic acid. The proposed structure consists of a major outer membrane protein cross-linked with disulfide bonds. It also contains cysteine-rich proteins (CRP) that may be the functional

equivalent to peptidoglycan. This unique structure allows for intracellular division and extracellular survival (Hatch, 1996).

2.1.3 Epidemiology of CT

Chlamydial genital infection leads all reportable bacterial infectious diseases (Eugene *et al.,* 2004). In contrast to Gonorrhea, the number of reportable chlamydial infections has tended to rise each year, probably a reflection of increased awareness of the disease and better diagnostic tests. Still only a fraction of the total cases, estimated at 4 million per year in the United States, is reported by physicians to public health departments. Globally, as of 2010, sexually transmitted chlamydial infection affects approximately 215 million people (3.1% of the population) (Vos, 2012). It is more common in women (3.8%) than men (2.5%) (Vos, 2012). In that year it resulted in about 1200 deaths down from 1500 in 1990 (Lozano, 2012). More than 14% of sexually active high school and college women were asymptomatic carriers of *C. trachomatis* in one study. Non-sexual transmission of this agent occurs, for example, in non-chlorinated swimming pools. Newborn babies of infected mothers often develop chlamydial ophthalmia neonatorum, also called inclusion conjunctivitis, and pneumonia from *C. trachomatis* infection contracted during passage through the birth canal (Eugene *et al.,* 2004).

2.1.4 Chlamydial Developmental Life Cycle

Chlamydia alternates between two morphological forms, the elementary body (EB) and the reticulate body (RB) (Moulder, 1991). EBs are extracellular, metabolically inert forms, responsible for dissemination of infection by their ability to attach to and invade susceptible cells. Upon infection, EBs are internalized in membrane bound vacuoles termed inclusions. EBs differentiates into metabolically active forms, termed RBs, and undergoes repeated cycles of binary fission leading to secondary differentiation back to EBs. The host cell then lyses, releasing EBs that infects neighbouring cells. Under stressful growth conditions,

imposed by immunological responses, antibiotics or nutrient deprivation, the developmental cycle is disrupted, resulting in the appearance of large, aberrant RBs (Hogan *et al*., 2004). This altered growth scheme appears to be associated with continued expression of genes associated with DNA replication but not with those genes involved with bacterial cell division.

2.1.4.1 Elementary Body (Morphology)

The term ‘‘elementary body’’ (EB) refers to the small (ca. 0.3 lm), round, electron dense, infectious form of the organism (e.g., (Matsumoto, 1973; Eb *et al*., 1976). The term, as with other developmental cycle terms, stems from viral nomenclature historically associated with chlamydial research. The EB is metabolically inert and has been likened to a ‘‘spore-like’’ form of the organism. The bacterial nucleoid is highly compacted in EBs due to the condensation of nuclear material by the bacterial histone-like proteins HctA and HctB (Barry *et al*., 1992; Brickman *et al*., 1993). The nucleoid has an eccentric location in the cell body suggesting an association with the bacterial inner-membrane or cell wall.

Chlamydial EBs are unusual in that little or no peptidoglycan is present in the cell wall. Structural rigidity is thought instead to be due to the highly cross-linked nature of the outer-membrane complex. Inter and intra molecular cystine bonds exist between the cysteine rich proteins of the outer envelope including OmpA, OmcB, and OmcA (Hatch, 1999). In addition, a hexagonally arrayed protein layer (predominantly OmcB) at the inner surface of the outer-membrane complex is thought to contribute to cellular stability in EBs.

Electron microscopic examination of EBs using freeze fracture/freeze etch procedures demonstrated the presence of hexagonally organized surface projections arranged regularly with a center to center spacing of approximately 50 nm (Matsumoto, 1982; Nichols *et al*., 1985). These ‘‘supramolecular structures’’ extend approximately 30 nm from the EB surface

and have a rotational symmetry corresponding to a 9-subunit composition. Several authors have speculated that these spike-like projections correspond to Type III secretion system (TTSS) ‘‘needle’’ structures, similar to those seen in Salmonella enterica serovar Typhimurium (Kimbrough and Miller, 2000). No conclusive evidence has been shown to confirm these hypotheses but Fields *et al*., 2003 have used an indirect argument that strongly supports the presence of TTSS in EBs. They have shown that inclusion membrane protein genes (Inc) are expressed early in the developmental cycle, much earlier than the genes encoding structural components of the TTSS. Furthermore, they showed that secreted Inc protein appeared in the inclusion membrane before TTSS proteins were produced. Since Inc proteins were TTSS substrates in heterologous *Yersinia* systems, they concluded that EBs must have pre-formed TTSS that function very rapidly upon the EB–host cell interaction (Clifton *et al*., 2004).

2.1.4.2 Attachment and Entry

As might be expected for an obligate intracellular parasite, chlamydiae have developed elaborate, and possibly redundant, mechanisms for host cell attachment and entry (Hackstadt, 1999). The ability to efficiently infect numerous non-phagocytic cell types from diverse animal species indicates that the bacterial adhesins involved must recognize conserved cellular receptors.

Despite the central importance of this interaction in bacterial pathogenesis, and the research effort directed towards this problem, no clear consensus exists as to the nature of the adhesin/receptor molecules involved. Bacterial adhesin candidates include OmpA, OmcB, and Hsp70 (Hackstadt, 1999). The polymorphic membrane protein (Pmp) family of auto transporters has also been suggested to play a role in attachment (Everett and Hatch, 1995; Grimwood and Stephens, 1999; Grimwood *et al*., 2001). This family of related proteins (21

members in *C. pneumoniae*) contains highly repetitive motifs of 4 amino acids (GGAI and FxxN, (Everett and Hatch, 1995)) that are found in other bacterial adhesins such as rOmpA from the obligate intracellular *Rickettsia* spp. (Grimwood and Stephens, 1999). Wehrl *et al*., 2004 demonstrated that PmpD of *C. pneumoniae* was indeed processed as an auto transporter and that antisera raised to the N-terminal passenger domain blocked chlamydial infectivity.

Recent studies indicate the interaction of EBs with host cells occurs in a two-stage process. The initial attachment occurs through electrostatic interactions of the bacteria with heparan sulfate containing glycosaminoglycans (Stephens *et al*., 2001; Taraktchoglou *et al*., 2001). This interaction is reversible and has been shown to occur through binding to the surface exposed OmpA protein (Su *et al*., 1996), although clear differences can be found between chlamydial species and serovars. A second, irreversible, binding stage was demonstrated using chemically mutagenized cell lines (Carabeo and Hackstadt, 2001) but the receptor responsible has yet to be identified. Davis *et al*., 2002 have shown that *C. trachomatis* serovar E is closely associated with protein disulfide isomerase (PDI), a component of the estrogen receptor complex, during attachment and entry of polarized HEC-1B cells.

Chlamydial attachment was previously shown to be dramatically enhanced in estrogen dominant primary human endometrial epithelial cells (Maslow *et al*., 1988) and the receptor complex is located at the apical surface of polarized cells, an important consideration for entry from the luminal surface of the genital epithelium. Furthermore, the authors suggest that the disulfide isomerase activity of PDI may play a role in the reduction of the highly disulfide cross-linked EB OMC, a requirement for productive attachment and entry (Raulston *et al*., 2002).

Clifton *et al*., 2004 have recently shown that, immediately following the irreversible binding step, a TTSS exported protein is delivered into the host cell. This protein, termed Tarp

(Translocated actin-recruiting phosphoprotein, CT456), is rapidly phosphorylated at tyrosine residues and phosphorylation correlates spatially and temporally with actin recruitment.

Further evidence for the direct involvement of Tarp comes from expression studies with a Tarp-EGFP fusion in which the product expressed in the cell cytoplasm is tyrosine-phosphorylated and recruits actin. Tyrosine-phosphorylated Tarp is detected on the cytoplasmic face of the cell membrane while the EB is still extracellular (i.e. prior to chromosome decondensation). These results indicate that chlamydial EBs have a functional TTSS that delivers important signaling molecules to the host cell prior to differentiation. The preformed nature of the TTSS and early effector molecule(s) such as Tarp, suggests that the expression of important proteins for attachment and entry, and their assembly into functional complexes, must be part of the secondary differentiation process in which RBs are converted to EBs.

2.1.4.3 Primary Differentiation

The chlamydial developmental cycle has a singular enigmatic stage, the differentiation of the infecting EB to a metabolically active RB. The process of differentiation can be blocked by the addition of antibiotic inhibitors of transcription or translation, suggesting that de novo protein expression is required to begin intracellular growth (Scidmore *et al*., 1996). This finding is at odds with the generally held understanding that the presence of the bacterial histone-like proteins HctA and HctB render the EB transcriptionally incompetent. This paradox has been at least partially addressed by the findings of Grieshaber *et al*., 2004 Chlamydial histone–DNA interactions are disrupted upon germination by a small metabolite in the non-mevalonate pathway (MEP) pathway of isoprenoid biosynthesis. The metabolite is thought to be 2-C-methylerythritol 2,4-cyclodiphosphate and is involved in functional antagonism of HctA. Findings showed that an *E. coli* HctA expression system was rescued

from the lethal effects of HctA by ispE; ispE is an intermediate enzyme in the MEP pathway. Stepwise reconstruction of this pathway showed convincing evidence thatthe aforementioned metabolite is the candidate to be the functional antagonist of HctA. Paradoxically, experimental data adds another question, the *E. coli* orthologue of ispE could not protect *E. coli* from the lethal effects of HctA expression. The question arises whether it is special enzyme kinetics attributed to chlamydial IspE or the transcriptional activity of the chlamydial ispE in the presence of HctA, or another unknown function that chlamydial ispE has that helps in the rescue from HctA lethality. Chlamydia possess another histone-like protein HctB, it is still unknown what antagonizes its function during the differentiation step (Grieshaber *et al*., 2004).

2.1.4.4 Reticulate Body (Morphology)

The ‘‘reticulate body’’ (RB) arises from the internalized EB following primary differentiation, as discussed above (figure 2). RBs are larger than EBs (ca. 1 lm) and the cytoplasm appears granular with diffuse, fibrillar nucleic acids, in contrast with the highly condensed nucleic acid content of the EB (Rockey and Matsumoto, 2000). RBs are non-infectious and are bounded by an inner and outer-membrane, resembling other, Gram-negative, eubacteria. The surface of RBs is covered with projections and rosettes that extend from the bacterial surface through the inclusion membrane (proposed TTSS needle structures), similar to those found on EBs but at a higher density (Matsumoto, 1982).

RBs undergo binary fission throughout the middle part of the developmental cycle. In certain species, the RBs tend to be closely associated with the inner face of the inclusion membrane throughout the period of rapid growth, but this may not apply to all species (i.e. *C. pneumoniae* appears to completely fill the interior of the inclusion and *C. caviae* tends to

grow in an ‘‘articulated’’ form of the inclusion, without a large internal space (Rockey and Matsumoto, 2000).

2.1.4.5 Cell Division

Cell division takes place during the RB stage (figure 1). Genome sequencing indicated that chlamydia lack an identifiable ftsZ ortholog, which encodes a protein centrally involved in bacterial cell division and found in all other sequenced eubacteria. Another surprising finding in the genomic analyses was the presence of a complete set of genes for the synthesis of peptidoglycan (Read *et al*., 2000; Read *et al*., 2003).

Numerous studies had reported that Chlamydia lacked peptidoglycan, with a single study reporting trace amounts in EBs (Su *et al*., 1985). Similarly, attempts to identify peptidoglycan in RBs were unsuccessful (Barbour *et al*., 1982). Somewhat surprisingly, penicillin and other b-lactams are inhibitory to chlamydial growth (e.g., (Moulder, 1993) and probably target the high molecular weight penicillin binding proteins. This paradoxical situation has been referred to as the ‘‘chlamydial anomaly’’ (Ghuysen and Goffin, 1999). RBs may synthesize small amounts of peptidoglycan that play a role in bacterial cell division, perhaps by substituting for the lack of FtsZ in the formation of nascent division septa (Chopra *et al*., 1998).

McCoy *et al*., 2003 have shown that the chlamydial MurA ortholog (UDP-N-acetylglucosamine enopyruvyl transferase), which catalyzes the first committed step in peptidoglycan biosynthesis, is functional in *E. coli*. The chlamydial MurA was found to encode a fosfomycin resistant form of the enzyme and this resistance was imparted to *E. coli* expressing the chlamydial enzyme. Expression studies indicated that chamydia are naturally fosfomycin resistant and that murA is expressed at the point in the developmental cycle

immediately preceding cell division (Belland *et al*., 2003; McCoy *et al*., 2003), providing circumstantial evidence for the involvement of peptidoglycan synthesis in cell division.

2.1.4.6 Secondary Differentiation (Late Gene Expression)

Following the period of rapid cell division, RBs begin to redifferentiate to EBs, here termed ‘‘secondary differentiation’’. The signal for this process is unknown and efforts to identify quorum sensing type pathways are not been supported by genomic analyses. Speculative mechanisms have been proposed that involve the dissociation of dividing RBs from the inclusion membrane as a trigger for secondary differentiation. The removal of the TTSS system needle apparatus from the inner surface of the inclusion membrane has been suggested as a critical event in this process (Bavoil *et al*., 2000). This hypothetical mechanism has many attractive features, including an explanation for the asynchronous nature of secondary differentiation, but much work remains to provide experimental evidence supporting it.

Expression of a number of late-cycle genes occurs during secondary differentiation (Hatch, 1999), including genes that encode components of the outer membrane complex (e.g., OmcA and B) and proteins involved in the condensation of the chromosome (e.g., HctA and B). Microarray analyses have extended the number of genes expressed during this stage of development. Belland *et al*. 2003 identified 26 late genes including a number of genes previously characterized as late genes based on other molecular biological studies (e.g., omcAB, hctAB, ltuB, lcrH.1) and a number of new genes that encode proteins with speculative and unknown function. Several newly identified late genes encoded proteins with functions predicted to be important in the formation of the highly disulfide-cross-linked outer membrane complex. These include two predicted thioredoxin disulfide isomerases (CT780 and CT783) and two membrane thiol proteases (mtpA and mtpB). The membrane thiol

proteases share homology with adenoviral proteases that play a role in the maturation of viral particles (Greber, 1998). Interestingly, expression of some late cycle genes may be directed towards the arming of EBs with proteins necessary during the early stages of the next infectious cycle (e.g., the TTSS protein LcrH.1), hence ‘‘late’’ genes may encode ‘‘early’’ proteins.

Nicholson *et al*. identified 70 late genes, classified as Stage III in that they were not expressed until 24 h PI. Two clusters were identified in this stage, the late temporal cluster and the very late cluster, the latter consisting of genes that show increased expression levels between 24 and 36 h PI. The late cluster included previously identified late genes (e.g., omcAB, hctAB, ltuB, lcrH.1), genes potentially involved in chromosome condensation (e.g., CT643 and CT660 in addition to hctAB), and a large number of genes (31) with unknown function and found only in chlamydia. The very late temporal cluster consisted of six genes that encode chlamydia-specific proteins of unknown function, including a gene (CT147) identified as an immediate-early gene in the Belland *et al.*, study.

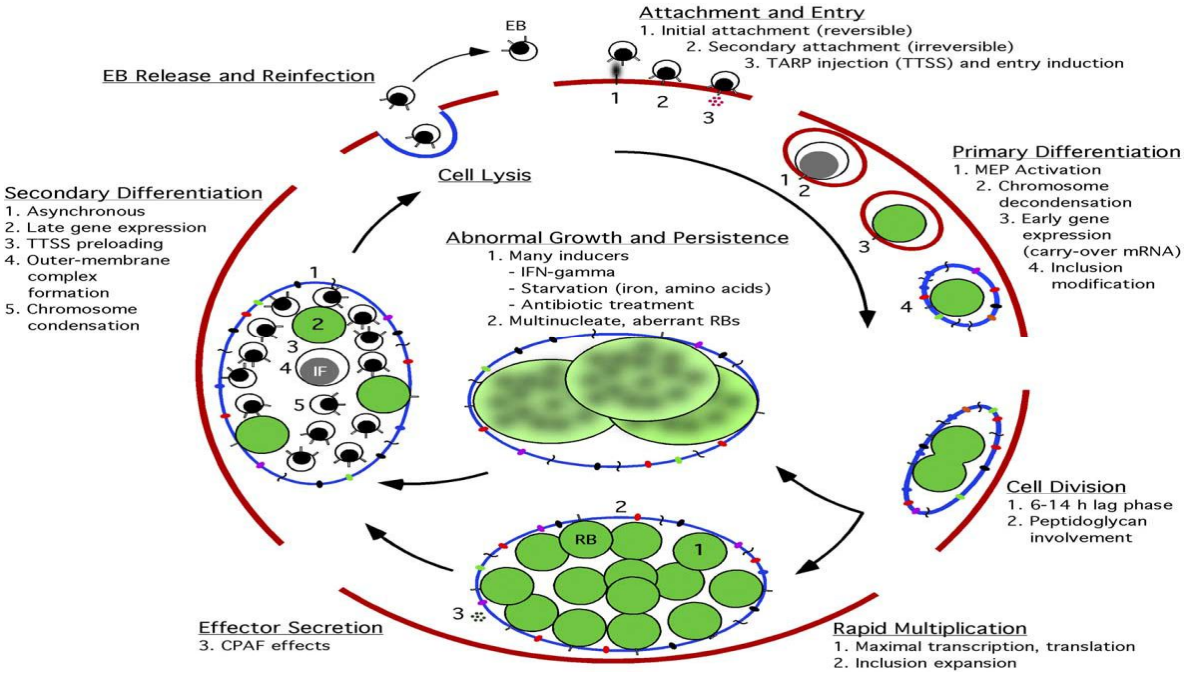


Figure 1: The Chlamydial Developmental Cycle (Belland *et al*., 2005)

2.1.5 Transmission of CT

C. trachomatis is naturally found living only inside human cells. Transmission among adults mainly occurs via sexual contact (horizontal transmission) and the transfer of infected secretions which primarily affect mucosal membranes such as the cervix, urethra, rectum, conjunctiva and pharynx. Transmission can also occur via genital-ocular auto-inoculation eventually leading to trachoma. Importantly, during passage through an infected birth canal *C. trachomatis* can be spread to newborns, who subsequently may become infected (vertical transmission) (Schachter *et al.,* 1986; Hammerschlag, 1989).

2.1.6 Symptoms of CT

Symptoms due to this contact are quite variable. The symptoms of *C. trachomatis* infection generally appear 7 to 14 days after exposure. In men, the main symptom is a thin, gray-white discharge from the penis, sometimes with painful testes with burning and pain during urination, and tingling or itching sensations. Women most commonly develop an increased vaginal discharge, accompanied by painful urination, abnormal vaginal bleeding, and upper or lower abdominal pain as well as irritation of the area around the vagina, bleeding after intercourse. Many infections of men and women are asymptomatic (Eugene *et al.,* 2004). Infection in women usually begins at the cervix. In men, non-gonococcal urethritis is the main symptom (Gale, 2012).

2.1.7 Pathogenesis and Immunity of CT

The infectious form of *C. trachomatis*, called an elementary body, attaches specifically to receptors on the surface of the host epithelial cells, thereby signaling the cell to take the bacterium by endocytosis. In the endocytic vacuole, the bacterium enlarges and becomes a non-infectious form called a reticulate body. The reticulate body divides repeatedly by binary fission, resulting in numerous elementary bodies that are released from the host cell and

infect nearby cells. The infected cells release cytokines that provoke an intense inflammatory reaction. Much of the tissue damage results from the cell-mediated immune response. The infection usually involves the urethra in both men and women. In men, it spreads to the tubules that collect sperm from the testicles, causing acute pain and swelling. In women, the infection commonly involves the cervix, making it bleed easily, often with sexual intercourse. The uterus is commonly involved, causing pain and bleeding. From the uterus, the infection spreads to the fallopian tubes, causing PID, and like gonorrhea, it can exit the fallopian tubes and infect the surface of the liver. Subsequent scar tissue formation is responsible for many of the serious consequences of the disease such as sterility and ectopic pregnancy (Eugene *et al.,* 2004). Infection does not stimulate long lasting immunity and reinfection results in an inflammatory response and subsequent tissue damage (Gene, 2010).

2.1.8 Alternate Growth Modes and Persistence

Many chlamydial diseases are associated with a long term or chronic infectious state. In most cases, it is difficult to establish whether chronic or recurrent infections arise through the inability of the host to resolve the infection or the occurrence of repeated infections with similar species or genotypes. Despite the unresolved nature of the disease etiology, persistence models of chlamydial infection have been studied to provide insight into the nature of chronic disease (Hogan *et al*., 2004). Persistence is defined as a long-term association between Chlamydia and their host cell in which these organisms remain in a viable but culture-negative state. The in vitro persistence systems often share altered Chlamydial growth characteristics for example, many studies have described enlarged, and pleomorphic RBs that neither undergo binary fission, nor differentiate to EBs, but nevertheless continue to replicate their chromosomes. These changes are generally reversible upon removal of the growth inhibitory factor (Hogan *et al*., 2004). Persistent in vitro infections have been induced by penicillin treatment (Matsumoto and Manire,1970), amino

acid starvation (Coles *et al*., 1993), iron deficiency (Raulston,1997), IFN-c exposure (Beatty *et al*., 1993), monocyte infection (Koehler *et al*., 1997), phage infection (Hsia *et al*., 2000), continuous culture (Kutlin *et al*., 2001). This subject has been thoroughly reviewed in Hogan *et al*., 2004. IFN-c mediated inhibition of intracellular chamydial replication occurs by depletion of the essential amino acid tryptophan, via the induction of indoleamine-2,3-dioxygenase (IDO) (Beatty *et al*., 1994). The effect of IFN-c on chlamydial infection could be reversed by addition of tryptophan (Byrne *et al*., 1986). Belland *et al*. have studied the induction of persistence with IFN-c and the subsequent reactivation, using microarray analysis. Persistent growth, characterized by large aberrant RBs, led to the up-regulation of genes involved in tryptophan utilization, DNA repair and recombination, phospholipid biosynthesis and translation. Up-regulation of the repressible trp BA operon (Wood *et al*., 2003) confirms the previous observations that IFN-c treatment reduces intracellular concentrations of tryptophan. In addition, a number of early genes were up-regulated, particularly the euo gene (30-fold increase) which encodes a DNA-binding protein that has been shown to bind to a late gene promoter region (i.e. omcAB (Zhang *et al*., 1998)).

Down-regulation of genes involved in RB to EB differentiation (late genes such as hctAB and omcAB), proteolysis and peptide transport, and cell division were seen during persistent growth. The transcriptional analyses were consistent with the biological properties associated with aberrant RBs in that cells were blocked in cytokinesis and the developmental cycle was arrested at a point preceding late gene expression. Removal of IFN-c and supplementation with added tryptophan led to a rapid reactivation from persistent growth. During reactivation the expression differences rapidly returned to control levels, i.e. euo expression dropped 20-fold in 12 h. The transcriptional changes in the presence of IFN-c that result in persistent growth appear to constitute a persistence stimulon. This coordinated biological response appears to have evolved to allow the organism to rapidly respond to immunological pressure

in a manner that allows for a period of resistance followed by rapid recovery after the waning of the host response.

2.1.9 Chlamydial Toxin

A putative chlamydial cytotoxin has been described (Belland *et al*. 2001), although original cytotoxic reports (Rake and Jones, 1994) involved lymphogranuloma venereum (LGV) isolates that apparently do not possess the actual putative cytotoxin gene. On the other hand, the putative chlamydial cytotoxin does share amino acid sequence similarities to the clostridial toxin B protein (Fan *et al*., 2008), and a study (Thalman *et al*., 2008) demonstrated that the ectopically expressed chlamydial protein glycosylates the small guanosine triphosphatase Rac1 in HeLa cells and causes actin reorganization in a manner similar to ectopically expressed authentic clostridial toxin B. Significantly, cytotoxin gene arrangements have been useful in defining *C. trachomatis* disease phenotypes (Belland *et al*. 2001), in that genital biovars contain a single gene with a large central deletion, LGV strains lack the toxin gene, and the closely related *C. muridarum* strain has 3 copies of the full-length putative toxin gene. In addition, cytotoxin gene polymorphisms have been useful in distinguishing ocular and genital *C. trachomatis* isolates (Carlson *et al*., 2004). Thus far, extensive comparisons among clinical genital isolates have not been made, nor has the actual function of the protein or cellular localization been established with certainty. The putative chlamydial cytotoxin is a protein that warrants further study to establish whether causal links to disease severity may emerge among strains with differing toxic potential, although current evidence suggests that little selective advantage is provided by cytotoxin bearing strains in human populations, because the trend in toxin-coding region of the chromosome (the so-called plasticity zone) is for loss of function (Carlson *et al.*, 2004; Fan *et al*., 2008).

2.1.10 Metabolic Processes of CT

Similar to other bacteria, *C. trachomatis* has the capacity to recognize and respond to environmental changes by a variety of mechanisms, including the classic 2-component system comprising an environmental sensor coupled to a transcriptional regulator designed to modulate gene expression in response to environmental changes (Koo and Stephens. 2003), which is probably responsible for transcriptional regulation during late-stage chlamydial development. Other putative chlamydial regulators also have been identified (Koo *et al*., 2006), including ≥1 species of small, noncoding RNA (Grieshaber *et al*., 2006). It is unclear how broad or narrow the repertoire of transcriptional regulators is across the genus and whether clues to disease severity phenotypes may be revealed by characterizing the repertoire of regulators of gene transcription; however, clearly, similar molecules have been associated with virulence phenotypes for many other important human pathogens, and there is no reason to suspect that *C. trachomatis* is any different in this regard.

Hypoferremia and transferrin receptor modulation are known consequences of the inflammatory response (Tacchini *et al*., 2008), and chlamydiae respond to iron limitation in a regulated way, typical of many pathogenic prokaryotes (Rau *et al*., 2005; Dill and Raulston, 2007). Iron in usable form is often limiting in any niche, and this may present more acutely in the female genital tract. Thus, the capacity of chlamydiae to sequester iron effectively may be an important virulence consideration in genital tract infections.

