TYPE – SPECIFIC, SEROPREVALENCE OF HERPES SIMPLEX VIRUS TYPE 1 AND 2 AND ASSOCIATED RISK FACTORS IN WOMEN OF CHILDBEARING AGE IN KOGI STATE

BY

DRISU,UTENO ITANYI
PG/BMS1513012

DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN
BENIN CITY

DECEMBER, 2018.

TYPE – SPECIFIC, SEROPREVALENCE OF HERPES SIMPLEX VIRUS TYPE 1 AND 2 AND ASSOCIATED RISK FACTORS IN WOMEN OF CHILDBEARING AGE IN KOGI STATE

BY

DRISU, UTENO ITANYI
PG/BMS1513012

BEING A THESIS IN THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE SUBMITTED TO THE SCHOOL OF POSTGRADUATE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS DEGREE IN MEDICAL LABORATORY SCIENCE (MEDICAL MICROBIOLOGY) IN UNIVERSITY OF BENIN BENIN CITY, EDO STATE, NIGERIA.

DECEMBER, 2018.

CERTIFICATION

This is to certify that this thesis was carried out by DRISU, Uteno Itanyi with Matriculation Number PG/BMS1513012, under my supervision: Rev. Prof. F.E. Oronsaye of the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

_____ _____

REV. PROF. F.E. ORONSAYE **DATE**
Project Supervisor

_____ _____

DR. F.O. AKINBO **DATE**
Head of Department

_____ _____

PROF. E.I. IKEH **DATE**
External Examiner

DEDICATION

This work is dedicated to God Almighty for his love and provision for life. Also to my late Father Mr. Joseph Idris Itanyi and Mother Mrs. Matha Alugbe Joseph.

ACKNOWLEDGEMENTS

My sincere gratitude and indebtedness goes to my Supervisor Prof. F.E. Oronsaye for the opportunity to carry out this research work under his invaluable guidance and also for his encouragement, patience, suggestions, corrections and rewarding criticism. My appreciation goes to the Head of Medical Laboratory Science Department Dr. F.O. Akinbo, Dr. M.A. Emokpae and Prof. H.B. Osadolor for their fatherly advice and encouragements. My unreserved gratitude goes to my Co-Supervisor Dr. B.I.G. Adejumo for his invaluable advice, support, and encouragement. My special thanks go to the Director, the secretary Dr. Isah Barnabas and the entire Health Research Ethical Committee Ministry of Health, Lokoja for granting me permission to carry out this research work in the state.

I also extend my gratitude to various Head of Department of Medical Laboratory unit of General Hospitals Idah, Okene, Kabba and Specialist Hospital, Lokoja Mrs. Stella Okenyi, Mr. Paul I., Mr. Ohikere B, Mr. Baba O. respectively, my thanks also goes to Dr. Abraham B for his contribution to the success of this work. I am also grateful to Mr. Ogho O. of Clinical Biochemistry Department of University of Benin Teaching Hospital for his assistance during the course of the analysis of this work. My thanks also go to Dr. E. Ekele for the statistical analysis of this research work.

Infact, I am grateful to all the staff of the Department of Medical Laboratory Science, University of Benin for their various roles and assistance towards the

success of my post graduate programme. My appreciation also goes to my friends and colleagues Mrs. Mercy Ehimika, Mrs. Isoke P., Mrs. Iyobo and others too numerous to be mentioned for their assistance throughout the programme.

My special thanks goes to my lovely wife Mrs. Esther A. Idris for her love, encouragement and moral support during the course of my study, to my lovely children, Mrs. Mercy Idris Ameh, Miss Gloria Idris, Mr. Samuel Idris, Mr. David Idris and Miss Charity Idris for their understanding, prayers and moral assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