Other immune-related environmental changes that are significant to the chlamydiae are a reflection of the immune regulated modulation of tryptophan availability resulting from the induction by interferon-g of the tryptophan-decyclizing enzyme indoleamine-2,3-dioxygenase (Shaw *et al*., 2000). Genital strains of *C. trachomatis* have evolved a unique method of surviving under these nutrient-limiting conditions by activating tryptophan biosynthetic genes

(Byrne *et al*., 1986). The unique feature of this method of acquiring tryptophan is that indole is required as a substrate, and neither the host nor the pathogen is capable of producing this compound. The current thought is that genital *C. trachomatis* must acquire indole from other bacterial flora (eg, vaginal flora) and, thus, be metabolically poised to complete the developmental cycle and be transmitted. Of interest, it has been found that all genital isolates examined at the molecular level possess functional partial tryptophan operons, whereas none of the tested ocular strains do (Caldwell *et al*., 2003). This is significant because it provides a metabolic distinction between ocular and genital strains that is nearly axiomatic with tissue-specific virulence. This important finding also provides a clear rationale to suggest that other metabolic pathways may be equally important in defining disease severity phenotypes among genital isolates.

2.1.11 Complications and Long Term Reproductive Outcomes of CT

Serious complications may result if chlamydia continues undiagnosed and untreated. When sex partners are not notified and properly treated, there is high risk of re-infection as having multiple infections also increases a woman's risk of serious reproductive health complications.Actual rates of progression of chlamydial infection to reproductive sequelae are unknown (Tiitinen *et al.,* 2006).

2.1.11.1 Chlamydial Genital Tract Infections in Men

Urethritis: Both *C. trachomatis* and *Neisseria gonorrheae* produce clinical signs and symptoms due to their preference to infect columnar or transitional epithelium. Urethritis is the commonest presentation for both infections. The inflammation produced by *C. trachomatis* is revealed as a discharge appearing at the urethral meatus with or without meatal inflammation. Symptoms experienced include discharge, burning on urination, itching sensation meatally or in the urethra. The discharge tends to be less acute, profuse and

purulent than in men with gonococcal urethritis and has a longer incubation period of 7-21 days usually. Also, frequent and urgent nocturia, haematuria, perineal pain, scrotal swelling, inguinal lymphadenopathy, pain on opening the bowels and fever can occur but very unusually (Moss, 2001). If symptoms are present they are generally mild (Takahashi *et al.,* 2006), compromising early identification which may lead to non gonococcal urethritis (Gaydos *et al.,* 2009). *C. trachomatis* is responsible for about 30-40% of the 4-6 million visits each year for non-gonococcal urethritis (NGU) (Atkins, 1996).

Epididymitis: Urethral infection with *C. trachomatis* can ascend from the lower genital tract and cause inflammation of the epididymis, a structure on the back of the testicle where sperm is stored, a condition known as epididymitis (Trojian *et al.,* 2009). Approximately 45%–85% of men with epididymitis have had prior *C. trachomatis* infections and/or gonococcal infections (Berger *et al.,* 1979; Melekos and Asbach, 1988) and many patients become infertile as a result. Over half 150000 cases of acute epididymitis are associated with Chlamydia (CDC, 1993). Symptoms include swelling and tenderness of the infected area, pain that may become constant and severe, fluid around the testes (hydrocele), and sometimes a fever rarely an abscess (collection of pus) that feels like a soft lump develops in the scrotum (Mark *et al.,* 2003).

Proctitis: *C. trachomatis* is also associated with proctitis an inflammation of the rectal mucosa in both lymphogranuloma venereum (LGV, systemic *C. trachomatis* infection caused by serovars L1–L3), and non-LGV strains (genital infection caused by serovars B and D–K) (Stamm *et al.,* 2008). Once, rarely identified in industrialized nations, reports of LGV proctitis among men who have sex with men have recently been increasing throughout Europe, North America and Australia (Martin-Iguacel *et al.,* 2010). Because symptoms of

LGV proctitis are nonspecific and unfamiliar to many physicians in industrialized countries, diagnosis may be delayed (Martin-Iguacel *et al.,* 2010).

Prostatitis: Chlamydial infection may also cause prostatitis-pain and swelling of the prostate gland, although this relationship has not been completely delineated. This can result in a collection of pus in the prostate. Chronic prostatitis can impair fertility (Mark *et al.,* 2003). *C. trachomatis* has been detected in up to 30% of prostatic or semen secretion samples and in 2.2% to 33% of prostate tissue samples collected from prostatitis patients (Ouzounova-Raykova *et al.,* 2010).

Reiter Syndrome: Reiter syndrome, a reactive arthritis secondary to an immune-mediated response, has been associated with a primary chlamydial infection. It may present as asymmetric polyarthritis, urethritis, inflammatory eye disease, mouth ulcers, circinate balanitis, and keratoderma blennorrhagica. Its etiology may not be completely clear, but two strong associations are observed: Reiter syndrome usually follows an infectious episode, and 80% of affected patients are positive for human leukocyte antigen (HLA)-B27 (Gene, 2010). The syndrome exists both as a sexually acquired reactive arthrosis (SARA) and a less common epidemic dysenteric form, the latter having been associated with *Shigella flexnerii, Salmonella sp, Yersinia enterocolitica and Campylobacter jejuni* (Moss, 2001). Peak onset is in the third decade, but it may appear in children and the elderly and incidence rate is around 33 per 100,000 in males (Cush, 1993).

Lymphogranuloma Venereum (*C. trachomatis* biovar: LGV): *Chlamydia* lymphogranuloma venereum is a biotype of *trachomatis* causing a generalized infection transmitted venerally which is of worldwide distribution and has been described as 6th V.D. The primary lesion of LGV is a small painless and inconspicuous vesicular lesion that appears at the site of infection, often the penis or vagina. The patient may also experience

fever, headache and myalgia. The second stage of the disease called "supperative lymphadenopathy", presents as a marked inflammation of the draining lymph nodes. The enlarged nodes become painful 'buboes' that can eventually rupture and drain. Fever, headache and myalgia can accompany the inflammation of the lymph nodes (Gene, 2010). Finally, the tertiary stage is when rectal narrowing or draining of the sinuses occurs (Gale, 2012). Furthermore, re-infection rates in males have been reported to be approximately 11.3%, which is similar to re-infection rates in women (Fung *et al.,* 2007).

2.1.11.2 Chlamydial Genital Tract Infections in Women

Cervicitis: *C. trachomatis* can infect the columnar epithelial cells of the endocervix and cause cervicitis, (Stamm *et al.,* 2008) and is also associated with urethritis. Although chlamydial cervicitis is often asymptomatic, it does cause mucopurulent discharge in about 37% of women and hypertrophic ectopy (an area of ectopy that is edematous and bleeds easily) in 19% of women (Stamm *et al.,* 2008). However, there are no specific genital symptoms that are correlated with chlamydial infection of the cervix for diagnosis (Stamm *et al.,* 2008).

Lower genital chlamydial infection can ascend to the upper genital tract, leading to serious complications. There are several ways *C. trachomatis* may be able to ascend into the upper genital tract. First, *C. trachomatis* has been shown in vitro to be able to attach to spermatozoa (Wolner-Hanssen and Mardh, 1984; Friberg *et al.,* 1987), possibly promoting rapid ascension into the upper genital tract (Zervomanolakis *et al.,* 2007). Second, the characteristics and production of the mucus plug, which is important for the protection against microorganisms, can be changed by hormonal fluctuations during the menstrual cycle, possibly influencing ascension (Paavonen *et al.,* 2008). Hormone levels at menarche also increase cervical ectopy in young women (Paavonen *et al.,* 2008), which provides a larger area for bacterial

attachment. Lastly, sub endometrial contractions, which are amplified prior to ovulation, may also promote ascension (Ijland *et al.,* 1996).

Ectopic Pregnancy: Ascending of chlamydial infection to the fallopian tubes causes scarring of the tubes which may interfere with the passage of fertilized egg to the uterus during pregnancy and when this happens, the egg may attach itself to the fallopian tube and starts to develop resulting to ectopic pregnancy which can further result in miscarriage and can cause the death of the mother (Russel *et al.,* 1992). The risk of ectopic pregnancy in women who have had PID is 7-10 times greater than that for women without a history of PID (Gene, 2010). In a study by Picaud *et al.,* 1991 the increase in ectopic pregnancy of about 1 in 44 deliveries was accompanied by an increase in the prevalence of *C. trachomatis* in a group of subjects with ectopics.

Fitz-Hugh-Curtis Syndrome: The term Fitz-Hugh-Curtis syndrome (perihepatitis) is a rare complication of PID that is 5 times more likely to be caused by *Chlamydia* than by *N. gonorrhoeae*. It frequently presents without the typical examination findings associated with PID (ie, the pelvic examination is normal) (Gene, 2010). It occurs when the organism spreads from the fallopian tubes through the peritoneum to produce a localized peritonitis. Spread through the lymphatic system and haematogenous spread may also likely occur as this has been found in women who have had tubal ligation and may rarely occur in men with gonococcal urethritis (Dalakar, 1981). Infected persons present Jwith right upper quadrant pain which may occur alone or with symptoms of vaginal discharge or PID. Fever, nausea and vomiting may as well be present. This condition occurs in 5-15% of women with laparoscopically diagnosed salpingitis and symptoms suggestive of it are found in 20% (Moss, 2001).

Reiter's syndrome: Reiter's syndrome is a triad of symptoms that include conjunctivitis, polyarthritis and genital inflammation. The disease is associated with HLA-B27. Approximately 50 - 65% of patients have an acute *C. trachomatis* infection at the onset of arthritis and greater than 80% have serological evidence for *C. trachomatis* infection. Other infections (shigellosis or *Yersinia enterocolitica*) have also been associated with Reiter's syndrome (Gene, 2010).

Cervical cancer: Women with a chlamydial infection (especially one caused by serotype G) are at increased risk for the development of cervical cancer; the risk is as much as 6.5 times greater than it is in women without infection (Gene, 2010). Chlamydial infections also increase the risk of acquiring HIV infection by increasing genital mucosal inflammation (Gene, 2010).

Endometritis: *C. trachomatis* has been found to be associated with endometritis (Eckert *et al.,* 2002; Weisenfeld *et al.,* 2002). Nearly half of patients with chlamydial cervicitis will have also an endometritis. This can be asymptomatic or the patient may complain of menorrhagia (an excessive regular menstrual flow) or postcoital bleeding. Plasma cells in the stroma and polymorphonuclear leukocytes in the superficial epithelium is seen in endometrial biopsy but it is rarely undertaken (Moss, 2001).

Salpingitis: *C. trachomatis* has been found to be also associated with laparoscopically confirmed salpingitis (Taylor-Robinson *et al.,* 2009). This bacterium initially infect the cervix and the urethra in women and infection spreads from the cervix to the fallopian tubes if left untreated resulting in the scarring of the fallopian tubes preventing the eggs from being fertilized. This blockage of the fallopian tubes due to chlamydial infection is known as salpingitis and it leads to infertility. In U.S.A., salpingitis accounts for 150000 to 200000 per year of infertility in women (Gates *et al.,* 1991). It was estimated by Westrom in 1975 that

even with antibiotic treatment, tubal occlusion still occurs in 13% of women after one attack of salpingitis, in 36% after two attacks and in 75% after three attacks. More so, a woman with salpingitis is six times more likely to have an ectopic pregnancy.

Bartholinitis: Bartholin's glands are located besides the opening of the vagina. Bartholinitis- infection of one or both glands or their ducts may develop when bacteria from the vagina enter the glands. Rarely, bartholinitis is due to a sexually transmitted disease. The surrounding tissues (vulva) may swell. Pus accumulates in the gland, causing a painful abscess. Taking an antibiotic usually clears the infection in few days but the infection may recur. Analgesics may relieve the pain and an abscess or cyst needs to be drained (Mark *et al.,* 2003). If the ducts become blocked, the gland may swell but cause no pain- a disorder called Bartholin's cyst. In women younger than 50, a cyst that causes no symptoms does not require treatment but in women 50 and older, a biopsy of the cyst is recommended (Mark *et al.,* 2003).

Chronic Pelvic Pain: Moss in 2001 defined chronic pelvic pain as a pain that has been present for six months. The prevalence rate in Britain has been reported to be 21.5 per 1000 women which, is comparable to 21.5 for migraine and 41.5 for backache (Zondervan *et al.,* 1999). Approximately 350000 females of reproductive age suffer from chronic pelvic pain which accounts for 20% of all gynaecological referrals (Davies *et al.,* 1992).

Pelvic Inflammatory Disease: *C. trachomatis* is a frequent pathogen of pelvic inflammatory disease (PID) (Ness *et al.,* 2004), and is isolated in the upper genital tract of about a quarter of PID patients (Haggerty *et al.,* 2004). However, rates of progression vary widely between patients. Among untreated *C. trachomatis* positive women, studies have reported that 2.0%–4.5% developed clinical PID within approximately 14 days (Geisler *et al.,* 2008). However, studies with longer follow-up have reported lower rates of PID following untreated

chlamydial infection, ranging from 0%–9.5% (Oakeshott *et al.,* 2010). In the largest study conducted in Norway, between 1990 and 2005, 1.09% of women who tested positive for *C. trachomatis* were hospitalized for PID (Bakken and Ghaderi, 2009). Furthermore, severe PID resulting in hospitalization represents a small proportion of PID cases (Paavonen *et al.,* 2008) and most cases of PID are mild to moderate and treated as outpatients. In the randomized controlled Prevention of Pelvic Infection (POPI) trial, 9.5% of untreated Chlamydia positive women (determined by nucleic acid amplification test) developed clinical PID within 12 months of follow-up (Oakeshott *et al.,* 2010). However, only 17% of self-reported PID cases were verified by medical records. In a prospective study of 1170 women from five US sites, 19% of those with treated chlamydial infection at baseline (nucleic acid amplification test) developed clinical PID within three years of follow-up (Ness *et al.,* 2006). Repeated chlamydial infections are common (Evans *et al.,* 2009) and may increase the risk of sequelae (Skjeldestad *et al.,* 2009). Ness *et al*., 2008 reported that in 443 women with clinically suspected PID, recurrence was higher and pregnancy rates were significantly lower among women whose antibody titers to chlamydia elementary bodies were in the highest titer.

2.1.12 Trachoma

This is a contagious disease caused by *C. trachomatis* serotypes A-C. It is one of the oldest known infectious diseases of humans and is the greatest single cause of blindness throughout the world- over 500 million people are infected and 20 million blinded each year. In endemic areas, most children are chronically infected within a few years of birth. Active disease in adult over age twenty is three times as frequent in females as in males because of mother-child contact. Although trachoma is uncommon in the United States, except among Native Americans in the Southwest, it is widespread in Asia, Africa, and South America (Eugene *et al.,* 2004).

Trachoma is transmitted by contact with inanimate objects such as soap and towels, by hand-to-hand contact that carries *C. trachomatis* from an infected eye to an uninfected eye, or by flies. The disease begins abruptly with an inflamed conjunctiva. This leads to an inflammatory cell exudates and necrotic eyelash follicles. The disease usually heals spontaneously. However, with reinfection, secondary infections, vascularization of the cornea, or pannus (tissue flap) formation, scarring of the conjunctiva can occur. If scar tissue accumulates over the cornea, blindness results (Eugene *et al.,* 2004).

2.1.12.1 Inclusion Conjunctivitis

Inclusion conjunctivitis is caused by *C. trachomatis* (biovar: trachoma) associated with genital infections (serovars D - K). The infection is characterized by a mucopurulent discharge, corneal infiltrates and occasional corneal vascularization. In chronic cases corneal scarring may occur. In neonates infection results from passage through an infected birth canal and becomes apparent after 5 - 12 days. Ear infection and rhinitis can accompany the ocular disease (Gene, 2010).

2.1.12.2 Ocular Lymphogranuloma Venereum

Infection with the LGV serovars of *C. trachomatis* (biovar: LGV) can lead to oculoglandular conjunctivitis. In addition to the conjunctivitis, patients also have an associated lymphadenopathy (Gene, 2010).

2.1.13 Infant Pneumonia

Infants infected with *C. trachomatis* (biovar: trachoma; serovars: D - K) at birth can develop pneumonia. The children develop symptoms of wheezing and cough but not fever. The disease is often preceded by neonatal conjunctivitis (Gene, 2010). Neonatal pneumonia has been reported as a sequeala of ophthalmia neonatorum, but is of great concern now following observations made by Beem and Saxon that *C. trachomatis* could regularly be recovered from

nasopharyngeal and tracheobronchial aspirates collected from infants with distinctive pneumonia syndrome (Beem *et al.,* 1977).

2.1.14 *Chlamydia* and HIV

The consequences of chlamydia are not limited to pregnancy. Research has shown that women infected with chlamydia are up to five times more likely to acquire HIV if exposed to the virus. The reason for the increased risk may be that chlamydia causes a spike in the number of white blood cells at the site of infection. Some of these immune system cells, while needed to fight the infection, also happen to be the main target for HIV (Gale, 2012).

2.1.15 *Chlamydia* and Cervical Cancer

Some studies have shown an increased risk of cervical cancer in women who have had chlamydia. A study published in the January 2001 issue of the Journal of the American Medical Association (JAMA) suggested that certain strains of *C. trachomatis* may increase the risk of cervical cancer in infected women. Although infections with cancer-causing strains of human papillomavirus (HPV) remain the prime cause of cervical cancer, infection with certain subtypes of *C. trachomatis* may contribute to that risk, according to the study (Gale, 2012).

2.1.16 Chlamydial Infection in Pregnancy

Chlamydial infection in pregnant women is especially serious because it is related directly to miscarriage, stillbirth, inclusion conjunctivitis and infant pneumonia (Prescott *et al.,* 2005). It as well increases the risk of premature rupture of membrane, preterm delivery, premature contractions and small or gestational age infants (Cohen *et al*., 1990). It is also implicated in low birth weight and presence of meconium (discharge of a greenish-black, viscid substance) (Much and Yeh, 1991).

Transmission to the newborn results from exposure to the mother's infected cervix during birth. All women should be screened for *Chlamydia* as part of routine prenatal care. Infants with chlamydial infection may be born prematurely. They also may experience eye inflammation (conjunctivitis) and breathing problems. Chlamydial infection also can involve the oropharynx, genital tract and rectum. Infection sometimes can cause pneumonia during an infant's first months (Gale, 2012).

2.1.17 Laboratory Diagnosis of CT

There are several laboratory tests for diagnosis of *C. trachomatis* but the sensitivity of the tests will depend on the nature of the disease, the site of specimen collection and the quality of the specimen. Since Chlamydia are intracellular parasites, swabs of the involved sites rather than exudate must be submitted for analysis. It is estimated that as many as 30% of the specimens submitted for analysis are inappropriate (Gene, 2010).

Cytology: Examination of stained cell scrapings for the presence of inclusion bodies has been used for diagnosis but this method is not as sensitive as other methods.

Culture: Culture is the most specific method for diagnosis of *C. trachomatis* infections. Specimens are added to cultures of susceptible cells and the infected cells are examined for the presence of iodine-staining inclusion bodies. Iodine stains glycogen in the inclusion bodies. The presence of iodine-staining inclusion bodies is specific for *C. trachomatis* since the inclusion bodies of the other species of chlamydia do not contain glycogen and stain with iodine.

Antigen Detection: Direct immunofluorescence and ELISA kits that detect the group specific LPS or strain-specific outer membrane proteins are available for diagnosis. Neither is as good as culture, particularly with samples containing few organisms (e.g. asymptomatic patients).

Serology: Serological tests for diagnosis are of limited value in adults, since the tests do not distinguish between current and past infections. Detection of high titer IgM antibodies is indicative of a recent infection. Detection of IgM antibodies in neonatal infection is useful.

Nucleic Acid Probes: Three tests based on nucleic acid probes are available. These tests are sensitive and specific and may replace culture as the method of choice.

2.1.18 Treatment of CT

Treatment of *C. trachomatis* infection is considered to be effective in preventing transmission to sexual partners (CDC, 2010). Several drugs are active against *C. trachomatis* in tissue cultures, including rifampin, tetracyclines, macrolides, sulfonamides, some fluoroquinolones, and clindamycin (Stamm *et al.,* 2008). Rifampin is highly active in vitro, but resistance to this drug can be readily developed (Schachter, 1983). Thus, rifampin is not used to treat human chlamydial infections (Stamm *et al.,* 2008). *C. trachomatis* is also susceptible to sulfonamides, but they are not used for genital chlamydial infections (Schachter *et al.,* 2008). Currently, the US CDC recommends treatment with azithromycin (1g, single dose taken orally) or doxycycline (100mg, twice per day for seven days) (CDC, 2010). A recent meta-analysis revealed similar microbiological cure rates for azithromycin (97%) and doxycycline (98%) (Lau and Qureshi, 2002). Azithromycin is recommended when compliance may be an issue, such as in adolescents or those with low socioeconomic status due to its ability to be given in a single dose. Alternative regimens can include erythromycin base (500 mg four times per day for seven days), erythromycin ethylsuccinate (800mg four times per day for seven days), ofloxacin (300mg twice a day for seven days), or levofloxacin (500mg daily for seven days) (CDC, 2010). Erythromycin is effective in treating chlamydia, but has side effects, which may reduce compliance (CDC, 2010). Levofloxacin and ofloxacin are also effective, but are expensive (CDC, 2010). There is currently no solid evidence of any in vivo

antibiotic resistance for *C. trachomatis* genital tract infection (Rekart and Brunham, 2008). However, very few laboratories actually monitor chlamydial antibiotic resistance (Rekart and Brunham, 2008). Although recurrent infections are common in both men and women, it is unclear if they are due to treatment failures. In the US, it is likely that many treatment failures go unnoticed in asymptomatic infection, because test-of-cure (repeat testing three to four weeks after completing therapy) is not recommended, except in pregnant women (CDC, 2010).

2.1.19 Prevention of CT

Unfortunately, literature on Chlamydia stresses treatment instead of prevention. Because Chlamydia is a completely preventable disease, this should be the focus of health care and research facilities. Prevention strategies include personal strategies, community-based strategies, and health-care provider strategies (CDC, 1993).

Personal Strategies: Personally, it is important to see a health care provider regularly. Because chlamydia is markedly asymptomatic, routine visits to a health care facility may be the only way for detection. It is also essential to refer sexual partners for testing and treatment if one has been diagnosed with chlamydia. Other behavioral changes include using condoms during sex, delaying the age of first intercourse, monogamy, discussing sexual history with partners, and educating yourself about sexually transmitted diseases and sharing this information with friends.

Community-Based Strategies: Community-based strategies include public awareness, HIV and STD risk reduction programs, school involvement, and targeting out-of-school adolescents through vocational training centers, detention centers, and recreation programs. Schools can provide rates of the disease, its adverse symptoms and consequences, information on treatment for sex partners, and where and how to obtain care. Role playing in

health classes can also be used to stress how to tell a partner of infection and how to find care for the infection.

Role of Health Care Providers: Health care providers need to be more aware and recognize symptoms of Chlamydia. Because most chlamydial infections look like a bladder infection, this is difficult. Similarly, providers should arrange for treatment of sex partners, counsel patients on the risk of STDs, and screen at-risk patients.

Wisconsin's Control Program: Wisconsin currently has a control program organized by Madison's Laboratory of Hygiene, Milwaukee's Bureau of Laboratories, and Planned Parenthood Inc. This program features selective screening, low-cost and high-volume testing, notification and treatment of infected partners, education of health care providers, and epidemiological methods to identify at-risk groups. It emphasizes coordinating private facilities and public programs to control the disease. This includes case reporting and notification of infected partners by private facilities to the public sector. The program is also working to identify the most effective methods of sampling and detecting chlamydial infection (Hillis *et al.,* 1995).

Social Issues of *Chlamydia*: *Chlamydia* is a socially transmitted disease. Thus, medical intervention cannot be the only solution to control infection rates. Social factors, including behavioral changes and consistent access to quality health care, need to be included to eradicate this preventable disease.

2.1.20 Vaccines to Prevent Chlamydial Infection

The asymptomatic nature of *Chlamydia* makes diagnosis, treatment, and prevention of sequelae a challenge. Host immunity induced by chlamydial infections is not long-lasting, and may take several months or years to develop (Schachter *et al.,* 2008). Further, a single *C. trachomatis* infection does not adequately protect against reinfection (Schachter *et al.,* 2008).

In addition, it is hypothesized that aggressive treatment may blunt natural immunity (Brunham *et al.,* 2005; Rekart and Brunham, 2008). Therefore, control and management of chlamydia may depend on a safe and effective vaccine that can induce a better immune response than what occurs naturally (Schachter *et al.,* 2008). However, the pathogenesis of *C. trachomatis* has not been completely elucidated, and the role of host immunology is unclear. Coupled with a lack of a suitable animal model and inability to manipulate chlamydia genetically, researchers have yet to develop a successful vaccine.

Chlamydial surface antigens are possible vaccine candidates (Schachter *et al.,* 2008). Several chlamydial vaccine trials have used the major outer membrane protein as a vaccine candidate. However, studies using the major outer membrane protein have been inconclusive and immunity is generally short-lived (Pal *et al.,* 2006). Furthermore, the major outer membrane protein is not serovar-specific. Thus, other antigens are currently being considered as vaccine candidates. The recently discovered surface-exposed protein, polymorphic membrane protein D, has been shown to act as an adhesion molecule (Wehrl *et al.,* 2003) and can stimulate pro-inflammatory cytokines through the nuclear factor-κB pathway (Niessner *et al.,* 2003). Crane *et al.,* (2006) found that polymorphic membrane protein can recruit host antibodies to the site of infection, making this protein a promising vaccine candidate. Antibody response was also neutralizing across different serovars. However, chlamydia may undergo a type of antigenic variation (Tan *et al.,* 2010). Thus, it has been suggested that a single antigen may not be optimal as a vaccine candidate. Further increasing our knowledge of chlamydial pathogenesis will bring researchers closer to developing a successful vaccine.

2.2 HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS) (Douek *et al.,* 2009), a condition in

humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive.

Aquired Immunodeficiency Syndrome (AIDS) was first clinically observed in 1981 in the United States (Dolin *et al.,* 2010). The initial cases were a cluster of injection drug users and gay men with no known cause of impaired immunity showed symptoms of Pneumocystis carinii pneumonia (PCP), a rare opportunistic infection that was known to occur in people with very compromised immune systems (Gottlieb, 2006). Soon thereafter, additional gay men developed a previously rare skin cancer called Kaposi's sarcoma (KS) (Friedman-Kien, 1981 and Hymes *et al.,* 1981). Many more cases of PCP and KS emerged, alerting U.S. Centers for Disease Control and Prevention (CDC) and a CDC task force was formed to monitor the outbreak (Basavapathruni and Anderson, 2007).