ABSTRACT

Viruses of the Herpetoviridae family infect 90% of the earth's population. Humans are the hosts of at least nine unique herpes viruses. This study is aimed at evaluating the seroprevalence of type specific Herpes simplex virus infection and the associated risk factors in a cross-section of asymptomatic healthy women of childbearing age in Kogi State, Nigeria. A total of 330 subject of 15 - 49 years were recruited from various local government areas of the three Senatorial districts of Kogi State. Blood samples were collected from participants and analyzed for HSV-1 and HSV-2 IgG antibodies quantitatively with IgG Type-Specific ELISA kit. In this research, the overall prevalence of HSV infection was 76.7%. Data further showed that majority (96.4%) of the population studied had HSV-1, while 77% had HSV-2 infections. Results also showed that the young adults age 15-25 years indicated prevalence of 93.7% for HSV-1 and 74.4% for HSV-2, while in the middle aged 26-35 years, the prevalence were 100% for HSV-1, and 78.4% for HSV-2. In the age group of 36-45 years the prevalence for HSV-1 and HSV-2 were 98.2% and 82.5% respectfully Among the married women the prevalence was 99.2% for HSV-1, while that of HSV-2 was 86.9% . For the unmarried women the prevalence was 94.5% for HSV-1, and 70.5% for HSV-2. The risk of HSV-1 or HSV-2 infections was not associated with age, age of first sexual exposure and number of sex partners before marriage. Married women were at greater risk for HSV-1 ($p = 0.03$) and HSV-2 ($p \leq 0.001$) infections compared to the unmarried women. The prevalence of HSV-1 and HSV-2 among educated subject were 96.2% and 79.9% respectfully while that of uneducated were 66.7% and 75%.The prevalence of HSV-1 and HV-2 among subject who had sexual intercourse below 15 years of age were 100% and 78.9% respectfully while those who had above 15 years of age were 98.4% for HSV-1 and 80.7% for HSV-2. The prevalence of HSV-1 and HSV-2 according to number of sexual partner before marriage were 100% for four partners, for three partners the prevalence was 100% and 83.3% respectfully. For two partners the prevalence for HSV-1 and 2 were 100% and 75% while that of one partner were 99% and 78.6% respectively. There were no significant difference ($p=0.611$) between the CD4 counts of HSV-1 and HSV-2 infected women and that of uninfected women. In conclusion, the prevalence of the HSV-1 and HSV-2 infections was high among asymptomatic healthy women of childbearing age in Kogi State, Nigeria. Efforts should be made to increase the awareness of HSV infection among women of child bearing populace.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Viruses of the Herpesviridae family infect 90% of the Earth's population (Wald and Corey, 2007). Humans are the hosts of at least nine unique herpes viruses. The most prevalent is herpes simplex virus type 1 (HSV-1), which causes latent infection but reactivates causing cutaneous or genital herpes, conjunctivitis, keratitis, encephalitis, or eczema herpeticum. HSV often co-infects HIV-infected patients, complicating treatment of AIDS. Herpes simplex virus type-1(HSV-1) is also involved in the pathogenesis of multiple sclerosis (Bello-Morales *et al.,* 2012) and result in male infertility (Shuppe *et al.,* 2008).

Herpes simplex virus-1 (HSV-1) and Herpes simplex virus type-2(HSV-2) infection is extremely common throughout the world (Gupta et al 2007).Epidemiological surveys demonstrate a rise in seroprevalence of HSV-1 up to 50-70% in developed countries and 100% in developing countries and HSV-2 seroprevalence from 10-40% and may reach 69-95% in HIV infected people and female sex workers (LeGoff *et al.,* 2014). This infection is the most common cause of genital ulcer disease in industrialized nations, and infections may be due to HSV types 1 or 2 (Corey, 1994). Although the majority of genital herpes is due to HSV-2, an increasing proportion is recognized as being due to HSV-1 (Wald, 2006). Although the clinical course

of acute first episode of genital herpes among patients with HSV-1 and HSV-2 infections is similar, the frequency and severity of recurrences is less with HSV-1 than with HSV-2 (Wald, 2006). In addition, the severity of clinically apparent first episodes and reactivation with HSV-2 infection are lower in those with prior HSV-1 (Corey, 1994). Despite increased awareness of these infections, they remain under diagnosed because the majority of infections are asymptomatic or unrecognized (Corey and Spear, 1986).

Symptomatic infections may present in unusual or atypical ways, increasing the diagnostic challenge (Ashley, 1998). Most transmission to partners, or less commonly to the neonate, occurs while the infected person is asymptomatic (Mertz et *al.,* 1992, Prober *et al.,* 1992). Infection with HSV has also been shown to increase the risk of acquisition or transmission of HIV infection (Fleming *et al.,* 1999). Genital herpes, an infection commonly caused by human herpes simplex virus 2 (HSV-2) in Nigeria and in most regions of the world, has been described as a silent pandemic with different countries being at different stages of the epidemic (Looker *et al.,* 2008, Oni *et al.,* 1996). Humans are the only natural host (Dordevic, 2006). The fact that transmission through viral shedding continues, also in the frequent asymptomatic cases, has sustained the worsening of the pandemic (Corey and Handsfield*,* 2000). The HSV-2 pandemic is further reinforced by the HIV pandemic and vice versa; and it is becoming clear that the efforts at controlling the spread of HIV may remain ineffective if control of HSV-2 infection, along with other sexually

transmitted infections (STI) is not integrated (Corey and Handsfield, 2000, Kolawole *et al.,* 2008).

Furthermore, HSV-2 infection has been reported to produce a wide range of deleterious obstetric effects, ranging from neonatal herpes, neonatal death, neuronal deficits, mental handicap, congenital malformations, intrauterine fetal death, preterm labour, low birth weight babies, and spontaneous abortion (Kalu *et al.,* 2014, Garland Steben, 2014).

Accurate and timely diagnosis of all patients with genital HSV infection is far from achieved in clinical practice. The majority of genital herpes patient are misdiagnosed of not formally diagnosed at all (Corey, 1994), hence they remain untreated, without counseling, and they continue to suffer.

1.2 Justification for the Study

The diagnosis of HSV based only on the subject's medical history and physical examination is frequently inadequate (Corey *et al*., 2008). Most primary genital infection with HSV-1 and HSV-2 are asymptomatic and all are followed by latent infection of neuronal cells in the dorsal root ganglia and only 10-25% of people with HSV-2 antibodies are aware of their genital herpes. However, a large proportion of seropositive patients present asymptomatic shedding episodes that contribute to the spread of these infection (Wald, 2011). The evidence suggesting that HSV is facilitating the spread of the global human immunodeficiency virus (HIV) epidemic and the

risk posed by these synergies to neonates in developing countries informed this study (Olantuji *et al.,* 2016).

1.3 Statement of the Problem

The infection of HSV is under diagnosed, hence no proper record for determination of its prevalence in Kogi State.

1.4 Aim of Study

The aim of the study was to evaluate the prevalence of HSV infection among women of reproductive age in Kogi State and associated risk factors.

1.5 Specific Objectives

1) To carry out IgG type-specific analysis in order to determine the seroprevalence of HSV-1 and 2 infections among women in Kogi State
2) To determine the risk factors of the infection.
3) To determine the prevalence of genital ulcer among women infected with HSV.
4) To determine CD_4^+ T cell count of the women infected with HSV.

CHAPTER TWO

LITERATURE REVIEW

2.1 History and Classification of Human Herpes Viruses

The identification of human herpes virus (HHV) infections can be traced back to ancient Greece where Herpes Simplex Virus (HSV) infections in humans were first documented. Hippocrates used the word "herpes", meaning to creep or crawl, to describe spreading skin lesions. Although the vesicular nature of lesions associated with HSV infections had been well documented in the late eighteenth century, person-to-person transmission of HSV was only first recognised by Vidal in 1893 (Roizman, 1996). HSV was first isolated in 1919 (Lowenstein, 1919) but decades passed before it was demonstrated that there were two serotypes of HSV belonging to the Herpetoviridae family, alphaherpetovirunae sub-family, HSV-1 and HSV-2 (Whitley and Roizman, 2001). Over time, several other members of the HHV family have been identified. The latest member, HHV-8, was only identified last decade (Chang *et al.,* 1994). The major laboratory advances of the past 20 years have enabled a torrent of new insights into the biological properties of HHV.