In 1983, two separate research groups led by Robert Gallo and Luc Montagnier independently declared that a novel retrovirus may have been infecting AIDS patients, and published their findings in the same issue of the journal Science (Barré-Sinoussi *et al.,* 1983; Gallo *et al.,* 1983). Gallo claimed that a virus his group had isolated from an AIDS patient was strikingly similar in shape to other human T-lymphotropic viruses (HTLVs) his group had been the first to isolate. Gallo's group called their newly isolated virus HTLV-III. At the same time, Montagnier's group isolated a virus from a patient presenting with swelling of the lymph nodes of the neck and physical weakness, two classic symptoms of AIDS. Contradicting the report from Gallo's group, Montagnier and his colleagues showed that core proteins of this virus were immunologically different from those of HTLV-I. Montagnier's group named their isolated virus lymphadenopathy-associated virus (LAV) (Basavapathruni and Anderson, 2007). As these two viruses turned out to be the same, in 1986, LAV and HTLV-III were renamed HIV (Aldrich and Wotherspoon, 2001).

2.2.1 Classification of HIV

HIV is a member of the genus Lentivirus (International Committee on Taxonomy of Viruses, 2002 (ICTV)), part of the family of Retroviridae (ICTV, 2002). Lentiviruses have many morphologies and biological properties in common. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period (Lévy, 1993).

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. It is more virulent, more infective (Gilbert *et al.,* 2003), and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa (Reeves and Doms, 2002).

2.2.2 Structure and Genome of HIV

HIV is different in structure from other retroviruses (figure 3). It is roughly spherical (McGovern *et al.,* 2002) with a diameter of about 120 nm, around 60 times smaller than a red blood cell, yet large for a virus (Fisher *et al*., 2007). It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24 (Various, 2008). The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle (Various, 2008).

This is, in turn, surrounded by the viral envelope that is composed of two layers of fatty molecules called phospholipids taken from the membrane of a human cell when a newly

formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein that protrudes through the surface of the virus particle (Various, 2008). This protein, known as Env, consists of a cap made of three molecules called glycoprotein (gp) 120, and a stem consisting of three gp41 molecules that anchor the structure into the viral envelope (Chan *et al*., 1997). This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle (Chan *et al*., 1997). Both these surface proteins, especially gp120, have been considered as targets of future treatments or vaccines against HIV (National Institute of Health, 1998).

The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (gag, pol, and env, tat, rev, nef, vif, vpr, vpu, and sometimes a tenth tev, which is a fusion of tat env and rev), encoding 19 proteins. Three of these genes, gag, pol, and env, contain information needed to make the structural proteins for new virus particles (Various, 2008). For example, env codes for a protein called gp160 that is broken down by a cellular protease to form gp120 and gp41. The six remaining genes, tat, rev, nef, vif, vpr, and vpu (or vpx in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease (Various, 2008).

The two Tat proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element. The TAR may also be processed into microRNAs that regulate the apoptosis genes ERCC1 and IER3 (Ouellet *et al*., 2008; Klase *et al*., 2009). The Rev protein (p19) is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the RRE RNA element. The Vif protein (p23) prevents the action of APOBEC3G (a cell protein that deaminates DNA: RNA hybrids and/or interferes with the Pol protein). The Vpr protein (p14) arrests cell division at G2/M. The Nef protein (p27) down-regulates

CD4 (the major viral receptor), as well as the MHC class I and class II molecules (Stumptner-Cuvelette *et al*., 2001).

Nef also interacts with SH3 domains. The Vpu protein (p16) influences the release of new virus particles from infected cells (Various, 2008). The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral genome packaging and recognized by Gag and Rev proteins. The SLIP element (TTTTTT) is involved in the frameshift in the Gag-Pol reading frame required to make functional Pol (Various, 2008).

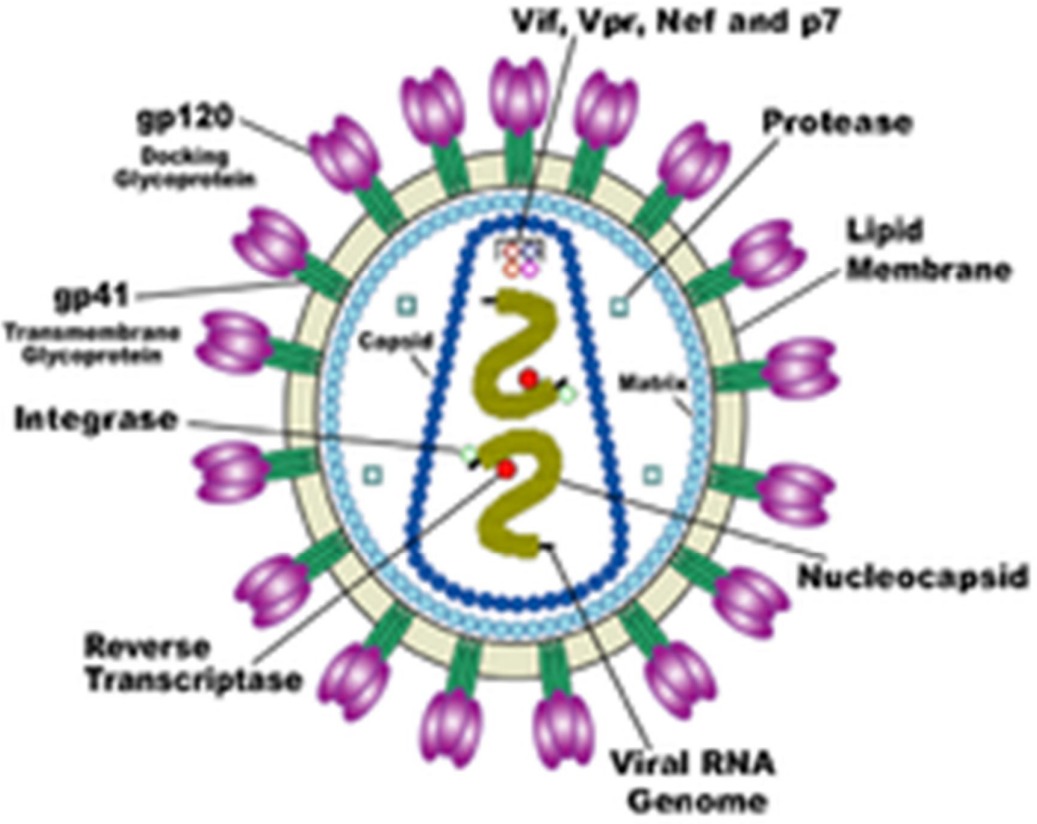


Figure 2: Structure of HIV (http://en.wikipedia.org/wiki/file:HIV_Virion-en.png)

2.2.3 Replication Cycle of HIV

HIV replicates through three processes which includes entry to the cell, replication and transcription and finally through assembly and release.

2.2.3.1 Entry to the cell

HIV enters macrophages and CD4+ T cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell (Chan and Kim, 1998; Wyatt and Sodroski, 1998).

Entry to the cell begins through interaction of the trimeric envelope complex (gp160 spike) and both CD4 and a chemokine receptor (generally either CCR5 or CXCR4, but others are known to interact) on the cell surface (Chan and Kim, 1998; Wyatt and Sodroski, 1998). gp120 binds to integrin α4β7 activating LFA-1 the central integrin involved in the establishment of virological synapses, which facilitate efficient cell-to-cell spreading of HIV-1 (Arthos *et al.,* 2008). The gp160 spike contains binding domains for both CD4 and chemokine receptors (Chan and Kim, 1998; Wyatt and Sodroski, 1998).

The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor (Chan and Kim, 1998; Wyatt and Sodroski, 1998). This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane (Chan and Kim, 1998; Wyatt and Sodroski, 1998). Repeat sequences in gp41, HR1, and HR2 then interact, causing the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membranes close together, allowing fusion of the membranes and subsequent entry of the

viral capsid (Chan and Kim, 1998; Wyatt and Sodroski, 1998). After HIV has bound to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease, and protease are injected into the cell (Chan and Kim, 1998). During the microtubule-based transport to the nucleus, the viral single-strand RNA genome is transcribed into double-strand DNA, which is then integrated into a host chromosome.

HIV can infect dendritic cells (DCs) by this CD4-CCR5 route, but another route using mannose-specific C-type lectin receptors such as DC-SIGN can also be used (Pope and Haase, 2003). DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs (Pope and Haase, 2003). The presence of FEZ-1, which occurs naturally in neurons, is believed to prevent the infection of cells by HIV (Haedicke *et al.,* 2009).

2.2.3.2 Replication and Transcription

Shortly after the viral capsid enters the cell, an enzyme called reverse transcriptase liberates the single-stranded (+) RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule (Zheng *et al.,* 2005). The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the antisense cDNA (Doc Kaiser, 2008). Together, the cDNA and its complement form a double-stranded viral DNA that is then transported into the cell nucleus. The integration of the viral DNA into the host cell's genome is carried out by another viral enzyme called integrase (Zheng *et al.,* 2005).

This integrated viral DNA may then lie dormant, in the latent stage of HIV infection (Zheng *et al.,* 2005). To actively produce the virus, certain cellular transcription factors need to be present, the most important of which is NF-κB (NF kappa B), which is upregulated when T-cells become activated (Hiscott *et al.,* 2001). This means that those cells most likely to be killed by HIV are those currently fighting infection.

During viral replication, the integrated DNA provirus is transcribed into mRNA, which is then spliced into smaller pieces. These small pieces are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev. As the newly produced Rev protein accumulates in the nucleus, it binds to viral mRNAs and allows unspliced RNAs to leave the nucleus, where they are otherwise retained until spliced (Pollard and Malim, 1998). At this stage, the structural proteins Gag and Env are produced from the full-length mRNA. The full-length RNA is actually the virus genome; it binds to the Gag protein and is packaged into new virus particles.

HIV-1 and HIV-2 appear to package their RNA differently; HIV-1 will bind to any appropriate RNA, whereas HIV-2 will preferentially bind to the mRNA that was used to create the Gag protein itself. This may mean that HIV-1 is better able to mutate (HIV-1 infection progresses to AIDS faster than HIV-2 infection and is responsible for the majority of global infections).

2.2.3.3 Assembly and Release

The final step of the viral cycle, assembly of new HIV-1 virons, begins at the plasma membrane of the host cell. The Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by protease and processed into the two HIV envelope glycoproteins gp41 and gp120. These are transported to

the plasma membrane of the host cell where gp41 anchors the gp120 to the membrane of the infected cell. The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell. Maturation occurs either in the forming bud or in the immature virion after it buds from the host cell. During maturation, HIV proteases cleave the polyproteins into individual functional HIV proteins and enzymes. The various structural components then assemble to produce a mature HIV virion (Gelderblom, 1997). This cleavage step can be inhibited by protease inhibitors. The mature virus is then able to infect another cell.

2.2.4 Epidemiology of HIV

When HIV was first recognized in 1981, UNAIDS and the WHO estimated that AIDS killed more than 25 million people from 1981 to 2005 (UNAIDS, 2006), making it one of the most destructive pandemics in recorded history (Douek *et al.,* 2009). These estimates mask the dynamic nature of this evolving epidemic in relation to temporal changes, geographic distribution, magnitude, viral diversity, and mode of transmission. Today, there is no region of the world untouched by this pandemic (Inciardi and Williams, 2005). UNAIDS estimated that 33.3 million people were living with HIV at the end of 2009, up from 26.2 million people in 1999 (UNAIDS, 2010). Also it was estimated that AIDS-related deaths in 2009 was 1.8 million, down from a peak of 3.2 million in 1997 and 2.1 million in 2004 (Cunningham *et al.,* 2010).

The most affected region is sub-Saharan Africa with an estimated 22.5 million people currently living with HIV with 1.3 million deaths and 1.8 million new infections (Cunningham *et al.,* 2010). However, the number of new infections declined by 19% across the region between 2001 and 2009, and by more than 25% in 22 sub-Saharan African

countries during this period (Douek *et al.,* 2009). Asia is the second-affected region, with 4.9 million people living with HIV (UNAIDS, 2010).

Heterosexual transmission remains the dominant mode of transmission and accounts for about 85% of all HIV infections (Inciardi and Williams, 2005). Southern Africa remains the epicenter of the pandemic and continues to have high rates of new HIV-1 infections (Hayes and Weiss, 2006). Outside of sub-Saharan Africa, a third of all HIV-1 infections are acquired through injecting drug use, most (an estimated 8·8 million) of which are in Eastern Europe and Central and Southeast Asia (Siegfried *et al.,* 2003). The rapid spread of HIV-1 in these regions through injecting drug use is of importance, since it is a bridge for rapid establishment of more generalized epidemics (Abdool-Karim, 2002).

A defining feature of the pandemic in the current decade is the increasing burden of HIV infections in women which has additional implications for mother-to-child transmission (Quinn and Overbaugh, 2005). Women now make up about 42% of those infected worldwide and over 70% of whom live in sub-Saharan Africa (UNAIDS, 2006). Overall, a quarter of all new HIV-1 infections are in adults aged younger than 25 years (Pettifor *et al.,* 2005). HIV infection rates are three to six times higher in female adolescents than in their male counterparts and this difference is attributed to sexual coupling patterns of young women with older men (Shisana and Davids, 2004). Population prevalence of HIV infection include concurrent sexual relationships, partner change, sexual practices, the presence of other sexually transmitted diseases and population mobility patterns(Bloom *et al.,* 2002; Korenromp *et al.,* 2005), others include economic, natural disasters and wars (Luire *et al.,* 2003; Hayes and Weiss, 2006).

2.2.5 Pathogenesis

HIV can attack a variety of types of cells in the human body, but most critically a subset of lymphocytes, T-helper (Th) cells. These cells possess the CD4 surface protein to which the virus attaches, and they are referred to as $CD4^+$ cells. In order for the virus to enter the cell, it must also attach to certain cytokine receptors on the cell surface. Following entry of the virus, a DNA copy is made using reverse transcriptase (Eugene *et al*., 2004). This copy integrates itself into the cell's genome and hides there, inaccessible to antiviral chemotherapy. If the cell becomes activated, however, the virus leaves the cell genome, replicates, and destroys the cell, releasing virions to infect additional cells. Despite the body's ability to replace hundreds of billions of $CD4^+$lymphocytes, the number of these lymphocytes slowly declines over many months or years. Infection of macrophages contributes to the decline in cellular immunity. Macrophages, also vitally important to the immune system, are also CD cells. Although they are generally not killed, their function is impaired both by the viral infection and the lack of interaction with Th lymphocytes. Eventually, the immune system becomes so impaired it can no longer respond to infections or cancers (Eugene *et al*., 2004).

2.2.6 Mode of HIV Transmission

The three major routes for HIV transmission include:

2.2.6.1 Sexual Route of Transmission

Sexual intercourse is the major mode of HIV transmissionand majority of HIV infections are acquired through unprotected sexual relations (CDC, 2010). Sexual transmission can occur when infected secretions of one sexual partner come into contact with the genital, oral, or rectalmucous membranes of another sexual partner (Boily *et al.,* 2009). In countries with high-income, the risk of female-to-male transmission is 0.04% per act and male-to-female transmission is 0.08% per act. These rates for various reasons are 4 to 10 times higher in

countries with low-income (Milett *et al.,* 2009). The rate also for receptive anal intercourse is much higher with 1.7% per act (Boily *et al.,* 2009).

A meta-analysis studies of the use of condom in 1999 showed that consistent use of latexcondoms reduces the risk of HIV via sexual transmission by about 85% (Nyindo, 2005). However, spermicide may actually increase the transmission rate (Mboto *et al.,* 2009).

Randomized, controlled trials in which uncircumcised men were assigned randomly to be medically circumcised in sterile conditions and given counseling and other men were not circumcised have been conducted in South Africa (Williams *et al.,* 2006) Kenya (Boily *etal.,* 2009) and Uganda (Gray, 2007) to show reductions in female-to-male HIV sexual transmission of 60%, 53%, and 51%, respectively. As a result, a panel of experts convened by WHO and the UNAIDS Secretariat has "recommended that male circumcision now be recognized as an additional important intervention to reduce the risk of heterosexually acquired HIV infection in men (WHO, 2007). Among men who have sex with men (MSM), there is insufficient evidence that male circumcision protects against HIV infection or other Sexually Transmitted Infections (STIs) (Milett *et al.,* 2009). HIV Studies among women who have undergone female genital cutting (FGC) have also reported mixed results with some evidence of increased risk of transmission (Maslovskaya *et al.,* 2009).

Observational studies also highlighted the relation between abnormal vaginal flora and increased risk of HIV-1 infection (Van derstraten, 2005). The high prevalence of vaginal infections such as bacterial vaginosis (30–50%), vulvovaginal candidosis (10–13%), and *Trichomonas vaginalis* (7–23%) in African women is found to be associated with a substantial risk of HIV-1 acquisition (Schwebke, 2005).

According to a 2007 Cochrane review studies programmes that aim to encourage sexual abstinence while also encouraging and teaching safer sex strategies for those who are

sexually active can reduce short- and long-term HIV risk behavior among young people in countries with high-income (Underhill *et al.,* 2008).

2.2.6.2 Transmission via Blood and Blood Products

Generally, HIV may be transmitted if infected blood comes into contact with any open wound (Mboto *et al.,* 2009). Infections in intravenous drug users, hemophiliacs, and recipients of blood transfusions (though most transfusions are checked for HIV in the developed world) and blood products (CDC, 2010) can be account for via this route of transmission. It is also of concern for persons receiving medical care in regions where there is prevalent substandard hygiene in the use of injection equipment, such as the reuse of needles in Third World Countries (Lifson, 1988). Health careworkers such as nurses, laboratory workers, and doctors have also been infected, although this occurs more rarely (Maslovskaya *et al.,* 2009). Since HIV transmission by blood and blood products became known, medical personnel are required to protect themselves from contact with blood by the use of universal precautions (Underhill *et al.,* 2008). HIV has been found at low concentrations in the saliva, tears, and urine of infected individuals, but there are no recorded cases of infection by these secretions and the potential risk of transmission is negligible (Lifson, 1988).

2.2.6.3 Mother-to-Child (MTC)

Mother-to-child transmission (MTCT) of HIV represents an especially tragic dimension of the burden of HIV/AIDS, particularly in resource constrained settings, where fragile and poorly funded health care systems hamper care and prevention efforts. The impact of this disease is best captured by data suggesting that at least one-third of HIV-infected children in developing countries die within their first year of life (Dabis *et al.,* 2000). MTCT has become a critical children's health problem in Africa, contributing to severe morbidity and significant mortality and undermining the impact of programs that had significantly reduced child mortality in previous decades.

The transmission of this virus from MTC can occur inutero (during pregnancy), *intrapartum* (at childbirth), or via breast feeding (Luzuriaga and Sullivan, 2005; UNAIDS, 2009). The rate of transmission at birth between MTC is around 25% in the absence of treatment (Coovadia, 2004). However, in situation where combinations of antiretroviral therapy and Caesarian section are available, transmission risk can be reduced to as low as one percent (CDC, 2010). Postnatal MTC transmission may largely be prevented by complete avoidance of breast feeding, however, this has significant association with morbidity (Smith *et al.,* 2005). Exclusive breast feeding and the provision of extended antiretroviral prophylaxis to the infant are also efficacious in avoiding transmission (Gottlieb *et al.,* 2004). UNAIDS in 2008 estimated that 430,000 children were primarily infected worldwide (19% of all new infections) by this route. Furthermore, 65,000 infections were averted through the provision of antiretroviral prophylaxis to HIV-positive women (UNAIDS, 2009).

2.2.7 HIV Transmission during Pregnancy

The sperm and the ovum lack the $CD4^+$ receptors and therefore cannot be infected by HIV. This implies that at fertilization, the resulting zygote does not contain the HIV genome. In most HIV-infected women, HIV does notcross the placenta from mother to foetus instead the placenta acts as a physical barrier that shields the developing foetus from circulating viruses in the mother's blood (Anderson, 1997). This protection from the placenta may break down, however, if the mother has a viral, bacterial, or parasitic placental infection during pregnancy, the mother becomes infected with HIV during the pregnancy and hence develops a high level of the virus for a short time or the mother has a severe immune deficiency associated with AIDS.

Thus, maternal conditions including untreated placental infections (particularly malaria), recent HIV infection, and advanced HIV disease have been cited as risk factors for MTCT

(WHO, 1999). Similarly, malnutrition during pregnancy may indirectly contribute to MTCT (Semba, 1997). However, one study suggests that vitamin A may increase the risk of vertical transmission (Fawzi *et al.,* 2002).

2.2.8 Sign and Symptoms

Infection with HIV-1 is associated with a progressive decrease of the $CD4^+$ T cell count and an increase in viral load (Grabar *et al.,* 2009). The patient's $CD4^+$ T cell count and viral load can be measured to determine the stage of infection (Blankson, 2010). HIV infection has three stages namely acute infection stage (also known as primary infection), latency and AIDS (Grabar *et al.,* 2009).

2.2.8.1 Acute Infection Stage

Generally, infection with HIV occurs by the introduction of bodily fluids from an infected person into the body of an uninfected person (Pantaleo *et al.,* 1997). A period of rapid viral replication ensues, leading to an abundance of virus in the peripheral blood. The level of HIV may reach several million virus particles per milliliter of blood during the primary stage of infection (Piatak, 1993). This response is accompanied by a marked drop in the numbers of circulating $CD4^+$ T cells (Grabar *et al.,* 2009). This acute viraemia developed is associated in virtually all patients with the activation of $CD8^+$ T cells, which kill HIV-infected cells, and subsequently with production of antibody or seroconversion (Daar *et al.,* 2001). The $CD8^+$ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the $CD4^+$ T cell counts rebound. A good $CD8^+$ T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus (Pantaleo *et al.,* 1997).

During this period (usually 2–4 weeks post-exposure) most individuals (80 to 90%) develop an influenza or mononucleosis-like illness called acute HIV infection (Kahn and Walker

1998) and the most common symptoms may include fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, mouth and esophageal sores, and may also less commonly include headache, nausea and vomiting, enlarged liver/spleen, weight loss, thrush, and neurological symptoms (Smith *et al.,* 2005). Individuals infected may experience all, some, or none of these symptoms. The duration of symptoms varies, averaging 28 days and usually lasting at least a week (Kahn and Walker 1998). They are often not recognized as signs of HIV infection because of the nonspecific nature of the symptoms. Even if patients go to their doctors or a hospital, they will often be misdiagnosed as having one of the more common infectious diseases with the same symptoms.

2.2.8.2 Chronic Infection Stage

A strong immune defense reduces the number of viral particles in the blood stream, marking the start of secondary or chronic HIV infection (Smith *et al.,* 2005). This stage of HIV infection can vary between two weeks and 20 years and during this phase of infection, HIV is active within lymph nodes, which typically become persistently swollen, in response to large amounts of virus that become trapped in the follicular dendritic cells (FDC) network (Burton *et al.,* 2002). The surrounding tissues that are rich in $CD4^+$ T cells may also become infected, and viral particles accumulate both in infected cells and as free virus (Blankson, 2010). Individuals who are in this phase are still infectious and at this time, $CD4^+$ $CD45RO^+$ T cells carry most of the proviral load (Clapham and Mcknight, 2001). Early initiation of antiretroviral therapy significantly improves survival, as compared with deferred therapy during this stage of infection (Kitahata *et al.,* 2005).

2.2.8.3 Acquired Immune Deficiency Syndrome (AIDS)

Cell-mediated immunity is lost when $CD4^+$ T cell numbers decline below a critical level of 200 cells per μL and infections with a variety of opportunistic microbes appear (Clapham and

Mcknight, 2001). Often, the first symptoms include moderate and unexplained weight loss, recurring respiratory tract infections (such as sinusitis, bronchitis, otitis media, pharyngitis), prostatitis, skin rashes, and oral ulcerations (Smith *et al.,* 2005). Common opportunistic infections and tumors, most of which are normally controlled by robust $CD4^+$ T cell-mediated immunity then start to affect the patient (Burton *et al.,* 2002). Resistance is typically lost early to oral *Candida* species and to *Mycobacterium tuberculosis*, which leads to an increased susceptibility to oral candidiasis (thrush) and tuberculosis. Later, reactivation of latent herpes viruses may cause worsening recurrences of herpes simplex eruptions, shingles, Epstein-Barr virus-induced B-cell lymphomas, or Kaposi's sarcoma(Blankson, 2010).

Pneumonia caused by the fungus *Pneumocystis jirovecii* is common and often fatal. In the final stages of AIDS, infection with cytomegalovirus (CMV, another herpes virus) or *Mycobacterium avium* complex is more prominent (Pantaleo *et al.,* 1997). Not all patients with AIDS get all these infections or tumors, and there are other tumors and infections that are less prominent but still significant.

2.2.9 Diagnosis of HIV Infection

Many HIV-positive people are unaware that they are infected with the virus (Kumaranayake and Watts, 2001). For example, in 2001 less than 1% of the sexually active urban populations in Africa have been tested and this proportion is even lower in rural populations (Kumaranayake and Watts, 2001). Furthermore, in 2001 only 0.5% of pregnant women attending urban health facilities are counselled, tested or receive their test results (Kumaranayake and Watts, 2001). Again, this proportion is even lower in rural health facilities (Kumaranayake and Watts, 2001). Since donors may therefore be unaware of their infection, donor blood and blood products used in medicine and medical research are routinely screened for HIV (Kleinman, 2004).

The diagnosis of HIV-1 infection is based on the detection of specific antibodies, antigens, or both, and many commercial kits are available (Greenwald *et al.,* 2006). Serological tests are used generally for screening (Perez *et al.,* 2005). A major advance has been the availability of rapid HIV-1 antibody tests (Cho *et al.,* 2006). These assays are easy to perform and provide results in as little as 20 minutes (Greenwald *et al.,* 2006), enabling specimen collection and proper diagnosis at the same visit. Rapid tests are important tools for surveillance, screening, and diagnosis, and can be reliably done on plasma, serum, whole blood, or saliva by health-care providers with little laboratory expertise (Nisole *et al.,* 2005). The two limitations of these serological tests are detection of infection during primary infection when antibodies are absent, and in infants younger than 18 months who might bear maternal HIV-1 antibodies. In these instances direct virus detection is the only option (eg, quantification of viral RNA [standard] or p24 antigen in heat denatured serum) (Stremlau *et al.,* 2006).

HIV-1 testing is initially by an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV-1. Specimens with a nonreactive result from the initial ELISA are considered HIV-negative unless new exposure to an infected partner or partner of unknown HIV status has occurred. Specimens with a reactive ELISA result are retested in duplicate (CDC, 2001). If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (e.g., Western blot or, less commonly, an immunofluorescence assay (IFA)). Only specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by Western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate Western blot result, which may be either an incomplete antibody response to HIV in an infected person or nonspecific reactions in an uninfected person (Celum *et al.,* 1991).

Although IFA can be used to confirm infection in these ambiguous cases, this assay is not widely used. In general, a second specimen should be collected more than a month later and retested for persons with indeterminate Western blot results. Although much less commonly available, nucleic acid testing (e.g., viral RNA or proviral DNA amplification method) can also help diagnosis in certain situations (CDC, 2001). In addition, a few tested specimens might provide inconclusive results because of a low quantity specimen. In these situations, a second specimen is collected and tested for HIV infection.

Modern HIV testing is extremely accurate. A single screening test is correct more than 99% of the time (Chou *et al.,* 2005). The chance of a false-positive result in standard two-step testing protocol is estimated to be about 1 in 250,000 in a low risk population (Chou *et al.,* 2005). Testing post exposure is recommended initially and at six weeks, three months, and six months (Tolle and Schwarzwald, 2010).

For staging purposes, measurement of CD4+ cells and viraemia is required (Cho *et al.,* 2005). Plasma viral load is widely used to monitor therapeutic success on antiretroviral treatment (CDC, 2010). Several commercially available tests provide sensitive quantification of plasma HIV-1 RNA copies. The newer versions of the Amplicor and Quantiplex (Roche, Indianapolis, IN, USA, and Bayer Diagnostics, Walpole, MA, USA, respectively) assays have overcome initial suboptimum performance for non-B subtypes (Berger *et al.,* 2005). While the viral load determines the rate of destruction of the immune system, the number of CD4+ cells reveals the degree of immunodeficiency and is, therefore, used to assess the stage of infection (Celum *et al.,* 1991). The CD4+ cell counts together with clinical manifestations (eg, occurrence of opportunistic infections) are key criteria for HIV-1 disease classification (Wood *et al.,* 2000). Flow cytometry analysis is the standard method for CD4+ cells quantification (Radeh *et al.,* 2005).

Standard methods for quantifying viral load and CD4+ cell counts need advanced laboratory infrastructures, and assays require a specimen to be tested within a short time of collection (Mofenson *et al.,* 2003). These requirements pose challenges for resource-constrained settings (Ceffa *et al.,* 2005). The use of dried blood spot specimen has resolved some of the difficulties associated with transportation of samples needed for virological assessments (Uttagamakul *et al.,* 2005). Measurement of reverse transcriptase activity in plasma samples, simplification of gene amplification methods (eg, Taqman technology) (Lombart *et al.,* 2005) and paper-strip quantification (dipstick assays) might provide cost-effective alternatives for the future (Tuaillon *et al.,* 2004; Jennings *et al.,* 2005). Similarly microcapilliary flow-based systems, CD4+ chips, or total white counts (panleucocyte gating) provide alternatives for establishment of the level of immunodeficiency in resource-limited settings (Rodriguez *et al.,* 2005; Spacek *et al.,* 2006).

2.2.10 Treatment of HIV Infection

2.2.10.1 Antiretroviral Compounds

Antiretroviral treatment is the best option for long lasting viral suppression and subsequently, for reduction of morbidity and mortality (Simon *et al.,* 2006). Current drugs however, do not eradicate HIV-1 infection and lifelong treatment might be needed (Lombart *et al.,* 2005). A total of 20 of the 21 antiretroviral drugs currently approved by the US Food and Drug Administration target the viral reverse transcriptase or protease. Eight nucleoside/nucleotide analogues and three non-nucleoside reverse transcriptase inhibitors inhibit viral replication after cell entry but before integration (Simon *et al.,* 2005). The recently approved darunavir (June, 2006) is the first of its class that retains activity against viruses with reduced susceptibility to protease inhibitors (Lazzarin *et al.,* 2003). Enfuvirtide targets a gp41 region of the viral envelope and stops the fusion process before the cell is infected. This drug needs to be injected twice daily and its use is reserved for treatment of heavily drug-experienced

patients since it can help overcome existing drug resistance (Lalezari *et al.,* 2003). Development of new antiretrovirals focuses on molecules that target entry, reverse transcription, integration, or maturation (Lombart *et al.,* 2005). Compounds that have been designed to inhibit resistant viruses are urgently needed since many patients treated during the past decades harbour viral strains with reduced susceptibilities to many if not all available drugs (Mocroft *et al.,* 1998).

The goal of antiretroviral treatment is to decrease the morbidity and mortality that is generally associated with HIV-1 infection (Lalezari *et al.,* 2003). A combination of three or more active drugs is needed to achieve this aim in most patients (Lombart *et al.,* 2005). Effective treatment returns to near normal the turnover rates of both CD4+ and CD8+ T-cell populations (Mohri *et al.,* 2001). Potent but well tolerated drugs with long half-lives and simplified regimens improve the options for first-line and second-line chemotherapeutic interventions (Simon *et al.,* 2005).

2.2.10.2 Combination Antiretroviral Agents

High rate of viral replication, low fidelity of reverse transcription, and the ability to recombine are the viral characteristics that lead to the diversity of HIV-1 species (quasi-species) in chronically infected individuals (Mohri *et al.,* 2001). This high genetic variability provided the rationale for highly active antiretroviral treatments (HAART) (Hogg *et al.,* 1998). By combination of several potent antiretroviral agents, viral replication is suppressed to such low levels that emergence of drug resistant HIV-1 variants was, if not prevented, at least delayed (Calza *et al.,* 2004). By doing so, CD4+ T-lymphocyte numbers increase, leading to a decrease of immune reconstitution that is sufficient to reverse clinically apparent immunodeficiency (Simon *et al.,* 2005). Widespread introduction of HAART in industrialized countries resulted in a striking decrease in morbidity and mortality, putting

forward the hope that HIV-1 infection can be transformed into a treatable chronic disease (Palello *et al.,* 1998; Mocroft and Lundgren, 2004).

A set of criteria composed of plasma viraemia concentration, absolute or relative CD4+ cell counts, and clinical manifestations, is used to recommend initiation of HAART (Nolan and Mallal, 2005). The benefits of treatment clearly outweigh the potential side-effects in patients with clinical signs of immunodeficiency (eg, AIDS defining illnesses) or with CD4+ numbers less than 200 per μL (recommendation of US Department of Health and Human Services, October, 2005) (Wyatt and Klotman, 2006). However, the best time point to begin treatment remains controversial in asymptomatic patients with modest depletion of CD4+ T cells (eg, more than 350 per μL) and modest levels of viraemia (eg, less than 100 000 copies per mL) (Mocroft and Lundgren, 2004). Studies with clinical endpoints supporting the validity of early versus late interventions in asymptomatic patients are difficult to conduct and insufficient clinical data are currently available. Early depletion of gut CD4+ T lymphocytes, increasing viral diversity, and the poor regenerative abilities of key populations of the immune system provide arguments for beginning treatment as early as possible (Mehandru *et al.,* 2005). The wide application of this principle is restricted by long-term drug toxicities that lead to reduction of quality of life, and by treatment costs (French *et al.,* 2004). Toxicities (eg, renal, hepato, mitochondrial), metabolic changes (e.g lipodystrophy, diabetes mellitus), and immune reconstitution disease are some of the long-term problems that complicate decade-long HAART (Calza *et al.,* 2004; Nolan and Mallal, 2005).

One strategy addressing life-long daily compliance to HAART has been structured treatment interruptions (Dybul *et al.,* 2003). The rationale for this approach was based on the premise that the body's own immune system could keep the virus in check if exposed to a very modest level of viral replication. If successful, this strategy could limit drug toxicity and

reduce treatment costs (Hirschel, 2001). Although preliminary findings for this strategy were mixed in terms of benefits (Abbas and Mellors, 2002; Powers *et al.,* 2006), the recent early closure of the SMART trial was based on increased morbidity and mortality in the treatment interruption arm (El-Sadr and Neaton, 2006). Thus, in the absence of clinical benefits, most Investigators strongly discourage treatment interruptions except as needed to address treatment intolerance (Mehandru *et al.,* 2005).

2.2.11 Prevention of HIV Infection

2.2.11.1 Mother-To-Child Transmission

Prevention of MTCT has seen advances in both industrialized and resource-constrained settings (Luzuriaga and Sullivan, 2005; Newell, 2006). Intrapartum transmission has been reduced by increasing access to interventions such as one dose of nevirapine to mother and newborn baby (Ekouevi *et al.,* 2005). Concerns about drug-resistant viral strains have led to several trials with combination treatments to reduce transmission during the intrapartum period (Chaix *et al.,* 2006; Mclntyre, 2006). In some settings, elective delivery by caesarean section can further reduce HIV-1 transmission during the intrapartum period (Cressey *et al.,* 2005), but the benefits of the intervention could be countered by post-partum sepsis and increasing maternal mortality (Read and Newell, 2005).

Because HIV-1 can be transmitted by breastfeeding, replacement feeding is recommended in many settings (Simon *et al.,* 2005). Poor access to clean running water precludes, however, the use of formula feeding under these circumstances (Magoni *et al.,* 2005) and exclusive breastfeeding with abrupt weaning is one option for reducing transmission (Rollins *et al.,* 2004). A potential novel intervention still being tested is the daily use of antiretrovirals during breastfeeding (Duerr *et al.,* 2005). More attention is starting to focus on the pregnant mother, especially initiation of antiretroviral therapy in mothers with low CD4+ counts

during pregnancy and thereafter (Welty *et al.,* 2005). Only limited data are available regarding the health of uninfected children born to HIV-1-positive mothers (Tuomala *et al.,* 2002). In a European cohort of exposed-uninfected children, no serious clinical manifestations were apparent, at least in the short term to medium term (median follow-up 2 years).

2.2.11.2 Sexual Transmission

Reduction of heterosexual transmission is crucial for control of the epidemic in many parts of the world (Chan, 2005) and prevention is achieved through reduction in the number of discordant sexual acts or reduction of the probability of HIV-1 transmission in discordant sexual acts (UNAIDS, 2009). The first can be achieved through abstinence and sex between concordantly seronegative individuals (Siegfried *et al.,* 2003). Abstinence and lifelong monogamous relationships might not be adequate solutions for many people and therefore several interventions aimed at lowering the risk of transmission per discordant sexual act are in the process of clinical testing (Chan, 2005). Male and female condoms provide a proven and affordable prevention option (Weller and Davis, 2002). In combination, these options are also more commonly referred to as the ABC (abstinence, be faithful, condom use) approach (UNAIDS, 2009).

Other biomedical prevention interventions include male circumcision, antiretroviral for prevention (eg, pre-exposure or post-exposure), chemoprophylactic treatment of herpes simplex virus-2 (HSV-2), microbicides, and vaccines (Garenne, 2006). Results from one of three independent phase III male circumcision trials underway in South Africa, Kenya, and Uganda has helped to allay some of the ambivalence around the protective effect of male circumcision (Siegfried *et al.,* 2005). The findings from the South African trial show a 60% protective effect of male circumcision (Auvert *et al.,* 2005). The possible mechanism relates

to the fact that the foreskin has apocrine glands that secrete lysozymes but also Langerhans cells expressing CD4 and other receptors (Szabo and Short, 2000). These skin-specific dendritic cells can uptake virus and are believed to play a part in transport of the virus to susceptible T cells (Halperin *et al.,* 2005). Immunofluorescence studies of foreskin mucosa suggest that these tissues might be more susceptible to HIV-1 infection than cervical mucosa (Patterson *et al.,* 2002).

Post-exposure prophylaxis is recommended after occupational (eg, needle stick) (Puro *et al.,* 2004) and non-occupational (eg, rape, sexual abuse) (Winston *et al.,* 2005) exposure, although data for efficacy and optimum drug combinations are few (Bassett *et al.,* 2004). Some clinical trials assessing the benefits of once daily pre-exposure chemoprophylaxis with antiretroviral compounds with long biological half-life (eg, tenofovir) have been put on hold or stopped (Blower *et al.,* 2000; Gray *et al.,* 2001). Neither the overall idea of pre-exposure prophylaxis nor the drug itself, which is well tolerated, was at the root of the protests. Concerns were centered on clinical trials in resource-poor settings and the perceived scarcity of adequate interventions protecting these vulnerable populations (Grosskurth *et al.,* 2000).

Herpes simplex virus-2 (HSV-2) might increase both the risk of transmitting and acquiring HIV-1 (Corey *et al.,* 2004). Antivirals (eg, acyclovir, valaciclovir) are effective in reducing viral shedding (Nagot *et al.,* 2005) and HSV-2 transmission in discordant heterosexual couples (Gupta *et al.,* 2004). The future of HSV-2 prevention might reside in the vaccine that is currently under development (Hosken, 2005). Whether prophylactic use of acyclovir in populations with high HSV-2 prevalence and incidence rates results in reduced HIV-1 incidence rates remains unresolved but several trials addressing this issue are underway, including HPTN039 (Corey *et al.,* 2004).

Gender disparities lie at the centre of women's vulnerability. Prevention options need to be provided that can be used by women independently of their male sexual partner's knowledge or consent (Duffy, 2005). Notwithstanding that redressing these disparities are a long-term challenge, several preventive interventions can be implemented in the interim on the basis of our incomplete understanding at a biological level of HIV-1 risk for women (Gray *et al.,* 2005). For example, there seems to be a correlation between levels of sexual hormones (eg, progesterone) and transmission risk (Gray *et al.,* 2005).

In addition to increasing access to female condoms and treatment of other sexually transmitted infections, trials are underway to assess the use of other barrier methods such as cervical caps, invisible condoms, diaphragms, and diaphragms combined with microbicides (Van derstraten, 2005). The control of vaginal infections is a potentially important method for decreasing HIV-1 acquisition that is yet to be tested (Auvert *et al.,* 2005). Periodic presumptive treatment for vaginal infections is being explored as an HIV-1 prevention strategy (Myer *et al.,* 2005).

2.2.12 Drug Resistance

The most common reason for treatment failure is emergence of drug resistance (Simon *et al.,* 2006) and viral resistance has been described to every antiretroviral drug and therefore poses a serious clinical as well as public health problem (Deeks, 2002). HIV-1 subtypes differ in the sequence of mutations leading to drug resistance, and some naturally occurring polymorphisms might actually modulate resistance (Parkin and Schapiro, 2004 and Kantor *et al.,* 2005). HIV-1 drug resistant is transmissible and can be detected in up to 20% of newly infected individuals in countries with broad access to antiretrovirals (Steain *et al.,* 2004). Drug resistance prevalence in the untreated population remains low in regions with poor access to treatment (Wensing and Boucher, 2003).

Short term antiretroviral based interventions are effective in prevention of MTCT (Flys *et al.,* 2006). These interventions however, could result in drug resistant viral variants in the mother, baby, or both (Toni *et al.,* 2005). Around half the women who received one dose of nevirapine to prevent MTCT harbour viruses resistant to non nucleoside reverse transcriptase inhibitors (NNRTI) (Eshleman and Jackson, 2002). These resistant viruses replicate efficiently and can be transmitted by breast milk (Lee *et al.,* 2005), and minor resistant populations present long after the intervention can possibly decrease the effectiveness of subsequent NNRTI-based treatment regimens (Palmer *et al.,* 2006). The combination of short course zidovudine, lamivudine, and nevirapine prevents peripartum transmission while reducing the risk of nevirapine resistant viruses (Chaix *et al.,* 2006).

2.2.13 Vaccines

A safe, protective, and inexpensive vaccine would be the most efficient and possibly the only way to curb the HIV pandemic (Ho and Huang, 2002). Despite intensive research, development of such a candidate vaccine remains elusive. Safety concerns prohibit the use of live-attenuated virus as immunogen (Sheppard, 2005). Many different approaches with recombinant technologies have been pursued over the past two decades (Pantophlet and Burton, 2006). Efforts initially, were focused on generating neutralizing antibodies with recombinant monomeric envelope gp120 (AIDSVAX) as immunogen (Wyatt and Sodroski, 1998). This vaccine did not induce neutralizing antibodies and, not unexpectedly, the phase III trials failed to show protection (Cohen, 2003). Antibody mediated HIV-1 neutralization is complicated by the high genetic diversity of the variable *Env* regions, epitopes masked by a carbohydrate shield (glycosylation), and conformational or energetic constraints (Garber *et al.,* 2004). Since CD8 T-cell responses control to some extent viral replication *in vivo*, recent vaccine development has focused on eliciting cellular immune responses (Francis *et al.,* 2003).

Overcoming pre-existing immunity against replication incompetent immunogenic vectors (e.g. recombinant adenovirus type 5) is one of the challenges (Roberts *et al.,* 2006). Safety and immunogenicity studies using replication defective vaccine vectors are continuing after preliminary studies in non-human primates showed some protection (Garber *et al.,* 2004). The immune system generally fails to spontaneously clear HIV-1 and the true correlates of protection continue to be ill defined (Jamieson *et al.,* 2006) However, the general belief is that approaches aimed at eliciting both humoral and cell mediated immunity are most promising to prevent or at least control retroviral infection (Ho and Huang, 2002).

2.3 CT CO-INFECTION WITH HIV

Additional attention is being paid to a number of non-viral, sexually transmitted infections (STIs) because of recent thinking that they predispose to heterosexual transmission of HIV infection by a variety of mechanisms. For example, the lesions created by the ulcerative STIs, like syphilis, are believed to serve as portals that facilitate the entry of HIV during heterosexual intercourse between an infected person and an uninfected partner (Laga *et al.,* 1993). On the other hand, the non-ulcerative STIs, for example, trichomoniasis, gonorrhoea and chlamydia, elicit localised inflammations and immune responses characterised by the infiltration and accumulation of immune cells expressing CD4 surface proteins essential for the binding of HIV prior to entry, thus also facilitating the entry of HIV (Altes *et al.,* 2002; Joyee *et al.,* 2005; Wordaz and Hamer, 2007). By a combination of these mechanisms, both the ulcerative and non-ulcerative STIs are thought to serve as co-factors in the heterosexual transmission of HIV. Consequently, the risk of HIV transmission by these mechanisms increases when either the ulcerative or/and non-ulcerative STIs are left untreated for extended periods of time. The relatively high prevalence of these STIs in populations of low socioeconomic status in parts of Asia and sub-Saharan Africa has been blamed in part for the

high rate of spread of HIV during heterosexual intercourse in these regions (Ghys *et al.,* 1997; Sorvillo *et al.,* 2000).

Whereas syphilis, trichomoniasis and gonorrhoea have since been known and treated as serious public health problems amongst sexually active people, *Chlamydia trachomatis* infection has recently grown from a relatively obscure infection to become the commonest sexually transmitted bacterial infection in many countries (WHO, 2008), where it is repeatedly associated with a variety of health complications including, infertility (Siemer *et al.,*2008), pelvic inflammatory disease, ectopic pregnancy (Peipert, 2003) and a number of equally serious complications in men (Lin *et al.,*1998). These complications of *C. trachomatis* have prompted a number of developed countries, notably the United Kingdom, to offer free screening and treatment to sub-sections of the population associated with the highest prevalence of the disease.

The association of untreated non-viral sexually transmitted infections with some long-term health implications as well as the heterosexual transmission of HIV has resulted in an upsurge of interest on the prevalence of these diseases, especially *C. trachomatis*, whose global prevalence has been on the increase in recent times.

Nevertheless, *C. trachomatis* infection and HIV infection have interrelationship independent of the sexually transmissible risk factors. The possible relationships between HIV infection and *C. trachomatis* infection are:

i. The invasive intracellular pathogenesis of *C.trachomatis* can cause substantial damage to the genital epithelial layer which may facilitate HIV infection (Hitchcock, 1999).

ii. Immunological changes due to HIV infection may favour *C. trachomatis* infection (Debattista *et al*., 2002). The interrelationship between the two infections and mutually associated transmission pattern remain to be understood in greater detail.

Epidemiological studies have linked genital chlamydial infection to an increased risk for acquisition of HIV disease (Ghys *et al*., 1997). On the other hand, immunosuppression due to HIV may lead to more aggressive chlamydial disease conditions like PID in those who are infected with *C. trachomatis* (Thomas and Simms, 2002).

When Chlamydia pre-exists before HIV infection, it predisposes the subjects to HIV infection through the initiation of inflammatory and immune responses which facilitate the entry of HIV (Wodarz and Hamer, 2007). When HIV pre-exists before Chlamydial infection, the immune suppression typical of HIV infection increases the risk of Chlamydial infection occurring as an opportunistic infection. In subjects concurrently infected by both infections, the inflammation caused by untreated Chlamydia will increase the population of HIV-bound lymphocytes and macrophages present. This will in turn increase the viral sheding in the HIV-infected individuals, thereby increasing the probability of HIV transmission during intercourse with an uninfected partner (Sorvillo *et al.,* 2000). The possibility that HIV and any such concurrently existing STIs can be co-transmitted in the affected individuals has also been recognised (Cohen, 2004; McClelland *et al.,* 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted among pregnant and non pregnant women attending clinics in Zaria metropolis, Kaduna State, Nigeria. Zaria lies on $11^{0}2^{1}$ latitude (North) and $7^{0}43^{1}$ (East) and it is about 300km North of Abuja, the Central City of Nigeria. It is predominantly a Hausa speaking town in Nigeria, however it is quite heterogenous in population in terms of religion, ethnic groups and culture. Zaria is also comprised of two Local Government Areas; Zaria and Sabongari. The indigenous Hausa live predominantly in the walled city which is homogenous and has Emir's palace while Sabongari has a mixed population of both indigenous and non indigenous groups, hence heterogenous.

Zaria is an educational centre with many academic institutions among which is the Ahmadu Bello University, the largest University in the South of the Sahara. It also has a College of Aviation Technology, a Federal College of Education, a Nigerian Railway Training School, an Institute of Transport Technology, and a State Polytechnic. There is also the Nigerian Military School and the Nigerian Army Depot comprising of barracks and training units for the Nigerian army. It also has many primary and post primary institutions including a Federal Government Girls College. It has many healthcare centres ranging from primary, secondary to tertiary. There are government general hospitals, many private hospitals and clinics and a University Teaching Hospital. Zaria also has a good number of industries among which is the Nigerian Tobacco Company with many expatriates and Nigerians as its employees, the Zaria Industry which manufactures tarpaulin and an Industrial Development Centre with a training institute for craftmen as well as Nigerian Institute of Leather and Science Technology.

The town is densely populated with students, civil servants and business men and women. Its socioeconomic lifestyle is also very similar to that of other urban cities like Kaduna, Zaria, therefore presents itself as an interesting location for the conduct of this study with its socioeconomic set up and the resultant population.

The study area includes A.B.U. Sick bay, Major Ibrahim B.A. Memorial Hospital, Sabo and St. Luke's Anglican Hospital, Wusasa.

3.2 Study Design

The study was a descriptive cross-sectional survey which combines the use of administered structured questionnaires and the analysis of serum samples collected from consented patients. Quasi design was adopted for the purpose of this study.

3.3 Ethical approval

Approval was obtained from the ethical committee of the various health care centres before the commencement of the research in August 2012 (appendix I).

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion criteria

Women receiving antenatal care and non-pregnant women at the various health centres during the period of study who gave consent to participate in the study aged <15-50 years with or without HIV were eligible for inclusion (appendix II).

3.4.2 Exclusion criteria

Women receiving antenatal care and non-pregnant women at the various health centres during the period of study who do not give their consent to participate in the study aged <15-50 years with or without HIV were not eligible for inclusion (appendix II).

3.5 Sample size

The sample size was determined using the following equation as described by Naing *et al*., (2006).

$$n = \frac{z^2 p (1 - p)}{d^2}$$

Where n = sample size

z = z score for a level of 95% confidence interval = 1.96

p = prevalence rate at 10.5% Zaria, Nigeria (Muhammad *et al*., 2009).

d = allowable error = 5% = 0.05

Therefore:

$$n = \frac{(1.96)^2 \times 0.105 \times (1 - 0.105)}{(0.05)^2}$$

= 144.406

= 144 samples

Attrition rate = 10% of 144 sample size

= 158.4

= 158 samples

A total of 270 samples were collected (equal number of samples were collected to make good comparism between the subjects).

3.6 Questionnaire Administration

A structured questionnaire was administered after informed consent (appendix II) was obtained from the study population in order to access the risk factors associated with the prevalence of *Chlamydia trachomatis* co-infection with HIV among pregnant and non-pregnant women. Parameters considered included age, occupation, husband's occupation,

educational level, marital status, family type, pregnancy status, gravidity and parity status, stillbirth, miscarriage, preterm delivery and infertility (Appendix III).

3.7 Sample Collection

Blood samples were collected from women of reproductive age <15-50 years who gave their consent. Approximately two hundred and seventy (270) blood samples were collected from one hundred and thirty five (135) pregnant and one hundred and thirty five (135) non-pregnant women attending clinics from each of the centres under study in Zaria.

All materials required for the collection of venous blood was assembled and labelled appropriately with the subject's identification number (ID) and date. Five mililitre of blood was collected through venepuncture using sterile 5ml needles and syringes (CHANGZHOU HUICHUN MEDICAL EQUIPMENT CO., LTD, CHINA - Expiry date: 02-2016). The blood sample in the syringe was released into the labelled container and allowed to clot for 30 minutes. This was followed by centrifugation of the collected blood sample to separate the serum at 3000 r.p.m. for 5 minutes. The serum was thereafter carefully separated with a transfer pipette in order to avoid extracting red cells and aseptically transferred into a sterile labelled serum storage screw-capped container. The serum was stored in the Microbiology laboratory in a freezer at a temperature of -20°C until analysed.

3.8 CT Screening Using Enzyme Linked Immunosorbent Assay (ELISA)

3.8.1 Principle of the Assay

Purified *Chlamydia trachomatis* (CT) antigen is coated on the surface of microwells (WKEA MED SUPPLIES, CHANGCHUN, CHINA - Expiry date: 08-2013). Diluted patient serum is added to wells, and the *Chlamydia trachomatis* human immunoglobulin G (IgG) specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and thiomethyl benzoate (TMB) Chromogenic Substrate is added.