Herpes viruses infect members of all groups of vertebrates, and the same host can be infected with multiple distinct and unique types. Initially classified on the basis of tissue tropism and more recently based on DNA sequence homology, the herpes viruses have been typically grouped into three subfamilies:

(1) The alpha-herpes viruses generally have a rapid replication cycle and a broad host cell range. Alpha-herpes viruses are further characterized by their neurotropic properties tendency to establish latent infections in neural tissue.

(2) The beta-herpes viruses, which differ in genome size and structure, have a much more restricted range of host cells and replicate more slowly in culture.

(3) The gamma-herpes viruses are lymphotropic, infecting predominantly T- or B-lymphocytes and causing either lytic or latent infection. Herpes viruses in this group are furthermore characterized by their oncogenic properties.

To date, eight distinct HHV have been described, each causing a characteristic disease. Among the alpha-herpes viruses, HSV -1 and -2 are the primary agents of recurrent facial and genital herpetic lesions, respectively, while varicella zoster virus (VZV) is the causative agent of chicken pox and shingles. The prototypic member of the group of beta-herpes virus is human cytomegalovirus (HCMV), which is linked to congenital infections of the nervous system. In contrast, infections with two other lymphotropic beta-herpes viruses, human herpes viruses -6 and -7 (HHV -6 and -7), generally cause mild early childhood diseases. Infections with two human gamma-herpes viruses, Epstein-Barr virus (EBV) and human herpesvirus-8 (HHV -8) are associated with human cancers. The most common disease caused by infection

with EBV is generally known as "infectious mononucleosis", while HHV-8 is believed to play a causative role in Karposi's sarcoma.

Herpes simplex virus (HSV) types one and two (HSV-1 and HSV-2) are the main cause of genital herpes, which is the main cause of genital ulcers worldwide. Most genital herpes is caused by HSV-2, but more recently there has been an increase in infections caused by HSV-1 (Garland, 2014 and Gupta, 2007). A serious complication of genital herpes is neonatal herpes, usually caused by HSV-2. It can lead to the death of a newborn, but fortunately this complication is rare. Infection is acquired most commonly via sexual activity (oral, vaginal, or anal). Most infections manifest with mild symptoms or are even asymptomatic, which increases the risk of transmission. The highest possibility of virus transmission is during outbreak periods when lesions are present**.**

2.2 Pathology

Transmission of HSV usually occurs through close contact with a person who is shedding the virus at a peripheral site, mucosal surface, or in genital or oral secretions. HSV penetrates susceptible mucosal surfaces or abraded cracks in the skin. After mucosal inoculation (in genital infection), the virus is transported along peripheral nerve axons to the nerve cell bodies' sacral ganglia. Virus remains latent indefinitely in the paraspinous ganglia.

Reactivation induces viral replication and is precipitated by multiple known factors (e.g., trauma, fever, ultraviolet light, stress, etc.), as well as unknown

factors. The reactivated virus migrates centrifugally to mucosal surfaces by way of the peripheral sensory nerves, where it may cause a cutaneous outbreak of herpetic lesions or subclinical viral shedding.

Histopathologic changes include focal necrosis, ballooning degeneration of cells, production of mononucleated giant epithelial cells, and eosinophilic intranuclear inclusions called Cowdry type A bodies.

Up to 90% of persons seropositive for HSV-2 antibody have not been diagnosed with genital herpes. However, many have mild or unrecognized asyptomatic disease and probably most, if not all, shed virus from the genital area intermittently (CDC, 2014).

2.3 Clinical Manifestations of Genital Herpes

The incubation period ranges from 2 to 10 days and most HSV infections are subclinical. In symptomatic infections, clinical manifestations of primary infection of the genital area with HSV-1 or HSV-2 are typically characterized by painful vesicular and ulcerative lesions. After acquisition of HSV infection at a mucocutaneous site, papules and macules appear which develop into pustular and ulcerative lesions. After 4 to 15 days, the lesions crust and re-epithelize (Gupta *et al., 2007,* Knipe, 2013). Primary infection can be associated with fever, dysuria, localized inguinal adenopathy, and malaise in both men and women. Paresthesias and dysesthesias that involve the lower extremities and perineum are common.