The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

3.8.2 Reagent Preparation

3.8.2.1 Wash buffer

The wash concentrate (WKEA MED SUPPLIES, CHANGCHUN, CHINA - Expiry date: 08-2013) was brought to room temperature (20-25^{0}C) from a temperature of 2^{0}C-8^{0}C and mixed gently until completely dissolved. Ten fold of the wash buffer concentrate was dissolved in distilled water to prepare 1000ml of wash buffer.

3.8.3 Assay Procedure

All reagents for the assay were brought to room temperature (20^{0}C-25^{0}C) from a temperature of 2^{0}C-8^{0}C and thoroughly mixed by swirling gently before use. Samples were numbered according to the microtitre wells. The desired number of coated strips was placed into the holder. Five microlitre (5µl) of the test samples, negative control, positive control and calibrator control were added to 200 µl of the sample diluents to make 1 in 40 dilutions. One hundred microlitres (100 µl) of diluted sera, calibrator and controls was dispensed into appropriate wells. For the reagent blank, 100µl sample diluents were dispensed into 1A well position. The holder was tapped so as to remove air bubbles, mixed well and incubated for 30 minutes at room temperature. The liquid from all the wells were removed and wells washed three times with washing buffer.

One hundred microlitres (100 µl) of the enzyme conjugate was also dispensed into each well and the plate incubated for 30 minutes at room temperature. Excess enzyme conjugate was removed by washing each well with washing buffer three times. Approximately, 100µl of TMB chromogenic Substrate was dispensed into each well and incubated at room

temperature for 30 minutes. One hundred microlitres (100 µl) of 2 N HCl was finally added to stop the reaction. There was a colour change from blue to yellow and within 15 minutes the plates were read at an absorbance of 450nm using a microwell reader (WKEA MED SUPPLIES, CHANGCHUN, CHINA – Expiry date: 08-2013).

3.8.4 Interpretation of Result

The calculation of cut off value was done using the manufacturer's specifications. A negative CT results was interpreted as any sample whose optical density was less than the calculated cut off value and samples with optical density greater than the calculated cut off value was reported as CT positive.

The cut-off optical density (OD) for Chlamydia IgG was obtained by multiplying the OD of calibrator by factor (f) printed on label calibrator. The Chlamydia IgG index was afterwards determined each by dividing the OD value of each sample by obtained OD value of cut-off. The results obtained were interpreted thus:

Negative: IgG index of 0.90 or less are seronegative for IgG antibody.

Equivocal: IgG index of 0.91 – 0.99 are equivocal and should therefore be retested.

Positive: IgG index of 1.00 or greater are seropositive for IgG antibody.

3.9 Human Immunodeficiency Virus Screening

Two different types of test kits were used for Human Immunodeficiency Virus **(**HIV) screening namely Determine® HIV 1/2 test kits and Uni-gold™ HIV test kits. All serum samples and test kits were brought to room temperature of about 20-25^{0}C from a temperature of between 2^{0}C-30^{0}C and labelled appropriately.

3.9.1 Determine® HIV 1/2 Test Kits

All test kits were brought to room temperature after which the sealed and protective foil were all removed from the test strips and placed on a flat working table. About 50µl of serum

sample was added to the sample pad and allowed to stand for 15 minutes. The serum migrates through the conjugate pad and mix with the selenium colloid-antigen conjugate through the solid phase. Antibody to HIV 1/2 in the serum binds to the antigen-selenium colloid (control bar) and to the antigen at the patient's bar, forming a red band at the patient bar site giving a positive test. Absence of antibody to HIV1/2 show no red band at the patient's bar giving a negative result (ALERE MEDICAL Co., Ltd. CHIBA, JAPAN – Expiry date: 03-2013).

3.9.2 Uni-Gold^{TM} HIV Test Kits

Samples reactive to Determine HIV 1/2 test were confirmed using Uni-gold HIV test kit. One of the sterile disposable pipettes was used to pipette 2 drops (60µl) of serum sample and then dispensed into the sample port, followed by addition of 2 drops of the wash buffer which was after wards allowed to react for 10 minutes. Antibodies of any immunoglobulin class specific to the recombinant HIV 1/2 proteins reacted with the gold linked antigens. The antibody protein-colloidal gold linked complex moves chromatographically along the membrane to the test and control region of the test device. After 10 minutes the result was immediately read. A positive reaction was visualized by a pink/red band in the test region of the kits. In the absence of human immunoglobulin antibodies to HIV in the analyzed specimen, a negative reaction occurs. Consequently, no visually detectable band develops in the test region of the device (TRINITY BIOTECH PLC, IRELAND – Expiry date: 25-05-2013).

3.10 Data Analysis

Results and data from questionnaires were presented on tables and figuress. All statistical analysis was done using a computer software program, SPSS Version 19. Associations and relationship between the various risk factors were obtained using chi-square, fisher's exact, analysis of variance (ANOVA) and the student t-test. Two tailed P values ≤ 0.05 was considered to be statistically significant.

CHAPTER FOUR

RESULTS

A total of 270 samples were collected out of which 135 samples were from pregnant women and 135 samples from non-pregnant women. In total, 32(11.9%) had chlamydial infection with 14(10.4%) being from pregnant women and 18(13.3%) from non-pregnant women (Table 4.1). A total of 7(2.6%) women were found to be HIV positive; 5(3.7%) were non-pregnant while 2(1.5%) were from pregnant women. In all, there was one case of HIV and chlamydial co-infection (Table 4.1).

The age distribution of chlamydial infection in both pregnant and non-pregnant women was assessed and presented in Figure 4.1. The highest percentage of chlamydial infection in pregnant women 3(11.5%) was recorded in age group 15–20 years followed by 4(10.5%) in the 26–30 age group. The least infection rate was found among women aged 31–35 years (9.7%) while those between 36–50 years had no chlamydial infection. Though there were slight variations in percentage occurrence of chlamydial infections with respect to age groups, those perceived variations were not statistically significant. In non-pregnant women, Chlamydial infection was highest in < 15 years (100.0%) followed by women aged 36–40 years while age group 31–35 years had 3(8.6%) infection among the infected people. Similarly, the infection showed no significant association with age.

Table 4.2 illustrates the distribution of chlamydial infection based on occupation and educational level. Though there was no statistically significant association between study subjects' occupation and chlamydial infection, there are variations in the various types of occupation with civil servants having 3(9.7%), self-employed 2(4.3%) and others 9(15.8%) had the highest chlamydial prevalence among the pregnant women. Among non-pregnant women, the highest occurrence rate was found among the self-employed 10(18.5%) closely

followed civil servants 4(18.2%) and then others with 4(6.8%). Among the women entirely, civil servants had the highest rate of chlamydial infection 7(13.2%).

A similar comparison of the study subjects' husbands' occupation revealed that there was no significant association between occupation and chlamydial infection. While civil servants had the highest prevalence of 12(17.6%) among the pregnant women, self-employed 7(17.5%) had the highest prevalence followed by civil servants self-employed 9(15.0%) among the non-pregnant women. The civil servants had the highest occurrence case 21(16.4%) of chlamydial infection among both pregnant and non-pregnant women (Table 4.2).

Perusal of the relationship between level of education and chlamydial infection showed that there was significant association between level of education and the infection among the women. Pregnant women with secondary education 7(12.7%) had the highest rate of infection followed by others with 1(12.5), those with tertiary education had 6(11.3%) whereas those with primary education 0(0.0%) had no chlamydial infection. Also among the non-pregnant women others, those with tertiary, secondary and primary education had prevalences of 4(26.7%), 6(14.0%), 7(11.7%) and 1(5.9%) respectively. There also was no significant association between level of education and chlamydial infection. Overall, others who had no education had a prevalence rate of 5(21.7%), those with tertiary education had 12(12.5%) followed by secondary education 14(12.2%) while individuals with primary education had prevalence of 1(2.8%) (Table 4.2).

A look at the marital status of the subjects showed that 14(10.4%) of the married pregnant women had chlamydial infection while 16(12.1%) married non-pregnant women had chlamydial infection and this was however found to be statistically significant among the non-pregnant women. Overall, there was statistically significant association between marital status and chlamydial infection (Table 4.3). No statistically significant association was found

between chlamydial infection and family type but those who practiced monogamy had prevalence of 13(11.8%) among the pregnant women and 17(15.2%) among the non-pregnant women. On the other hand, those that practiced polygamy had a prevalence of 1(4.0%) among the pregnant women and 1(4.3%) among the non-pregnant women. Those who practiced monogamy on the whole had the highest prevalence 30(13.5%) among the women.

There was statistically significant association ($\chi^2 = 13.025$, $p = 0.043$) between the number of years in marriage among the pregnant women with those who have stayed in marriage for 26–30 years having the highest prevalence 2(66.7%) of chlamydial infection. This was followed by 9(11.1%) for women who have spent 0-5 years in marriage and then 3(10.7%) for those who have spent 6-10 years im marriage. In non-pregnant women on the other hand, those that have been married for 26–30 years had the highest prevalence 3(42.9%) followed by 1(25.0%) for those that have stayed in marriage for 31–35 years. On the whole, women that have been married for 26–30 years had the highest prevalence 5(50.0%) followed by 1(25.3%) for women that have been married for 31–35 years; this variation was found to be significantly associated with chlamydial infection (Table 4.3).

Figure 4.2 reveals that there is no statistically significant difference between chlamydial infection and number of sexual partners. Only individuals with one sexual partner among the subjects had chlamydial infection. There were 14(10.7%) pregnant women with chlamydial infection while non-pregnant women with infection were 18(13.6%). Comparison of the infection rates between the pregnant and non-pregnant women showed that there was no statistically significant difference between pregnant and non-pregnant women.

In Figure 4.3, it was revealed that pregnant women who have no children had higher prevalence 5(10.4%) of Chlamydial infection than pregnant women 8(10.3%) that have children and it showed no significant association, while non-pregnant women that have

children had a prevalence of 18(13.7%) as opposed to non-pregnant counterparts 0(0.0%) that have had no children and was found to be significant statistically. Overall, the prevalence based on whether or not the subjects have children was 27(12.4%) and 5(9.6%) respectively and was not statiscally significant.

Table 4.1: *Chlamydia trachomatis* and HIV Co-infection among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

		No. +ve (%)		
Status	**No. of samples**	**CT**	**HIV**	**Co-infection**
Pregnant	135	14 (10.4)	2 (1.5)	0 (0.0)
Non-pregnant	135	18 (13.3)	5 (3.7)	1 (0.7)
Total	**270**	**32 (11.9)**	**7 (2.6)**	**1 (0.4)**

KEY

No. +ve = Number of positive samples

CT = *Chlamydia trachomatis*

HIV = Human Immunodeficiency Virus

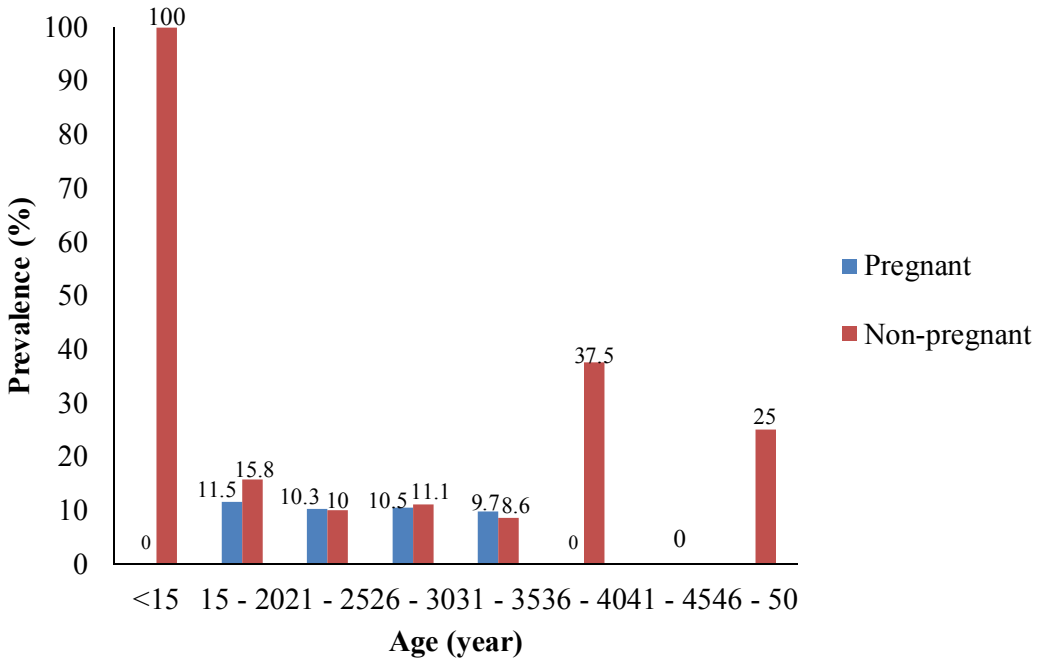


Figure 4.1: Percentage distribution of chlamydial infection according to age (t = 0.866, p = 0.242) among women attending clinics in Zaria metropolis, Kaduna State, Nigeria.

Table 4.2: Distribution Rate of Chlamydial Infection According to Demographic Factors among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Risk factor	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
Occupation of Subjects						
Civil servants	31	3(9.7)	22	4(18.2)	53	7(13.2)
Self-employed	47	2(4.3)	54	10(18.5)	101	12(11.9)
Others	57	9(15.8)	59	4(6.8)	116	6(11.2)
Statistics		$\chi^2 = 5.565$, p = 0.201		$\chi^2 = 6.665$, p = 0.222		$\chi^2 = 0.314$, p = 0.978
Husband's Occupation						
Civil servants	68	12(17.6)	60	9(15.0)	128	21(16.4)
Self-employed	32	1(3.1)	40	7(17.5)	72	8(11.1)
Others	35	1(2.9)	35	2(5.7)	70	3(4.3)
Statistics		$\chi^2 = 5.060$, p = 0.261		$\chi^2 = 4.123$, p = 0.326		$\chi^2 = 3.957$, p = 0.289
Level of education						
Primary	19	0(0.0)	17	1(5.9)	36	1(2.8)
Secondary	55	7(12.7)	60	7(11.7)	115	14(12.2)
Tertiary	53	6(11.3)	43	6(14.0)	96	12(12.5)
Others	8	1(12.5)	15	4(26.7)	23	5(21.7)
Statistics		$\chi^2 = 3.512$, p = 0.500		$\chi^2 = 8.567$, p = 0.167		$\chi^2 = 7.079$, p = 0.051*

KEY: No. +ve = Number of positive samples; * = Statistically significant association exists at $p \leq 0.05$

Table 4.3: Distribution Rate of Chlamydial Infection According to Risk Factors among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Risk factor	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
Marital status						
Married	134	14(10.4)	132	16(12.1)	266	30(11.3)
Separated	1	0(0.0)	2	1(50.0)	2	1(50.0)
Widowed	0	0(0.0)	1	1(100.0)	2	1(50.0)
Statistics		$\chi^2 = 0.117$, p = 0.733		$\chi^2 = 8.995$, p = 0.011*		$\chi^2 = 5.656$, p = 0.059*
Family type						
Monogamy	110	13(11.8)	112	17(15.2)	222	30(13.5)
Polygamy	25	1(4.0)	23	1(4.3)	48	2(4.2)
Statistics		$\chi^2 = 1.340$, p = 0.247		Fischer exact = 0.144		Fisher's exact = 0.069
Years in Marriage						
0 – 5	81	9(11.1)	61	7(11.5)	142	16(4.2)
6 – 10	28	3(10.7)	21	3(14.3)	49	6(12.2)
11 – 15	8	0(0.0)	23	2(8.7)	31	2(6.5)
16 – 20	11	0(0.0)	10	1(10.0)	21	1(4.8)
21 – 25	4	0(0.0)	7	1(14.3)	11	1(9.1)
26 – 30	3	2(66.7)	7	3(42.9)	10	5(50.0)
31 – 35	-	-	4	1(25.0)	4	1(25.0)
36 and above	-	-	2	0(0.0)	2	0(0.0)
Statistics		$\chi^2 = 13.025$, p = 0.043*		$\chi^2 = 7.275$, p = 0.507		$\chi^2 = 16.924$, p = 0.031*

KEY: * = Statistically significant association exists at $p \leq 0.05$; No. +ve = Number of positive samples

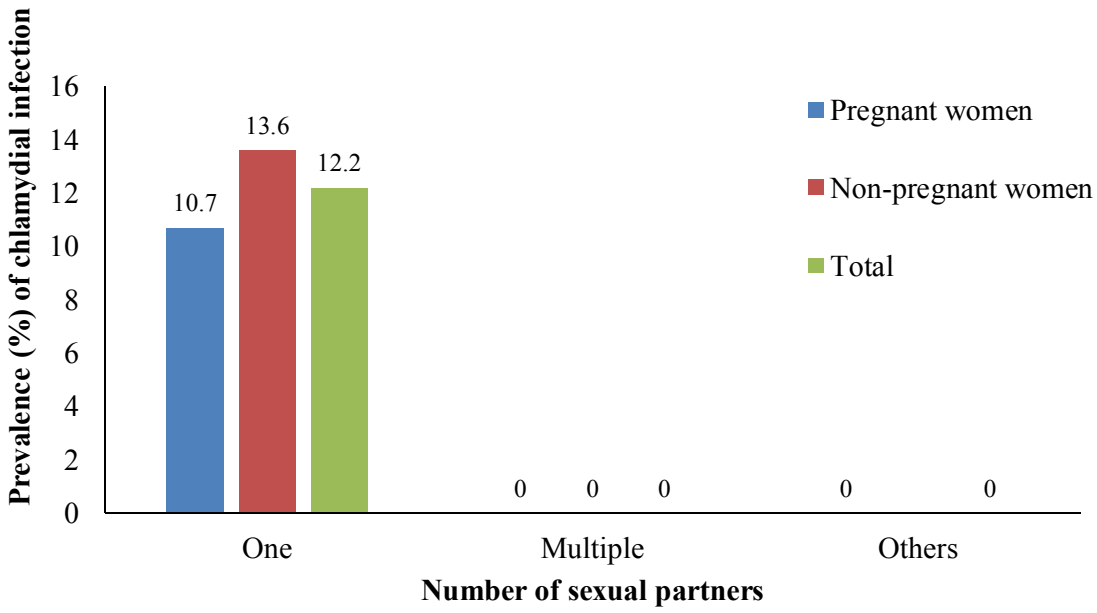


Figure 4.2: Prevalence of chlamydial infection according to number of sexual partners (t = 0.751, p = 0.453) among women attending clinics in Zaria metropolis, Kaduna State, Nigeria.

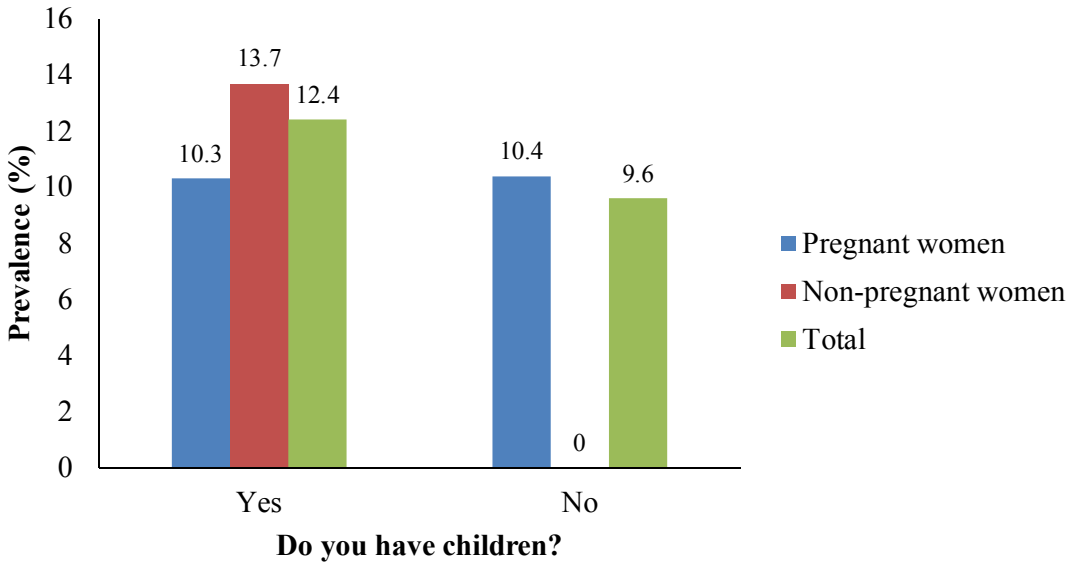


Figure 4.3: Prevalence of chlamydial infection according to whether or not the respondents (Pregnant women: $\chi^2 = 0.041$, $p = 0.999$; Non-pregnant women: $\chi^2 = 10.303$, $p = 0.001$**; Total: $\chi^2 = 1.311$, $p = 0.291$) attending clinics in Zaria metropolis, Kaduna State, Nigeria have children.

Table 4.4 shows the relationship between age of women at first childbirth and chlamydial infection. Generally, women who bore their first children at age 10–15 years had the highest prevalence 6(19.4%) of chlamydial infection closely followed by those aged 26–30 years 5(17.2%). Among the pregnant women, the highest prevalence was observed in age group 26–30 years 2(20.0%) followed by those that did not state their years at first childbirth 5(13.9%); while in the non-pregnant women, the highest prevalence was observed in the age group 10–15 years 6(30.0%). The observed data revealed that there is no statistically significant association.

The current pregnancy status of the pregnant women was compared with respect to their chlamydial infection status and the data showed no relationship between the pregnancy status and the infection. The relationship was further probed to identify the trimester most associated with the infection using analysis of variance (ANOVA) and it was revealed that there was statistically significant difference between the trimesters of pregnancy with third trimester having the highest prevalence 11(15.7%) followed by second trimester with 2(4.1%) prevalence of chlamydial infection (Figure 4.4).

Women with chlamydial infection were compared on the ground of whether or not they had sexual intercourse during pregnancy; 122 of them answered in the affirmative out of which 11(9.0%) had the infection. On the other hand, 13 of the women said they do not have sexual intercourse during pregnancy and 3(25.0%) of them had chlamydial infection. This difference between them was then compared using student t-test and it was established that there was no statistically significant difference in the infection between those that had sexual intercourse during pregnancy and those that do not (Figure 4.5).

Table 4.4: Prevalence of Chlamydial Infection Based on Age of Women at First Childbirth Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Age at first childbirth	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
Age at first childbirth						
10 – 15	11	0(0.0)	20	6(30.0)	31	6(19.4)
16 – 20	43	4(9.3)	51	6(11.8)	94	10(10.6)
21 – 25	26	2(7.7)	40	3(7.5)	66	5(7.6)
26 – 30	10	2(20.0)	19	3(15.8)	29	5(17.2)
31 – 35	9	1(11.1)	1	0(0.0)	10	1(10.0)
Not stated	36	5(13.9)	4	0(0.0)	40	5(12.5)
Statistics		$\chi^2 = 3.009$, p = 0.699		$\chi^2 = 6.963$, p = 0.223		$\chi^2 = 3.813$, p = 0.577

KEY: No.+ve = Number of positive sample

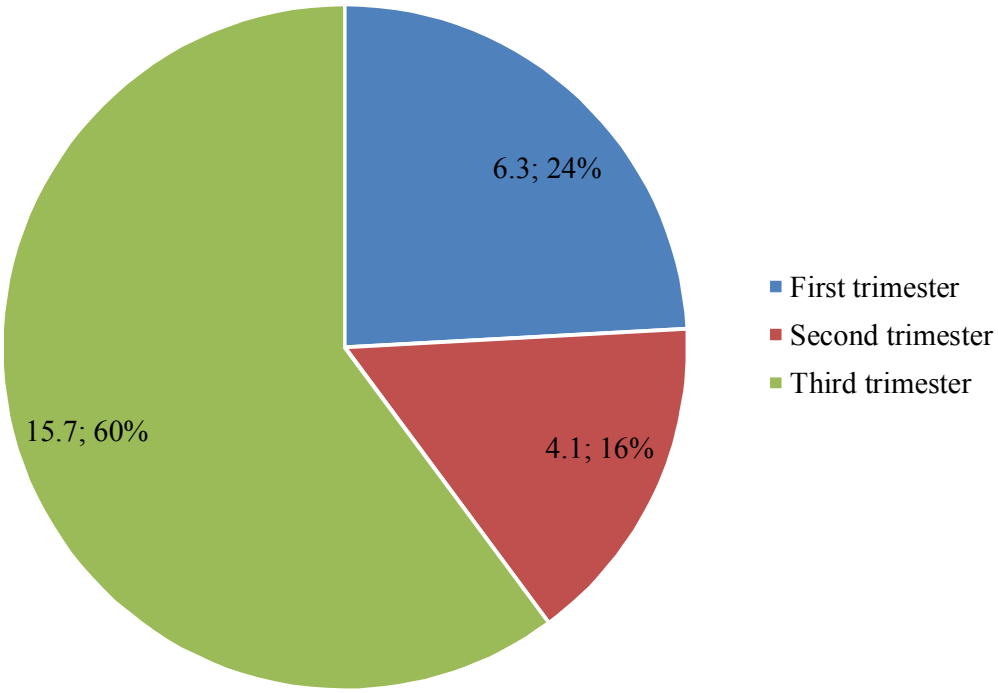


Figure 4.4: Prevalence of chlamydial infection based on pregnancy status (χ^2 = 4.528, p = 0.104) (F = 3.301, df = 2, p = 0.040) among women attending clinics in Zaria metropolis, Kaduna State, Nigeria.

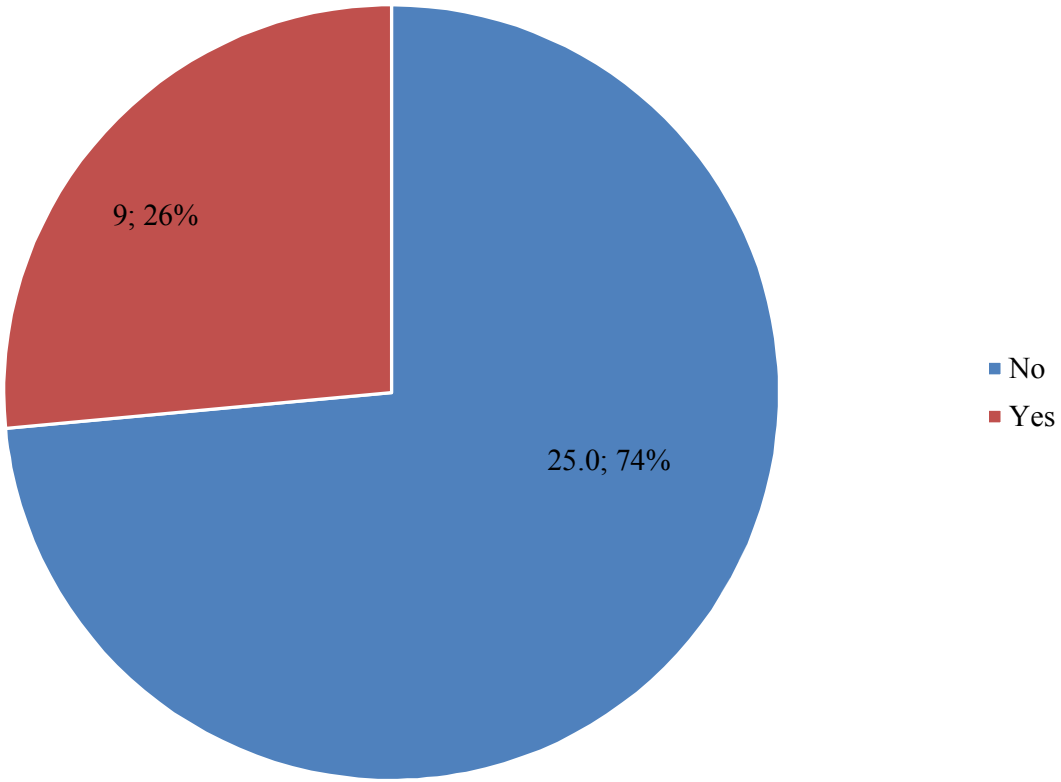


Figure 4.5: Prevalence of chlamydial infection based on sexual intercourse during pregnancy (t = 1.734, p = 0.085) among women attending clinics in Zaria metropolis, Kaduna State, Nigeria.

The study subjects were compared based on abnormal vaginal discharge and the result is as presented in Table 4.5. Women with abnormal vaginal discharge who tested positive for chlamydial infection had higher rate of occurrence 12(13.0%) than women without abnormal vaginal discharge 20(11.2%) who tested positive for chlamydial infection. Pregnant women with abnormal vaginal discharge had higher prevalence 5(12.5%) of chlamydial infection than their counterparts 9(9.5%) who do not have abnormal vaginal discharge. These observed differences were not statistically significant. Also, non-pregnant women with abnormal vaginal discharge had higher prevalence 7(13.5%) of chlamydial infection than their non-pregnant counterparts 11(13.3%) who had no abnormal vaginal discharge but the difference was not significant.

The prevalence of chlamydial infection of all the subjects were compared on the basis of their previous history of vaginal discharge and it was discovered that there was no significant association between vaginal discharge and the infection. The prevalence of Chlamydial infection among pregnant women was 8(17.8%) for those with previous history of vaginal discharge as against 5(5.6%) for those without previous history of abnormal vaginal discharge with statistically significant association between previous history of vaginal discharge and chlamydial infection. The non-pregnant women showed that there was no significant association between vaginal discharge and the infection (Table 4.5).

Assessment of the study subjects based on burning during urination showed that 1(7.1%) pregnant woman with chlamydia infection had burning sensation when urinating while 13(10.8%) of the pregnant women positive for chlamydial infection had no burning sensation when urinating. This difference was not statistically significant. Similarly, there was no association between burning on urination and chlamydial infection among non-pregnant women (Table 4.5).

A prevalence of 4(10.8%) of chlamydial infection was observed in pregnant women that experienced painful intercourse. This was not significantly different from the prevalence observed in pregnant women 10(10.2%) that do not experience painful intercourse. On the other hand, non-pregnant women that experience painful intercourse had higher prevalence 6(23.1%) of chlamydial infection than non-pregnant women 12(11.0%) that do not feel pain during intercourse. This perceived difference was not significant (Table 4.5). The relationship between lower abdominal pain as well as bleeding between periods were analysed and it was observed that there was no significant association between them. Also, test of association between bleeding between periods and chlamydial infection showed that there was no statistical association.

Table 4.6 shows that occurence of chlamydial infection in pregnant women with history of stillbirth was 3(15.0%) and 11(9.6%) in those without history of stillbirth. In non-pregnant women with history of stillbirth, the prevalence rate obtained was 3(15.8%) as opposed to 15(12.9%) in non-pregnant women without history of chlamydial infection. Overall, there was no statistically significant association between chlamydial infection and history of childbirth. Investigation of the relationship between history of premature birth, history of miscarriage and history of infertility also showed that there was no statistically significant association between these factors and chlamydial infection.

Table 4.5: Occurrence of Chlamydial Infection Based on Clinical Symptoms among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Clinical symptoms	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
Abnormal vaginal discharge						
Yes	40	5(12.5)	52	7(13.5)	92	12(13.0)
No	95	9(9.5)	83	11(13.3)	178	20(11.2)
Statistics		$\chi^2 = 0.277$, p = 0.598		$\chi^2 = 0.001$, p = 0.972		$\chi^2 = 0.190$, p = 0.663
Previous history of vaginal discharge						
Yes	45	8(17.8)	58	7(12.1)	103	15(14.6)
No	90	6(6.7)	77	11(14.3)	167	17(10.2)
Statistics		$\chi^2 = 3.985$, p = 0.046*		$\chi^2 = 0.141$, p = 0.708		$\chi^2 = 1.172$, p = 0.279
Burning on urination						
Yes	14	1(7.1)	17	2(11.8)	31	3(9.7)
No	121	13(10.8)	118	16(13.6)	239	29(12.2)
Statistics		$\chi^2 = 0.300$, p = 0.861		Fisher's exact = 0.598		$\chi^2 = 0.300$, p = 0.861
Painful intercourse						
Yes	37	4(10.8)	26	6(23.1)	63	10(15.9)
No	98	10(10.2)	109	12(11.0)	207	22(10.6)
Statistics		Fisher's exact = 0.569		$\chi^2 = 3.646$, p = 0.104		$\chi^2 = 1.272$, p = 0.259
Lower abdominal pain						
Yes	51	3(5.9)	49	10(20.4)	100	13(13.0)
No	84	11(13.1)	86	8(9.3)	170	19(11.2)
Statistics		Fisher's exact = 0.148		$\chi^2 = 3.332$, p = 0.068		$\chi^2 = 0.200$, p = 0.654
Bleeding between periods						
Yes	6	0(0.0)	36	7(19.4)	42	7(16.7)
No	129	14(10.9)	99	11(11.1)	228	25(11.0)
Statistics		Fisher's exact = 0.512		$\chi^2 = 1.587$, p = 0.208		$\chi^2 = 1.104$, p = 0.293

KEY: * = Statistically significant association exists at $p \leq 0.05$; No. +ve = number of positive samples

Table 4.6: Occurence of Chlamydial Infection Based on Clinical History among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Clinical history	No. of samples	Pregnant No. +ve (%)	No. of samples	Non-pregnant No. +ve (%)	No. of samples	Total No. +ve (%)
History of stillbirth						
Yes	20	3(15.0)	19	3(15.8)	39	6(15.4)
No	115	11(9.6)	116	15(12.9)	231	26(11.3)
Statistics		Fisher's exact = 0.343		Fisher's exact = 0.483		$\chi^2 = 0.545$, $p = 0.461$
History of premature birth						
Yes	11	1(9.1)	6	3(50.0)	17	4(23.5)
No	124	13(10.5)	129	15(11.6)	253	28(11.1)
Statistics		Fisher's exact = 0.681		Fisher's exact = 0.031*		$\chi^2 = 2.368$, $p = 0.124$
History of Miscarriage						
Yes	31	3(9.7)	34	3(8.8)	65	6(9.2)
No	104	11(10.6)	101	15(14.9)	205	26(12.7)
Statistics		Fisher's exact = 0.594		Fisher's exact = 0.282		$\chi^2 = 0.563$, $p = 0.453$
History of infertility						
Yes	22	0(0.0)	22	4(18.2)	44	4(9.1)
No	113	14(12.4)	113	14(12.4)	226	28(12.4)
Statistics		Fisher's exact = 0.072		Fisher's exact = 0.331		$\chi^2 = 0.384$, $p = 0.536$

KEY: * = Statistically significant association exists at $p \leq 0.05$; No. +ve = number of positive sample

The age distribution of HIV infection among both pregnant and non-pregnant women was also assessed and presented in fig. 4.6. The highest percentage of 3.2% HIV cases among pregnant women was recorded among age group 31-35 years followed by age group 26-30 years with 2.6%. The age groups 15-20, 21-25 and 36-40 years had no HIV 0.0%. Though there were slight variations in percentage occurrence of HIV infection with respect to age groups, those perceived variations were not statistically significant. On the other hand, the age distribution of HIV among non-pregnant women was highest in the age group 46-50 years 25.0%. Ages 30 years and below as well as 41-45 years had no HIV infection 0.0%. And it showed no significant association with age.

Table 4.7 revealed that there was statistically significant association between HIV infection among pregnant women and occupation. Pregnant women who are self-employed 1(2.1%) had the highest HIV infection followed by others 1(1.8%). In non-pregnant women, HIV infection was highest among civil servants, 2(9.1%) followed by those that did not state their occupation type 2(3.4%). The least rate of HIV infection 1(1.9%) was recorded among non-pregnant self-employed. There was no significant association between HIV infection among non-pregnant women and occupation. Overall, there was no significant association between occupation and HIV infection. Civil servants 2(3.8%) had the highest HIV infection followed by others 3(2.6%) while the self-employed had the least prevalence 2(2.0%).

Similar test of association between HIV infection and level of education showed that there was no statistically significant association between the infection and level of education (Table 4.7). Though in pregnant women, HIV infection was highest among those with primary education, 1(5.3%) followed by secondary education with 1(1.8%), tertiary and others without formal education had no HIV infection among while in non-pregnant women the highest prevalence was

recorded also among those with primary education 2(11.8%), secondary 2(3.3%) and tertiary level of education had 1(2.3%). There was no HIV infection among others without formal education (Table 4.7). Generally among the women, HIV infection was highest among those with primary education 3(8.3%) and no significant association exists between HIV among the women.

The association of marital status was compared with HIV infection in pregnant and non-pregnant women. In pregnant women, there was no significant association between HIV in pregnancy and marital status. On the other hand, HIV infection among non-pregnant women was significantly associated with marital status while married women had 3(2.3%) prevalence, widows had 2(100.0%) and those who were separated had no HIV infection 0(0.0%). Comparison of the infection with respect to marital status irrespective of pregnancy status showed that there was significant association between HIV and marital status with married women having HIV prevalence of 5(1.9%) and widows 2(100.0%) (Table 4.8).

The test of association of family type with HIV infection showed that there was no significant association between family type and HIV infection in pregnancy. Also, HIV infection in non-pregnant women was not significantly associated with family type (Table 4.8).

HIV infection in non-pregnant women was compared with respect to years in marriage. There was significant association with those who have stayed in marriage for 36 years and above had high prevalence of HIV 1(50.0%) and those who had stayed for 21-25 years in marriage had 1(14.3%) prevalence among the women. Similar comparison among the pregnant women showed no significant association with those who have spent 11-15 years 1(12.5%) having a high rate of HIV infection (Table 4.8). On the whole, significant association exists between HIV

infection and years spent in marriage with those who have stayed for 36 years and above having a high rate of infection 1(50%) among the women.

Prevalence of HIV infection according to number of sexual partners was assessed as reported in Figure 4.7. Two (1.5%) of pregnant women who had one sexual partner each were HIV positive. None of the women with multiple sexual partners had HIV infection. Chi-squared test of association showed that there was no significant association between HIV infection and number of sexual partners among pregnant women. On the other hand, there was significant association between HIV infection among non-pregnant women and number of sexual partners with prevalence of 4(3.0%) among non-pregnant women with one sexual partner and 1(33.3%) among non-pregnant women with multiple sexual partners.

The above comparison was done irrespective of whether or not the women were pregnant and it was found that there was no significant association between HIV infection and sexual partners; though women with one sexual partner had HIV prevalence of 6(2.3%) while those with multiple sexual partners had prevalence of 1(16.7%P).

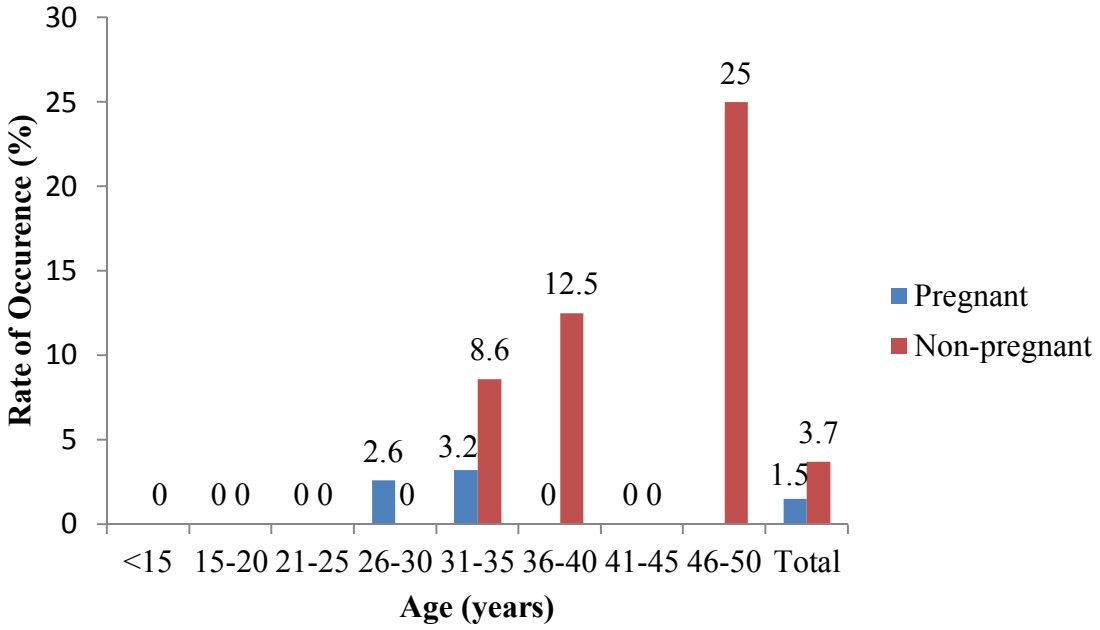


Figure 4.6: Age group distribution of HIV infection in pregnant (HIV: $\chi^2 = 1.983$, p=0.739) and non-pregnant women (HIV: $\chi^2 = 0.171$, p = 0.997) attending clinics in Zaria metropolis, Kaduna State, Nigeria.

Table 4.7: Distribution of Prevalence of HIV Infection According to Demographic Factors among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Risk factor	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
Occupation						
Civil servant	31	0(0.0)	22	2(9.1)	53	2(3.8)
Self-employed	47	1(2.1)	54	1(1.9)	101	2(2.0)
Others	57	1(1.8)	59	2(3.4)	116	3(2.6)
Statistics		$\chi^2 = 32.542$, $p = 0.031$*		$\chi^2 = 5.809$, $p = 0.398$		$\chi^2 = 8.641$, $p = 0.342$
Level of education						
Primary	19	1(5.3)	17	2(11.8)	36	3(8.3)
Secondary	55	1(1.8)	60	2(3.3)	115	3(2.6)
Tertiary	53	0(0.0)	43	1(2.3)	96	1(1.0)
Others	8	0(0.0)	15	0(0.0)	23	0(0.0)
Statistics		$\chi^2 = 2.822$, $p = 0.588$		$\chi^2 = 3.926$, $p = 0.416$		$\chi^2 = 6.225$, $p = 0.083$

KEY: * = Statistically significant association exists at $p \leq 0.05$; % = Percentage; No. +ve = number of positive samples

Table 4.8: Distribution of Prevalence of HIV Infection According to Risk Factors among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Risk factor	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
Marital status						
Married	134	2(1.5)	132	3(2.3)	266	5(1.9)
Separated	1	0(0.0)	1	0(0.0)	2	0(0.0)
Widowed	0	0(0.0)	2	2(100.0)	2	2(100.0)
Statistics		$\chi^2 = 0.015$, $p = 0.902$		$\chi^2 = 52.796$, $p < 0.001$**		$\chi^2 = 75.731$, $p < 0.001$**
Family type						
Monogamy	110	1(0.9)	112	4(3.6)	222	5(2.3)
Polygamy	25	1(4.0)	23	1(4.3)	48	2(4.2)
Statistics		Fisher's exact = 0.248		Fisher's exact = 0.857		Fisher's exact = 0.449
Years in Marriage						
0 – 5	81	1(1.2)	61	1(1.6)	142	2(1.4)
6 – 10	28	0(0.0)	21	0(0.0)	49	0(0.0)
11 – 15	8	1(12.5)	23	1(4.3)	31	2(6.5)
16 – 20	11	0(0.0)	10	1(10.0)	21	1(4.8)
21 – 25	4	0(0.0)	7	1(14.3)	11	1(9.1)
26 – 30	3	0(0.0)	7	0(0.0)	10	0(0.0)
31 – 35	0	0(0.0)	4	0(0.0)	4	0(0.0)
36 and above	0	0(0.0)	2	1(50.0)	2	1(50.0)
Statistics		$\chi^2 = 6.201$, $p = 0.357$		$\chi^2 = 18.410$, $p = 0.026$*		$\chi^2 = 25.002$, $p = 0.002$**

KEY: * = Statistically significant association exists at $p \leq 0.05$; ** = Statistically significant association exists at $p \leq 0.01$;
% = positive; No. +ve = number of positive samples.

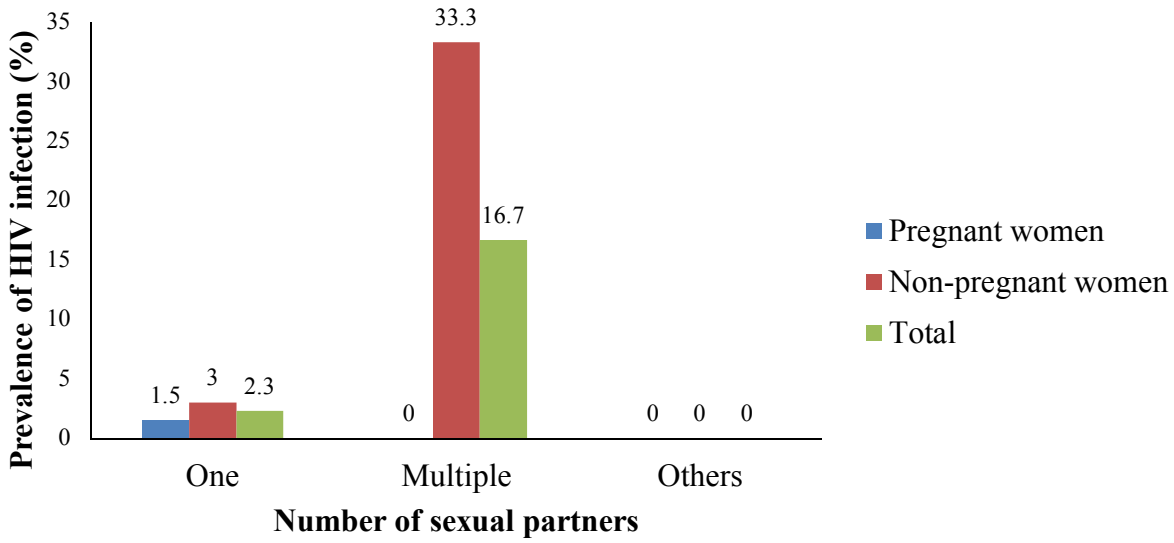


Figure 4.7: Prevalence of HIV infection according to number of sexual partners (Pregnant women: $\chi^2 = 0.062$, $p = 0.969$; Non-pregnant women: $\chi^2 = 7.552$, $p = 0.006$*; Total: $\chi^2 = 4.834$, $p = 0.089$).

Prevalence of HIV infection in both pregnant and non-pregnant women based on age of women at first childbirth was assessed and presented in Table 4.9 and no significant association between HIV infection and age of women at first childbirth was found.

Figure 4.8 presents the prevalence of HIV infection based on pregnancy trimester. Third trimester pregnant women had HIV prevalence of 1.4% as against 6.3% in women whose pregnancies were in first trimester. No women in second trimester had HIV infection.

Comparison of history of stillbirth with HIV in pregnant women and non-pregnant women was not statistically significant. Similar comparison using history of premature birth, history of miscarriage and history of infertility showed no significant association (Table 4.10).

Table 4.9: Prevalence of HIV Infection Based on Age of Women at First Childbirth among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Age at first childbirth	No. of samples	Pregnant No. +ve (%)	No. of samples	Non-pregnant No. +ve (%)	No. of samples	Total No. +ve (%)
Age at first childbirth						
10 – 15	11	0(0.0)	20	0(0.0)	31	0(0.0)
16 – 20	43	1(2.3)	51	3(5.9)	94	4(4.3)
21 – 25	26	0(0.0)	40	1(2.5)	66	1(1.5)
26 – 30	10	0(0.0)	19	0(0.0)	29	0(0.0)
31 – 35	9	0(0.0)	1	0(0.0)	10	0(0.0)
Not stated	36	1(2.8)	4	1(25.0)	40	2(5.0)
Statistics		$\chi^2 = 1.466$, p = 0.917		$\chi^2 = 7.466$, p = 0.188		$\chi^2 = 4.114$, p = 0.533

KEY: % = Percentage; No +ve = Number of positive sample

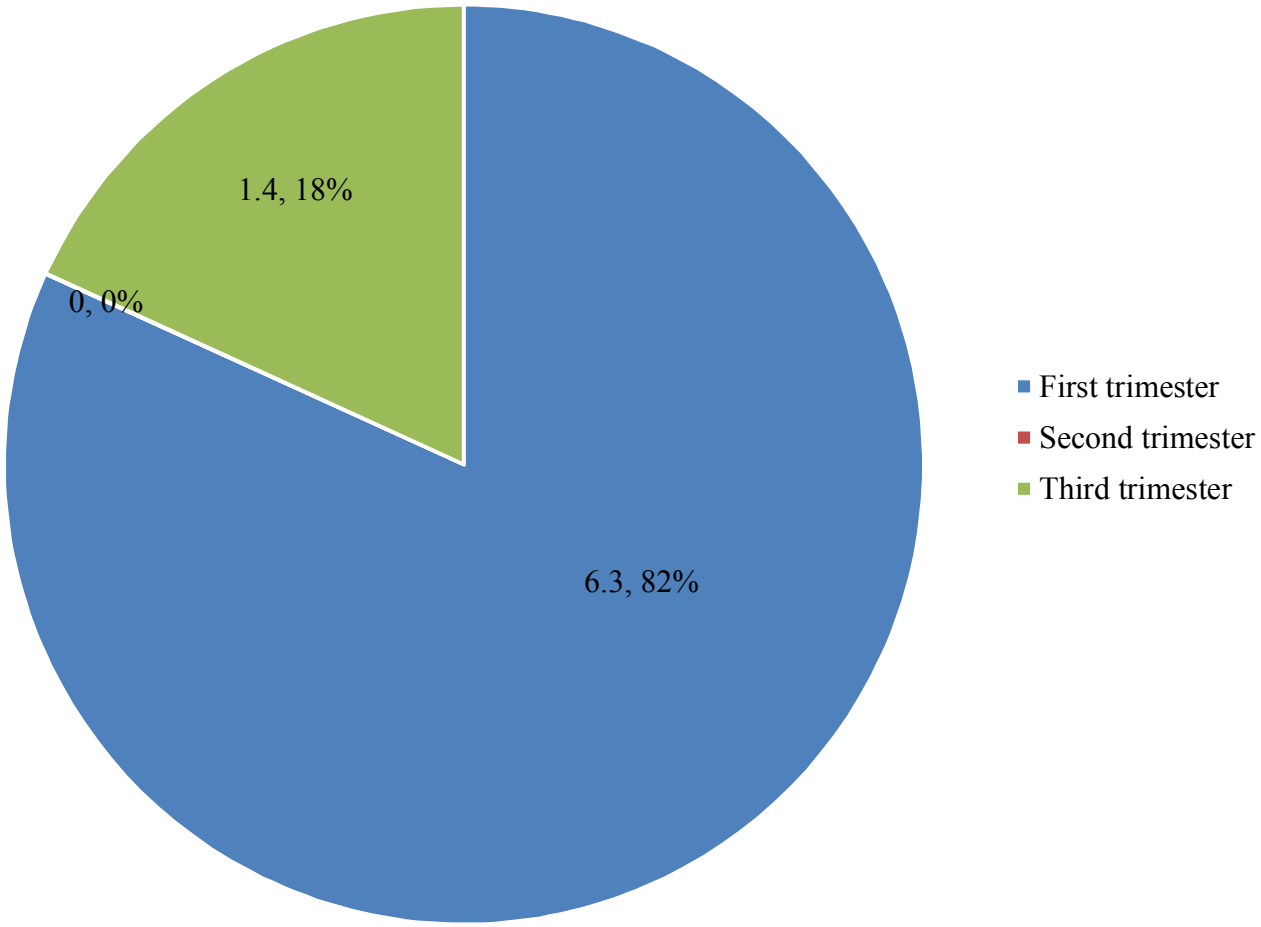


Figure 4.8: Prevalence of HIV infection based on pregnancy status among women (χ^2 = 5.828, p = 0.074) (F = 4.420, df = 2, p = 0.084) attending clinics in Zaria metropolis, Kaduna State, Nigeria.