In women with primary infection, lesions appear on the vulva and are usually bilateral (Knipe, 2007), with involvement of the cervix. Lesions may involve also the perineum, buttocks, and/or vagina. In men, primary infection results in vesicular lesion on the glans of penis or the penile shaft (Knipe, 2007). There is also a possibility of extra-genital lesions occurring in both sexes. Systemic complication in the form of aseptic meningitis can occur in both men and women. Viral excretion may persist for up to 3 weeks (Knipe, 2013).

Clinically, an acute episode of genital herpes is similar in infection regardless of the type of HSV causing infection, but recurrences are less frequent and less severe in HSV-1infection (Sing *et al*., 2005). Primary infection is associated with larger quantities of virus shedding in the genital tract and a longer period of viral shedding, on average 3 weeks (Gupta *et al.,* 2007 and Patel, 2014).

Vertical transmission from an infected mother to fetus is usually manifested with vesicular lesions on the skin of infected babies. Infection can result in a disseminated disease in the newborn with central nervous system involvement, neonatal death of untreated cases is up to 50% (Kimberlin *et al.,* 2001 and Patel *et al.,* 2010). The proportion of women with HSV-2 antibodies increased significantly with age, from 57 % of women aged16–23 years to 71 % of those aged >30 years, with higher parity and history of pregnancy termination also associated with increased probability of positive HSV-2 sero status (Aebi-Popp *et al.,* 2016)

2.4 Structure of HSV

As shown in Fig. 1A below, cryoelectron tomography has provided the most detailed data on the virion structure at resolution of 7 nm (Grunewald *et al.,* 2008). The virions are spherical particles 186 nm in diameter with glycoprotein spikes protruded from each virion, making their full diameter about 225 nm. The nucleocapsid occupies an eccentric position on one virion side (the proximal pole), it is close to the envelope; on the other side (the distal pole), it is 30-35 nm apart from it. The tegument is an amorphous layer with some structured regions containing 7-nm width filament sap posed to the membrane.

The virion consists of 40 proteins of viral and cellular origin, 10 of which are glycosylated. Eleven proteins are located on the virion surface. The *core* contains the linear double-stranded DNA genome wrapped as a toroid. A small fraction of the viral DNA appears to be circular. Host polyamines spermine and spermidine are found in the viral core, neutralizing the negative charges on the viral DNA and providing its proper packing. The virion contains 70,000 and 40,000 molecules of spermine and spermidine per virion, respectively.

The polyamines are strongly bound to the DNA and cannot be exchanged with added radioactively labeled polyamines. By the degradation of the outer envelope using detergents and urea, spermidine, but not spermine, can be removed from the virion. Recently, polyamines and modified polyamines have been considered as possible regulators or inhibitors of some viral infections.

Dextran-conjugated polyamines, in particular dextran-propan-1,3-diamine, inhibited HSV-1 growth in BS-C-1 cell line (Yudovin-Farber *et al.,* 2003).

The *tegument* is comprised of 26 proteins, some of them participating in capsid transport to the nucleus and other organelles (UL36, UL37, ICP0) (Radtke *et al* 2010), viral DNA entry into the nucleus (VP1-2, UL36) (Jovasovic *et al.,* 2008), activation of early genes transcription (VP16, encoded be *UL48* gene), (Ace *et al.,* 1989), suppression of cellular protein biosynthesis, and mRNA degradation (VHS, UL41) (Jovasovic *et al*., 2008). The tegument contains RNA-binding proteins US11, UL47, and UL49 presumably bound to viral and cellular transcripts packaged in the virion.