Table 4.10: Prevalence of HIV Infection Based on Clinical History among Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Clinical history	No. of samples	Pregnant No. +ve (%)	No. of samples	Non-pregnant No. +ve (%)	No. of samples	Total No. +ve (%)
History of stillbirth						
Yes	20	0(0.0)	19	0(0.0)	39	0(0.0)
No	115	2(1.7)	116	5(4.3)	231	7(3.0)
Statistics		Fisher's exact = 0.725		Fisher's exact = 0.463		Fisher's exact = 0.331
History of premature birth						
Yes	11	0(0.0)	6	0(0.0)	17	0(0.0)
No	124	2(1.6)	129	5(3.9)	253	7(2.8)
Statistics		Fisher's exact = 0.843		Fisher's exact = 0.794		Fisher's exact = 0.631
History of miscarriage						
Yes	31	0(0.0)	34	3(8.8)	65	3(4.6)
No	104	2(1.9)	101	2(2.0)	205	4(2.0)
Statistics		Fisher's exact = 0.592		Fisher's exact = 0.101		Fisher's exact = 0.222
History of infertility						
Yes	22	0(0.0)	22	1(1.5)	44	1(2.3)
No	113	2(1.8)	113	4(3.5)	226	6(2.7)
Statistics		Fisher's exact = 0.700		Fisher's exact = 0.595		Fisher's exact = 0.680

KEY: % = Percentage; No. +ve = Number of positive samples

CHAPTER FIVE

DISCUSSION

This study reports the seroprevalence of *C. trachomatis* and co-infection with Human Immunodeficiency Virus in women of reproductive age group attending clinics in Zaria. A prevalence of 11.9% was found in this study for CT with 10.4% being from pregnant women and 13.3% from non-pregnant women. This CT prevalence was higher than 3.7% reported in Gombe (Charanchi and Tahir, 2012), 4.7% reported in the United States (Miller *et al*., 2004) and in Australia, prevalence of 5.6% and 7.5% were also reported (Vajdic *et al*., 2005; Bandea *et al*., 2008). These marked differences could be as a result of reduced sexual risk-behaviour, increased awareness on chlamydial infection and other sexually transmitted diseases, easy access to laboratory diagnoses and treatment among others in the developed countries, the reverse is the case in developing countries Nigeria inclusive. The result obtained in this investigation is consistent with the earlier report of 10.5% recorded in Zaria, Nigeria (Muhammad *et al*., 2009), 11% in PortHarcourt (Wariso *et al*., 2012), 13.3% in Benin City (Isibor *et al.,* 2005), 11.0% in Abeokuta, Nigeria (Walker *et al*., 1989), 10% in Ibadan (Darougar *et al*., 1982), 9.8% in Abeokuta, Ogun State, Nigeria (Ogiogwa *et al*., 2012) and 9% in Maiduguri (Sanders *et al*., 1994). The reason could be that the infection remained endemic; its spread could be on the increase. It could also be as a result of increase in the sexual risk behavioural attitudes of individuals in the society.

Reports about the prevalence of *Chlamydia trachomatis* in various risk groups also recorded significant figures, for example, prevalence rate of 38.3% has been reported among patients with tubal infertility in Northern Nigeria (Tukur *et al*., 2006), also 38.18% among gynaecological patients in Zaria, Northern Nigeria (Jatau *et al*., 2009). A study in Enugu also recorded 26.4%

prevalence rate in adult women (Ikeme *et al*., 2011). Prevalence of 41% of *C. trachomatis* has also been reported from the South-Eastern part of Nigeria (Okoror *et al*., 2007), 45% prevalence in patients attending various gynaecological clinics in south east Nigeria (Okoror *et al*., 2007), a prevalence of over 51% among pregnant and non-pregnant women and their spouses reported by Okoror (Okoror *et al*., 2000) and an overall prevalence of 56.10% was found among gynaecologic clinic attendees in Jos, Nigeria which are all in contrast to the prevalence obtained from this study. Genital Chlamydia infection is generally considered a silent infection in women. This study consists of women both pregnant and non-pregnant alike and they could be considered asymptomatic since about 70-90% of infected women show no symptoms (Nelson and Helfand, 2001) and are usually not treated. It has previously been reported that relative frequencies in developing countries are scanty and infection could be higher in developing countries (Krivoshein, 1989). As diagnoses for chlamydial infections are not usually carried out, most infections caused by Chlamydia are usually misdiagnosed and taken for other infections. Also, since *Chlamydia* sp are usually found in latent infection, the infections will pass unnoticed and remain endemic in the population for a long time (Okoror *et al*., 2007).

Co-infection between CT and HIV was detected in 0.4% which shows that combined infection between CT and HIV does exist. Elsewhere, higher seroprevalence rate among different populations have been reported. For instance, 12.1% was recorded in Gombe (Charanchi and Tahir, 2012) and 29.4% obtained in Abuja (Izebe *et al*., 2008). This low co-infection prevalence therefore suggests that CT pre-existed before HIV infection that predisposes the subject to HIV infection through the initiation of inflammatory and immune responses which facilitate the entry of HIV (Wodarz and Hamer, 2007) and which shows that people are becoming more informed about HIV infection and preventive measures are also taken. The difference in the

seroprevalence of *Chlamydia trachomatis* infection in the different populations of women may not be unconnected with the differences in predisposing factors, cultural practices, sexual practices and access to improved sexually transmitted disease management as well as degree of public awareness. *Chlamydia trachomatis* is said to be associated with increased genital HIV shedding that may increase HIV transmissibility (Ghys *et al.,* 1997). In addition, in HIV infected individuals, infections with *C. trachomatis* and genital mycoplasmas are important biologic markers of behaviour that may expose others to HIV. The bacterium *Chlamydia trachomatis* as well as the Human Immunodeficiency virus-1 (HIV-1) are sexually transmitted pathogens and both can infect monocytes/macrophages and have an obligate intracellular replication cycle (Roters, 2009). It has been hypothesized that sexually transmitted infections (STIs), including genital Chlamydia, enhance HIV-1 transmission; yet, the underlying biological mechanisms remain unclear. The pathogen's common biology makes it possible that *C. trachomatis* and HIV mutually affect each other's replication cycle during monocyte/macrophage co-infection (Roters, 2009).

The prevalence of CT infection was found to increase with decreasing age in this study. The percentage recorded in the age group 15-20 years was 11.5% followed by 26-30 years having 10.5% and ages 31-35 years had 9.7% among pregnant women. Among the non-pregnant women, age group <15 years had the highest percentage 100% followed by ages 36-40 years 37.5% and ages 31-35 years having the least 8.6%. Though there were slight variations in percentage occurrence of chlamydial infection with respect to age groups in the women, those perceived variations were not statistically significant. This result is in agreement with Rassjo *et al*., (2006) who declared that younger age group was associated with active sexual practice and also associated with higher rates of chlamydial infection. It was also observed that in developing

countries, females may show higher prevalence in genital infections due to sexual assault which greatly increases the risk of STI/HIV transmission in women because protection is rarely employed, while physical trauma to the vaginal cavity frequently occurs and these facilitate infection (WHO, 2006). There was no chlamydia infection in ages 36-40 years. Ages 15-36 years are the sexually active groups of the community and are at a higher risk of being behaviourally more vulnerable to STI acquisition. There was also no chlamydia infection in the age group 41-45 years. This is in conformity with the findings of Okoror *et al*., (2007) who discovered no positive case in women of this age group. Radcliffe *et al*., (2001) also reported that old age was associated with low risk of chlamydial infection and was also in agreement with CDC (2004), which reported that the age group of 14-39 years accounted for over 95% of Chlamydia cases in the United States. This could as well be due to the fact that production of squamous epithelial cells (the actual reservoirs for *Chlamydia trachomatis*) is very low with old age and in menopause, hence viability may be low or absent (Dale *et al*., 2001).

There was no significant association of chlamydial infection with the subjects' occupation. Chlamydial infection was detected in women who were civil servants and self-employed. A similar comparison of the study subjects' husbands' occupation revealed that civil servants had the highest infection rate among the pregnant women while the self-employed had the highest prevalence among the non-pregnant group. The high prevalence among civil servants was surprising as one expects that they should be well informed, enlightened, have a healthy lifestyle so as to protect themselves from sexual health risks and in turn educate others. Hence this also calls for a closer attention since they daily interact with people from different works of life. The reason for the high prevalence among women who are self-employed could also be due to care-free lifestyle, ignorance and promiscuity among them.

The relationship between level of education and chlamydial infection showed that there was no significant association. It has however been shown from this study that the level of education as a socioeconomic factor has an effect on the study population. The reason for the high prevalence of CT infection among women with tertiary and secondary school education may probably be due to high sexual activities among such groups. Moreso, since most of the seropositive women had some level of education, it can therefore, be expected that they would benefit from educational awareness interventions of the disease and in the application of measures to prevent it.

Marital status of the study subjects revealed that 10.4% of the married pregnant women had chlamydial infection while married non-pregnant women had 12.1%. Overall, there was no statistically significant association between marital status and chlamydial infection. This may probably account for the cases of history of stillbirth 15.4%, premature birth 23.5%, miscarriage 12.7% and infertility 12.4% in this study. Moreso, *C. trachomatis* is an asymptomatic infection in both women and men and transfer of infection can occur easily between husband and wife (Okoror *et al*., 2007) and this indicates that marital status play a role in CT infection. Therefore in any attempt to control CT, both partners should be evaluated and treated because it has been shown that *Chlamydia trachomatis* can affect sperm health, contributing to male infertility and when infected with *Chlamydia trachomatis*, sperm show a dramatic decrease in motility, and viability (Eley, 2003).

Those who practice monogamy had prevalence of 11.8% among the pregnant women which is slightly lower than 15.2% among the non-pregnant women. On the other hand, those who practiced polygamy had a prevalence of 4.0% among the pregnant women and 4.3% among the non-pregnant women. Although no statistical correlation was found, sexual promiscuity among

partners does expose them more to risk of infection. This variability of results between different studies compared to ours may be explained by the variation in sexual activity between our population and other populations, also other religions may restrict sexual activity to one partner while in others there is no restriction and there are several partners (van Valkengoed *et al*., 2004).

Based on number of years in marriage, pregnant women who have stayed in marriage for 26–30 years have the highest prevalence of chlamydial infection 66.7% and it was found to be statistically significant than in non-pregnant women that have been married for 26–30 years having a prevalence of 42.9%. On the whole, women that have been married for 26–30 years had the highest prevalence 50.0% followed by those married for 31-35 years 25.3%. This can be attributed to the fact that previous sexual exposure to the chlamydial infection at adolescent age and anatomy of the female genital tract puts them at increased risk (Ogiogwa *et al.,* 2012). For those who have been recently married (0-5 years) that had chlamydial infection, it is likely that sexual relation within that period is usually active (Okoror *et al*., 2007). This variation was found to be significantly associated with chlamydial infection among the women.

With respect to number of sexual partners, it was discovered that 10.7% pregnant women and 13.6% non-pregnant women infected with *Chlamydia trachomatis* reported having one sexual partner. The important role of this factor cannot be underscored. For those that answered one partner to the question of 'Number of sexual partners' and tested positive, there is an uncertainty of whether or not their spouses maintained single sexual partner in addition to the practice of polygamy in this part of Nigeria, although there was no significant correlation between chlamydial infection and number of sexual partners. This is however in contrast to report by

CDC U.S. (2011) that the fewer sex partners you have, the lower your risk of being exposed to *Chlamydia* and other STIs.

The findings showed that pregnant women who have no children had higher prevalence 10.4% of chlamydial infection than pregnant women that have children 10.3%, while non-pregnant women that have children had a prevalence of 13.7% as opposed to non-pregnant counterparts 0.0% that had no children and it was statiscally significant. Overall, the prevalence based on whether or not the subjects have children was 12.4% and 9.6% respectively and it was not significantly associated. This therefore implies that bearing children probably changes the physiology of the organ that allows easier inhabitation of the bacterium.

Among the pregnant women, the highest prevalence of *Chlamydia trachomatis* infection was observed in age group 26–30 years 20.0% of women at first childbirth while in the non-pregnant women, the highest prevalence was observed in the age group 10–15 years 30.0%. Generally, women who bore their first children at age group 10–15 years had the highest prevalence 19.4% of chlamydial infection closely followed by those aged 26–30 years 17.2%. This implies that the earlier an individual is engaged in sexual activity, the higher the chances of being infected with *C. trachomatis* and may as well be a reflection of the reported higher incidence of sexual activities among the youths (age <25 years) which is in agreement with report from Mawak *et al*., (2011).

The current pregnancy status of the pregnant women was compared with respect to their chlamydial infection status to identify the trimester most associated with the infection and it was found that women at their third trimester had the highest prevalence 15.7% and the association was found to be significant. The possibility that pregnancy per se increases the risk of infection by *C. trachomatis* has been suggested by various authors, particularly in studies carried out with

pregnant adolescents who have higher rates of infection than non-pregnant teenagers (Simões *et al.,* 1998). This might be due to the involvement of pregnancy in inducing hormonal secretions that may make the females more vulnerable to infection. This also implied that *Chlamydia trachomatis* may be a source of complications in pregnancy which is in agreement with report by Okoror *et al*., (2008). Importantly also, during passage through an infected birth canal *C. trachomatis* can be spread to newborns, who subsequently may become infected leading to serious complications such as conjunctivitis and respiratory tract infection (Darville, 2005). If this trend is allowed to continue it would constitute a major national health disaster in no distant future. Thus, there is the need for concerted efforts at controlling it.

Women with chlamydial infection were compared on the ground of whether or not they had sexual intercourse during pregnancy, 122 of them answered in the affirmative out of which 9.0% had the infection. On the other hand, 13 of the women said they do not have sexual intercourse during pregnancy and 25.0% of them had chlamydial infection although the association was not significant. Relatively little is known about sexual behaviour in pregnancy in the general population. Pregnancy is an indication that unprotected sex has occurred and several studies in the general population have suggested that pregnancy is not a time of decreased sexual risk-taking (Niccolai *et al*., 2003).

High prevalence of abnormal vaginal discharge as well as previous history of abnormal vaginal discharge was found in both pregnant and non-pregnant mothers and the observed differences were not significant. Chlamydial infection was significantly associated with previous history of vaginal discharge among the pregnant women. Vaginal discharge is one of the symptoms associated with chlamydial infections (Black, 1997) and women with this symptom are at higher risk of its potential consequences. The presence of discharge in mothers may be

regarded as evidence of maternal genital infection (though the specific type of infection still requires evaluation). A report showed that babies from mothers with antenatal vaginal discharges were eight times more likely to develop conjunctivitis (Ernest *et al*., 2000). This was consistent with other previous reports (Gerdes, 1991). The presence of discharge increases the chances of getting infecting organism into the infant's conjunctiva especially during passage through the birth canal, where the number of organism can multiply rapidly and so we suggest that routine screening of women (pregnant and non-pregnant) especially those with vaginal discharges be carried out in order to prevent or reduce the incidence of PID and its adverse sequelae.

Assessment of prevalence of chlamydial infection based on clinical symptoms observed in both pregnant and non-pregnant women that experienced burning during urination, painful sexual intercourse, lower abdominal pain as well as bleeding between periods revealed that there was no significant difference. This may account for both the symptomatic and asymptomatic nature of CT in women which shows that CT is an implicated cause of these factors, hence a major threat to the reproductive health of women (Brunham *et al.*, 2005). Also, asymptomatic patients, unaware of their infection, may serve as a reservoir of infection to their partners.

The prevalence of CT based on history of stillbirth in the women was high compared to those without, though, not statistically significant. Similarly, the association between CT with history of miscarriage, infertility and premature birth was not significant. Premature birth represents a major problem for obstetrics and neonatology due to its increasing frequency and accompanying socioeconomic impact which we found chlamydial infection to be associated with as it indicates that *Chlamydia trachomatis* contributes relatively to early than late prematurity (Moreau *et al*., 2005). It should however be noted that the proportion of premature births attributable to

Chlamydia highly depends upon the *C. trachomatis* prevalence in a given population and that some residual confounding as a result of co-infection by other genitourinary pathogens cannot be excluded.

Miscarriage could be induced by a persistent asymptomatic *C. trachomatis* infection spreading to the foetal tissue or endometrium. Relatively few miscarriages occur during *C. trachomatis* primary infection (Baud *et al*., 2008). That several patients exhibited *C. trachomatis* positive serologic results without *C. trachomatis* DNA suggests that miscarriage might also occasionally be induced by damage from a past chlamydial infection or persistent *C. trachomatis* antibodies that might interfere with embryonic antigens (Baud *et al*., 2008). Women who responded to either yes or no had infection with CT which implies that the asymptomatic nature of the infection is endemic and this infection may therefore be responsible for quite a number of abnormalities faced by women and it becomes worse when both men and women harbour the infection which in most cases are asymptomatic. The finding of Muhammad *et al*., (2009) also supports this. This then points out that the risk of infection cannot be ruled out which calls for further exploration of the infection in both partners as *Chlamydia trachomatis* has previously been incriminated in low sperm count and female infertility and miscarriages (Hollows, 1985). It could also be a source of complications being experienced by women and their spouses attending pre-natal diagnosis (Okoror *et al*., 2008).

Therefore, it is also important to determine the epidemiology of repeated infections, particularly if they are caused by treatment failures, increased susceptibility to infection due to host immunity, or reinfection from an untreated partner.

In this study also, HIV prevalence was detected in 2.6% with 1.5% from pregnant women and 5 3.7% from non-pregnant women. This is higher than the 0.0% seroprevalence rate reported in Ibadan (Alli *et al*., 2010) and however lower than the values reported by other investigators such as Akouma *et al*., (2005) who reported a general prevalence rate of 81.0% among pregnant women receiving prenatal care at Sainte- Justine Hospital, Canada. This is quite alarming and it is apparent that pregnant women are involved in unprotected sexual intercourse leading to pregnancy which could account for the high prevalence rate among this group (Nwachukwu and Orji, 2008). It is also lower than the 17.5% reported in Ibadan, Nigeria (Okonko *et al*. 2012a), 13.5 % and 9.3 % reported in 2001 and 2003 respectively among women who attended antenatal clinics in Benue State (FMOH, 2002 and 2004), 11.3% recorded among women of child bearing age in Nasarawa State (Adoga *et* al., 2009), lower also than that reported among women who attended antenatal clinics in Nasarawa State (10.8 % in 1999, 8.1 % in 2001 and 6.5 % in 2004) (FMOH, 2002 and 2004). 10.0% reported in Benin City, Nigeria (Umolu *et al*., 2005), 10.0% reported among Children in Anyigba, Kogi State, Nigeria (Sule *et al*., 2010), 9.0% prevalence rate reported for HIV in Ibadan (Okonko *et al.*, 2012), the 7.0% reported in Benin City, Nigeria (Oronsaye and Oronsaye, 2004), the 6.0% reported among blood donors in Jos, Nigeria (Egah *et al.,* 2004), the 5.8% reported in Maiduguri, Nigeria (Chikwem *et al*., 1997), the 5.0% reported among adolescents in a high HIV and TB prevalence community (Middelkoop *et al*., 2011), 4.79% reported among fresh Nigerian Graduates (Nwachukwu and Orji, 2008), the 4.55% reported in Cameroon (Musi *et al*., 2004), the 3.8% seroprevalence of HIV reported at MNH in Dar es Salaam (Matee *et al*., 2006), the 3.5% seroprevalence reported for HIV in Enugu (Chukwurah *et al*., 2005) and the 3.1% reported by Buseri *et al*., (2009) among prospective blood donors in Osogbo, Nigeria. Cultural and geographical differences between these regions which may influence risk exposures may account for this. It was reported that the differences in

the spread of the epidemic can be accounted for by a complex interplay of sexual behaviour and biological factors that affect the chances of HIV transmission per sex act (Buve *et al*., 2002).

The prevalence of HIV infection also varied with age as the highest prevalence rate was recorded in age 31 years and above followed by ages 26-30 among pregnant women whereas among non-pregnant women HIV prevalence was highest in age group 46-50 years followed by ages 30 and above. This agrees with previous findings of Motayo *et al*., (2009) who reported a higher HIV seropositivity among age group 20-29 years and Anteyi *et al.*, (1994) who reported also that HIV is contracted during child bearing age in females. This supports the 2004 Nigerian Federal Government report that discovered a uniform pattern of highest prevalence among the 20-24 years age bracket (in three zones) and the 25-29 age group (in two zones) (FMOH,2002 and 2004). It is generally accepted that HIV infection in the younger age brackets is indicative of the level of newer infections. Akinjogunla and Adegoke, 2009 reported a significant difference in the age of the individuals with the HIV infection and the prevalence of the HIV in relation to age to be 13.6% for age group 31-45 years and Okonko *et al*., (2012a and 2002b) who also reported a significant difference in the age of the subjects and HIV seropositivity. The high prevalence in 46-50 years could also be due to increased susceptibility to some reproductive tract infections (RTIs) like Chlamydia and gonorrhoea, especially with advanced immune suppression (McClelland *et al*., 2005). However, this observation deviated from that of Macpherson *et al*., (2006) who reported in Canada that 36 out of 256, 970 children greater than 15 years of age were HIV positive. Age group 15-20, 21-25, and 36-40 had no HIV infection among pregnant women while age groups 30 years and below as well as 41-45 years had no HIV infection among non-pregnant women. This lack of infection in ages 41-45 may likely be due to fear of HIV and STI or due to high religious morale, lack of sexual ego, poverty or any other factor that may

discourage sexual relationship at old age as suggested by (Adome *et al*., 2005). The lack of infection within these age groups 15-20, 21-25, and 36-40 may be as a result of the small number of samples within the age brackets, and not necessarily a true reflection of the real situation.

Our results showed that there was statistically significant association between occupation and HIV infection among the pregnant women, though, the result showed no significant association among non-pregnant women. While occupation has been shown in previous studies to have a direct correlation to the prevalence of HIV infection, for example long distance drivers and commercial sex workers (Akinsete, 2000), the finding in this study of a higher incidence among civil servants calls for closer attention and since they are generally educated people having greater access to information than those who have and are more likely to make informed decisions and act on information given, they would greatly benefit from intervention programs. In addition, they have better jobs and greater access to money and other resources which can help them lead healthier lives. The rather high seroprevalence rate recorded in this study for these groups raises once again the questions of ethics, rights and professional duty as they relate to universal antenatal screening for HIV.

Similarly, there was no significant association between level of education and HIV infection among pregnant women and non-pregnant women. HIV infection was found to decrease with increase in education in this study and majority of the seropositive women have some level of education. This can therefore, be anticipated that they would benefit from educational interventions at raising awareness of the disease as well as in the application of measures to prevent it.

The association of marital status was compared with HIV infection in pregnant and non-pregnant women. In pregnant women, there was no significant association between HIV in pregnancy and

marital status. On the other hand, non-pregnant women with HIV infection was significantly associated with marital status while married women had 2.3% prevalence, widows had 100.0% and those who were living separate had no HIV infection 0.0%. Comparison of the infection with respect to marital status irrespective of pregnancy status showed that there was significant association between HIV and marital status with married women having HIV prevalence of 1.9% and widows, 100.0% (Table 4.9). This shows that infection of women of childbearing age with this silent infection portends serious danger for both heterosexual and vertical transmissions, thus a major health burden to the community at large. The widows were more likely to be infected than the married and those married but living separate, but the sample size in this class of people was not large enough to reach statistical significance. However, this may be a reflection of the actual situation since the widows in the study were more prone to having multiple sex partners.

The test of association of family type with HIV infection show.ed that there was no significant association between family type and HIV infection in pregnancy and also in women without pregnancy. This probably could be an indication of more extramarital sexual relationships among these husbands and wives in polygamous family than among their counterparts married into monogamous family relationship.

The association between HIV and years spent in marriage was assessed and found to be significant among non-pregnant women and also among the women entirely. In all, women who spent 36 years and above in marriage had the highest prevalence of HIV infection. This could probably mean that the longer the years spent in marriage, the more susceptible one becomes.

Prevalence of HIV infection according to number of sexual partners was associated as reported in Figure 4.7 and this supports data presented in global reports (UNAIDS/WHO, 2009). Two 1.5% of pregnant women who had one sexual partner were HIV positive. None of the women

with multiple sexual partners had HIV infection. Chi-squared test of association showed that there was no significant association between HIV infection and number of sexual partners among pregnant women. On the other hand, there was significant association between HIV infection among non-pregnant women and number of sexual partners with prevalence of 3.0% among non-pregnant women with one sexual partner and 33.3% among non-pregnant women with multiple sexual partners. The role of this risk factor in the women cannot be underscored as partners who remain unfaithful to each other are at greater risk of HIV acquisition. This finding is consistent with the report from UNAIDS (2001) that a variety of factors increase the vulnerability of women and girls to HIV. They include social norms that deny women sexual health knowledge, practices that prevent them from deciding the terms on which they have sex, limited access to economic opportunities and autonomy and the multiple household and community roles they are saddled with.

Prevalence of HIV infection in both pregnant and non-pregnant women based on age of women at first childbirth was assessed and presented in Table 4.10. Though there was no significant association between HIV infection and age of women at first childbirth, ages 16-20 had the highest prevalence rate. The situation is much worse in those parts of the country where young women, are forced to marry at earlier ages, and have minimal access to modern family planning methods and formal education. Low level of female enrollment in school, high drop-out rate, early pregnancy and unsafe sexual practices due to ignorance contribute to the acquisition of HIV (Montgomery et al., 2002). In such a situation of widespread promiscuity, sexually transmitted diseases, including HIV spread rapidly. Thus, a true reflection of the reported higher incidence of sexual activities among the youths aged <25 years (Gbadegesin *et al*., 2004). This agreed also with report by Workowski and Berman (2006) that young adult women with HIV are

more susceptible to STI due to regular changes in women; such as change in hormonal distribution, increased vaginal microbial ecology and other physiological changes that resulted in lower immune status in addition to HIV infection.

Figure 4.9 presents the prevalence of HIV infection based on pregnancy trimester. Third trimester pregnant women had HIV prevalence of 1.4% as against 6.3% in women whose pregnancies were in first trimester. No women in second trimester had HIV infection. It has been proposed that hormonal changes associated with pregnancy as well as the impact of these hormones on the vaginal mucosa account for the increased susceptibility to infection (Brabin, 2002; Garcia-Bujalance *et al*., 2004). One potential route of congenital HIV transmission may be direct placental infection by HIV as early as the first trimester, with subsequent transplacental spread of the virus (Maury *et al*., 1989). If the mother is infected with HIV the fetus has a 25% chance of being infected by the virus. The foetus is more likely to be infected if the mother has a high vireamia, has AIDS, or has a low CD4+ cell count (Chamberlain, 2004).

Comparison of history of stillbirth with HIV in pregnant women and non-pregnant women was not statistically significant. So also, history of premature birth, history of miscarriage and history of infertility showed no significant association (Table 4.11). Women are making up a larger proportion of newly HIV-infected persons than ever before (Hader *et al*., 2001). Spontaneous abortion, low birth weight, stillbirth, pre-term labour, premature rupture of membranes, bacterial pneumonia, urinary tract infection have been associated with pregnancies complicated by HIV infection (Gbadegesin *et al*., 2004).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

From this study, the overall prevalence rate of CT was found to be 11.9%. Pregnant and non-pregnant women had prevalence rates of 10.4% and 13.3% respectively. Co-infection between CT and HIV was also established with a prevalence rate of 0.4%. Transmission of *Chlamydia trachomatis* is principally sexual which makes it one of the high risk infections for easy transmission of HIV and also a major STI (Sexually transmissible infection) (Debattista *et al*., 2002).

It was equally noted in this study that sexually active age may have an impact on the incidence of *Chlamydia trachomatis* as highest prevalences (10.5%-100%) observed in both pregnant and non-pregnant women were young adults (<15-35 years) that are in their sexually active stage increased with increasing age. The high prevalence with a worse situation in the young age brackets underscores the necessity for sustainable intervention initiatives among women with the youth as a special focus.

The fact that heterosexual and vertical transmissions constitute the major modes of HIV infection in Nigeria calls for a concerted effort at establishing the magnitude of the problem in order to plan appropriate intervention.

It is obvious that the infection of women of childbearing age with these silent infections portends serious danger for both heterosexual and vertical transmissions, hence posing a significant threat to the global efforts at combating the epidemic. Hence, there is an urgent need for a national policy on routine screening for *Chlamydia trachomatis* as treatment is cheap and effective, while

the attendant morbidity resulting from delayed diagnosis is more difficult to manage and associated with severe sequelae.

6.2 RECOMMENDATIONS

Chlamydia infection remains largely undiagnosed and a silent disease in apparently healthy populations in developing countries and can be eradicated if behavioural modifications are implemented. By adopting strategies outlined below, not only will chlamydia rates begin to fall, but also rates of other sexually transmitted diseases. These modifications are essential in the current epidemic of the most severe sexually transmitted disease, AIDS.

1. It is imperative that studies that strive to understand further the natural history and pathogenesis of *C. trachomatis* be carried out as this will improve treatment and control measures, and aid in vaccine development since rates of progression vary widely between individuals.
2. Patient Education: Appropriate counseling of infected individuals must be performed to inform patients of the possible long-term risks and complications of their infection, including the possibility of infertility. Educate them regarding the risk of other STDs, to take steps to prevent re-infection. Hence, they should avoid sexual contact until their treatment is completed.
3. All sexual partners should be evaluated and treated and should also consider using latex condoms to minimize the chances of re-infection since re-infection is very common and is related to non-treatment of infected sexual partners or acquisition from a new partner.
4. Treatment of all sexual partners should be initiated early and the entire course of antibiotics be completed as this will make the prognosis excellent. This is because treatment of Chlamydial infections, save for being cheap and effective, have an added

benefit since treatment of Chlamydial infections could delay the spread of human immunodeficiency virus (HIV).

5. Antenatal screening should be considered an effective tool for the diagnosis, treatment and prevention of further transmission of STIs. The identification and treatment of STIs in this population will not only benefit the individual woman and her sexual partners, but also her infant with the protection from adverse outcomes including congenital infection.
6. It is therefore also imperative that HIV infected women receive adequate screening for STIs during the antenatal period and appropriate counselling and followup for treatment and prevention.
7. All sexually active non-pregnant young women as well as pregnant women aged 24 years or younger and for older non-pregnant and pregnant women who are at increased risk for chlamydial infection should be screened as recommended by the US Preventive Services Task Force.
8. Intensive counseling on the complications of recurrent infections is of great necessity since chlamydial infection is one of the leading causes of infertility and PID a serious disease that often requires hospitalization in women.
9. Other measures such as the use of barrier method during sexual intercourse, limiting the number of sexual partners, public awareness, school involvement, and peer educators may also help in reducing the incidence and prevalence of this infection.
10. Laboratories should provide improved techniques for the identification and confirmation of *Chlamydia trachomatis*.
11. While on the ongoing campaign against HIV, fight against sexual promiscuity should be intensified.

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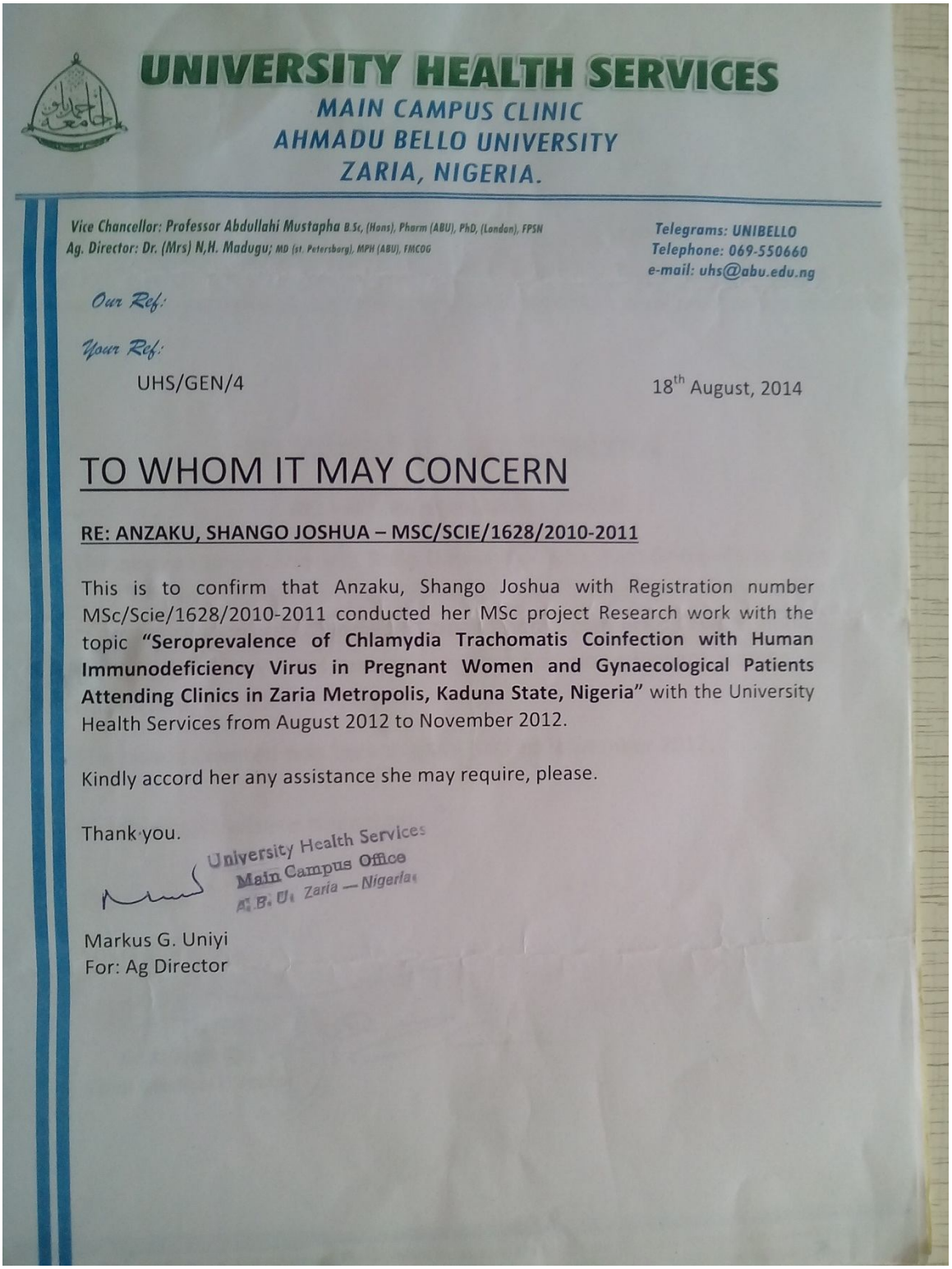
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APPENDIX I: ETHICAL APPROVAL



UNIVERSITY HEALTH SERVICES
MAIN CAMPUS CLINIC
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA.

Vice Chancellor: Professor Abdullahi Mustapha B.Sc, (Hons), Pharm (ABU), PhD, (London), FPSN
Ag. Director: Dr. (Mrs) N,H. Madugu; MD (st. Petersburg), MPH (ABU), FMCOG

Telegrams: UNIBELLO
Telephone: 069-550660
e-mail: uhs@abu.edu.ng

Our Ref:

Your Ref:
UHS/GEN/4

18th August, 2014

TO WHOM IT MAY CONCERN

RE: ANZAKU, SHANGO JOSHUA – MSC/SCIE/1628/2010-2011

This is to confirm that Anzaku, Shango Joshua with Registration number MSc/Scie/1628/2010-2011 conducted her MSc project Research work with the topic **"Seroprevalence of Chlamydia Trachomatis Coinfection with Human Immunodeficiency Virus in Pregnant Women and Gynaecological Patients Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria"** with the University Health Services from August 2012 to November 2012.

Kindly accord her any assistance she may require, please.

Thank you.

University Health Services
Main Campus Office
A.B.U. Zaria — Nigeria

Markus G. Uniyi
For: Ag Director

St. Luke's Anglican Hospital
(DIOCESE OF WUSASA)
P.M.B. 1145 Wusasa,
Zaria Kaduna State
E-mail: slahwusasa@live.com
:08174974470

Our:.....

Date: 15th August, 2014

TO WHOM IT MAY CONCERN

RE: - ANZAKU SHANGO JOSHUA

The above named Ahmadu Bello University Zaria, Post Graduate Student was given permission to conduct her research work titled "Seroprevalence of *Chlamydia trachomatis* coinfection with human immunodeficiency virus among pregnant and non-pregnant women" in this facility.

The period covered was from August 2012 to November 2012.

Kindly assist where necessary.

Thank you.

Yours Sincerely,

Dr. Gilbert U
Chief Medical Director

BOARD OF GOVERNORS
Prof. Jonathan George (Chairman), Prof. Ibrahim Musa, Mrs. Freda Gowon, Dr. Taylor Adeyemi, Mr. Ibrahim Mato

KADUNA STATE MINISTRY OF HEALTH

MAJOR IBRAHIM B. ABDULLAHI (MIBA) MEMORIAL HOSPITAL

Sabon Gari Zaria

Our Ref: _____ Your Ref: _____ Date: 4/8/2014

TO WHOM IT MAY CONCERN

RE: ANZAKU SHANGO JOSHUA

The above name Post Graduate Student of Ahmadu Bello University, Zaria. Has conducted research work in this facility.

The period covered was from August to November, 2012.

Thank you.

A. Abbas
Matron I/C

APPENDIX II: CONSENT FORM

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

This Informed Consent Form is for Malaria patients attending clinics in some hospitals in Kaduna State. We are inviting you to participate in this research work titled "Seroprevalence of *Chlamydia trachomatis* Coinfection with Human Immunodeficiency Virus among Women attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Principal Investigator: Anzaku, Shango Joshua

Collaborating Investigators: Prof. E.D. Jatau

Dr. I.O. Abdullahi

Name of Organization: Department of Microbiology,

Ahmadu Bello University, Zaria – Nigeria.

Name of Sponsor: Self

Name of Proposal: Postgraduate Research Proposal.

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: INFORMATION SHEET

Introduction

I am Anzaku, Shango Joshua a postgraduate student of the Department of Microbiology, Ahmadu Bello University, Zaria, carrying out a research work under the supervision of Prof. E.D. Jatau and Dr. I.O. Abdullahi.

We are doing a research work on Seroprevalence of *Chlamydia trachomatis* Coinfection with Human Immunodeficiency Virus among Women attending Clinics in Zaria Metropolis, Kaduna State, Nigeria. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.

Purpose of the research

The purpose of this research is to survey the seroprevalence of *Chlamydia trachomatis* coinfection with human immunodeficiency virus among women attending clinics in Zaria Metropolis, Kaduna State, Nigeria.

Participant selection

We are inviting all women (pregnant and non-pregnant) with or without human immunodeficiency virus who are attending clincs in Zaria metropolis, Kaduna State to participate in the research work on the Seroprevalence of *Chlamydia trachomatis* Coinfection with Human Immunodeficiency Virus among Women.

Do you know why we are asking you to take part in this study? YES..... NO……..

- *Do you know what the study is about? YES..... NO. .....*

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/hospital. You may change your mind later and stop participating even if you agreed earlier.

- *If you decide not to take part in this research study, do you know what your options are? YES…… NO……..*
- *Do you know that you do not have to take part in this research study, if you do not wish to? YES…… NO …….*
- *Do you have any questions? YES…… NO. ……*

Procedures and Protocol

When you have agreed to participate in this research, we will take blood from your arm using a syringe and needle. Plain vacutainer bottles, will be used to show you the amount of blood that we will collect from you). At the end of the research, any leftover blood will be destroyed.

Description of the Process

During the research you will make just one visit to the clinic.

In the visit, a small amount of blood will be taken from your arm with a syringe. This blood will be tested for the presence of *Chlamydia trachomatis* and human immunodeficiency virus and analysis will be carried out. We will also ask you a few questions about your general health and other measures so as to investigate the risk factors associated with the occurrence of *Chlamydia trachomatis* and human immunodeficiency virus.

Duration

The research will take place over two months. During that time, it will be necessary for you to come to the clinic just once. After that, the research will finish.

- *How many times are we asking you to come to the hospital to participate in the research?.....*
- *Can you tell me the number of time we will take your blood sample?.....*
- *Do you have any questions?.....*
- *Do you want me to go through the procedures again?.....*

Risks

There is a risk that you may share some personal or confidential information by chance, or that you may feel uncomfortable talking about some of the topics. However, we do not wish for this to happen. You do not have to answer any question or take part in the research if you feel the question(s) are too personal or if talking about them makes you uncomfortable.

Benefits

If you participate in this research, you will have the following benefits:

- ✓ You will have the opportunity of having tested for *Chlamydia trachomatis*.
- ✓ Your blood will be screened for human immunodeficiency virus.

- ✓ There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.
- ✓ Apart from these few benefits listed above, your participation is likely to help us find answer to the research questions.

Reimbursements

You will not be provided with any special incentive or travel allowance for you to take part in the research. But we advise that your visit to the clinic will have to be on your normal schedule clinic days. However, you will have the opportunity of doing free *Chlamydia trachomatis* test.

Can you tell me if you have understood correctly the benefits that you will have if you take part in the study?.....

- ➢ *Do you know that the study will not pay for your travel costs? .....*
- ➢ *Do you know the kind of benefit you can get by participating in this research?.....*
- ➢ *Do you have any other questions?.....*

Confidentiality

With this research, it is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research.

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the principal investigator will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except the principal investigator.

- ➢ *Did you understand the procedures that we will be using to make sure that any information that we as researchers collect about you will remain confidential?.....*
- ➢ *Do you have any questions about them?.....*

Sharing the Results

The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so, refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

This proposal has been reviewed and approved by Scientific Ethical Committee (SEC) of the Ministry of Health and other hospitals, which is a committee whose task is to make sure that research participants are protected from harm. If you wish to find out more about the SEC, contact the office of the Assistant Dean, School of postgraduate studies, ABU, Zaria.

- ➢ *Do you know that you do not have to take part in this study if you do not wish to? ……*
- ➢ *Do you know that you can ask me questions later, if you wish to? ……*
- ➢ *Do you know that I have given the contact details of the person who can give you more information about the study?.....*
- ➢ *You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?.....*
- ➢

PART II: CERTIFICATE OF CONSENT

I have read the foregoing information, or it has been read and translated to me in language that I understand. I have also talked it over with the doctor to my satisfaction. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional sheet to keep for myself. I therefore consent voluntarily to participate as a participant in this research.

Name of Participant: .....

Signature of Participant: .....

Date: .....

Day/month/year

Statement by Witness

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness: ..... AND Thumb print of participant

Signature of witness: _____

Date _____

Day/month/year

Statement by the Researcher/Person Taking Consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. *Blood sample will be taken*

I confirm that sufficient information, including about risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent: .....

Signature of Researcher /person taking the consent: _____

Date _____

Day/month/year

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF POSTGRADUATE STUDIES

AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

Serial No.: ……. ***Hospital No.: ……*** ***Age: ……..*** ***Phone No.: ……..……***

This Informed Consent Form is basically for men, women and children attending some selected Hospitals in Kaduna State. We are inviting you to participate in this research work titled "Sero-epidemiological survey of dengue virus infection in malaria patients in some parts of Kaduna State, Nigeria. Dengue virus is a small tiny organism (a virus) that causes diseases such as dengue fever, dengue hemorrhagic fever and dengue shock syndrome. The research will involve the collection of blood samples of participants. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this clinic/hospital.

CERTIFICATE OF CONSENT

I ……of …….. do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that blood sample will be taken for the test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant ……..…

Signature/right thumb print of participant ……. Date …….

Statement by Witness:

I have witnessed the accurate reading of the consent form and detail explanation of the study to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness……

Signature of witness…….…. Date …….

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher …….

Signature ……... Date.……

APPENDIX III: STRUCTURED QUESTIONAIRE

RESEARCH TOPIC: Seroprevalence of *Chlamydia trachomatis* (CT) Co-infection with Human Immunodeficiency Virus (HIV) among Women attending Clinics in Zaria Metropolis, Kaduna State, Nigeria.

Your maximum cooperation is highly needed in providing the correct data and it will be treated with utmost confidentiality. Thank you ma.

SECTION A: BIODATA

Serial Number: Hospital Number:

Age: 15-20 ( ) 21-25 ( ) 26-30 ( ) 31-40 ( ) 41-45 ( ) 46-50 ( )

Occupation: Civil Servant ( ) Self Employed ( ) Unemployed ( ) Retired ( ) Others ( )

Husband's Occupation: Civil Servant ( ) Self Employed ( ) Unemployed ( ) Retired ( ) Others ( )

Highest Educational Level: Primary ( ) Secondary ( ) Tertiary ( ) Quranic ( ) None ( )

SECTION B: EPIDEMIOLOGICAL RISK FACTORS

Marital Status: Single ( ) Married ( ) Widow ( ) Divorced ( ) Married but Living Separated ( )

Family Type: Monogamy ( ) Polygamy ( ) Others ( )

Number of Sexual Partner(s): One ( ) Multiple ( )

How Long Have You Been Married: 1-5( ) 6-10( ) 11-15( ) 16-20( ) 21-25( ) 26-30( ) 31-35( )

Others ( )

Do You Have Children: Yes ( ) No ( )

Age at Which You Had First Child: 10-15( ) 16-20( ) 21-25( ) 26-30( ) 31-35( ) 36 & above ( )

Current Pregnancy Status: First Trimester ( ) Second Trimester ( ) Third Trimester ( )

Sexual Intercourse during Pregnancy: Yes ( ) No ( )

Any Abnormal Discharge from Vagina: Yes ( ) No ( )

Any Previous History of:	a. Abnormal vaginal discharge	Yes ( ) No ( )
	b. Burning on urination	Yes ( ) No ( )
	c. Pain during intercourse	Yes ( ) No ( )
	d. Lower abdominal pain	Yes ( ) No ( )
	e. Bleeding between periods	Yes ( ) No ( )
Any history of:	a. Stillbirth	Yes ( ) No ( )
	b. Premature birth	Yes ( ) No ( )
	c. Miscarriage	Yes ( ) No ( )
	d. Infertility	Yes ( ) No ( )

APPENDIX IV: OD results for ELISA (CT IgG)

Plate 1		1	2	3	4	5	6	7	8	9	10	11	12
A	Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
	OD	0.195	0.115	3.204	4.00*	0.750	0.336	0.233	0.357	0.393	0.249	0.180	0.209
	Quant		0.115	3.204	*	0.750	0.336	0.233	0.357	0.393	0.249	0.180	0.209
	Quali												
B	Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
	OD	0.130	0.820	0.165	0.500	0.466	0.285	0.377	0.181	0.396	0.159	0.652	0.352
	Quant	0.130	0.820	0.165	0.500	0.466	0.285	0.377	0.181	0.396	0.159	0.652	0.352
	Quali												
C	Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
	OD	0.329	1.941	1.038	0.211	0.166	0.242	0.546	0.233	0.401	2.015	0.290	0.583
	Quant	0.329	1.941	1.038	0.211	0.166	0.242	0.546	0.233	0.401	2.015	0.290	0.583
	Quali												
D	Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
	OD	0.274	0.252	1.877	0.337	0.142	0.550	0.409	0.289	1.419	0.173	0.300	0.180
	Quant	0.274	0.252	1.877	0.337	0.142	0.550	0.409	0.289	1.419	0.173	0.300	0.180
	Quali												
E	Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
	OD	0.353	2.684	0.707	0.385	0.288	0.402	1.535	1.033	2.402	2.018	0.410	0.324
	Quant	0.353	2.684	0.707	0.385	0.288	0.402	1.535	1.033	2.402	2.018	0.410	0.324
	Quali												
F	Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
	OD	0.832	1.915	0.449	0.639	0.287	0.856	0.735	0.481	0.316	0.168	0.740	0.509
	Quant	0.832	1.915	0.449	0.639	0.287	0.856	0.735	0.481	0.316	0.168	0.740	0.509
	Quali												
G	Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
	OD	0.435	2.235	0.413	0.148	0.533	2.304	3.179	0.749	0.553	0.393	2.525	0.452
	Quant	0.435	2.235	0.413	0.148	0.533	2.304	3.179	0.749	0.553	0.393	2.525	0.452
	Quali												
H	Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
	OD	0.691	2.794	0.542	2.303	0.391	3.290	0.975	1.488	1.802	1.840	0.348	0.196
	Quant	0.691	2.794	0.542	2.303	0.391	3.290	0.975	1.488	1.802	1.840	0.348	0.196
	Quali												

Plate 2		1	2	3	4	5	6	7	8	9	10	11	12
A	Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
	OD	0.184	0.325	0.353	0.178	0.589	3.597	0.589	0.330	0.272	0.303	0.567	0.299
	Quant		0.325	0.353	0.178	0.589	3.597	0.589	0.330	0.272	0.303	0.567	0.299
	Quali												
B	Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
	OD	0.130	0.820	0.165	0.500	0.466	0.285	0.477	0.256	0.953	0.228	0.553	1.824
	Quant	0.130	0.820	0.165	0.500	0.466	0.285	0.477	0.256	0.953	0.228	0.553	1.824
	Quali												
C	Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
	OD	0.210	0.154	0.183	0.953	0.626	0.530	0.539	0.243	0.943	0.676	0.299	1.487
	Quant	0.210	0.154	0.183	0.953	0.626	0.530	0.539	0.243	0.943	0.676	0.299	1.487
	Quali												
D	Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
	OD	0.148	0.303	0.826	0.296	0.597	0.451	0.843	0.084	1.688	0.565	0.666	0.224
	Quant	0.148	0.303	0.826	0.296	0.597	0.451	0.843	0.084	1.688	0.565	0.666	0.224
	Quali												
E	Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
	OD	0.453	0.484	0.494	0.304	0.911	0.804	0.713	0.371	1.602	0.415	2.965	0.327
	Quant	0.453	0.484	0.494	0.304	0.911	0.804	0.713	0.371	1.602	0.415	2.965	0.327
	Quali												
F	Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
	OD	0.177	0.377	0.837	0.151	0.337	0.443	0.399	0.833	0.508	1.146	0.205	0.393
	Quant	0.177	0.377	0.837	0.151	0.337	0.443	0.399	0.833	0.508	1.146	0.205	0.393
	Quali												
G	Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
	OD	0.226	0.200	0.460	1.176	0.744	1.149	0.525	0.470	0.228	0.554	1.677	1.276
	Quant	0.226	0.200	0.460	1.176	0.744	1.149	0.525	0.470	0.228	0.554	1.677	1.276
	Quali												
H	Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
	OD	0.096	0.463	2.604	0.233	0.286	0.913	1.474	0.213	0.733	0.451	0.330	0.307
	Quant	0.096	0.463	2.604	0.233	0.286	0.913	1.474	0.213	0.733	0.451	0.330	0.307
	Quali												

Plate 3		1	2	3	4	5	6	7	8	9	10	11	12
A	Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
	OD	0.175	0.104	0.377	0.127	0.170	0.220	0.295	0.512	0.353	0.268	0.351	0.506
	Quant		0.104	0.377	0.127	0.170	0.220	0.295	0.512	0.353	0.268	0.351	0.506
	Quali												
B	Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
	OD	0.225	0.168	0.183	0.179	0.074	0.181	0.245	0.333	0.157	0.255	0.484	1.900
	Quant	0.225	0.168	0.183	0.179	0.074	0.181	0.245	0.333	0.157	0.255	0.484	1.900
	Quali												
C	Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
	OD	1.302	0.169	0.177	0.931	0.177	0.446	1.098	0.925	0.359	0.674	0.605	1.668
	Quant	1.302	0.169	0.177	0.931	0.177	0.446	1.098	0.925	0.359	0.674	0.605	1.668
	Quali												
D	Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
	OD	0.218	0.440	0.155	0.981	1.779	0.612	0.240	1.129	0.188	0.237	1.690	0.298
	Quant	0.218	0.440	0.155	0.981	1.779	0.612	0.240	1.129	0.188	0.237	1.690	0.298
	Quali												
E	Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
	OD	0.122	0.424	0.204	0.810	0.152	0.570	0.280	2.269	1.324	0.291	0.440	1.097
	Quant	0.122	0.424	0.204	0.810	0.152	0.570	0.280	2.269	1.324	0.291	0.440	1.097
	Quali												
F	Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
	OD	0.137	0.104	0.102	0.195	0.077	0.264	0.383	0.268	1.597	0.141	3.195	0.453
	Quant	0.137	0.104	0.102	0.195	0.077	0.264	0.383	0.268	1.597	0.141	3.195	0.453
	Quali												
G	Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
	OD	0.192	0.189	0.312	0.118	0.886	0.308	0.796	1.396	3.025	0.732	1.546	0.511
	Quant	0.192	0.189	0.312	0.118	0.886	0.308	0.796	1.396	3.025	0.732	1.546	0.511
	Quali												
H	Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
	OD	0.212	3.290	0.140	0.214	0.178	0.513	0.668	0.279	0.054	0.037	0.066	0.082
	Quant	0.212	3.290	0.140	0.214	0.178	0.513	0.668	0.279	0.054	0.037	0.066	0.082
	Quali												