The capsid has icosahedral configuration and is composed of 162 capsomeres (Fig. 1A below) – 150 hexons and 12pentons.Three types of capsids can be isolated from infected cells: A-capsids (procapsids) lack both scaffold proteins and viral DNA; B-capsids do not contain viral DNA but contain the protein scaffold for it; C-capsids contain the viral genome (Gibson and Roizman, 1972, Sheaffer *et al.,* 2001). Capsids of any type consist of four principal proteins the major capsid protein UL19 (VP5), VP26 accessory protein (UL35), and also UL18 (VP23) and UL38(VP19C) proteins, whose functions are not well studied.

Six copies of the major capsid protein, VP5, form the hexons, and five copies form the pentons. Six copies of VP26 occupy the outer surfaces of the hexons formed byVP5. A single molecule of VP19C and two copies of VP23 form a

triplex that binds surrounding capsomeres to form connections between them. In the center of every capsomere, there is a channel joining the virion outer surface and core. The channels in hexamers are 4 nm in diameter, and in pentamers they are slightly narrower, and in B capsids these channels are completely closed. The capsid contains UL6 protein, which forms the portal on the vertex of one of the 12 capsid axes, through which the viral genome is presumably packed into the capsid (Brown and Newcomb, 2011), and VP24 (UL26) protease, breaking the scaffold during DNA packaging.

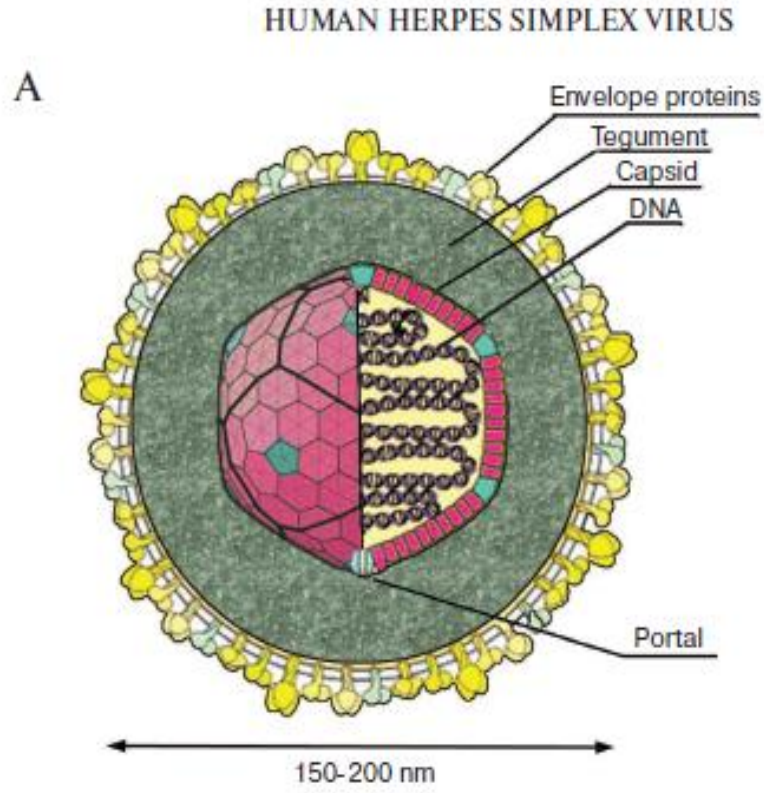


Figure 2.1: Human Herpes Simplex Virus (Grunewald *et al*., 2003)

The outer envelope of the virion consists of lipid bi-layer and 11glycoproteins (gB, gC, gD, gE, gG, gH, gI, gJ,gK), membrane gL, and gM (Figure 2.1) (Chowdhury *et al*., 2013), and at least two unglycosylated membrane proteins (UL-2 and US-9). The lipid bi-layer is formed by cell membrane during virus egression by exocytosis. The function of glycoproteins in virus entry into the cell is now studied extensively.

HSV-1 is a double-stranded DNA-enveloped virus, with a central core containing the viral DNA, an inner core surrounded by envelope derived from both host cellular membranes and viral glycoproteins, and an icosahedral capsid composed of viral proteins (Riley, 1998). Between the capsid and the envelope lies the tegument, a proteinaceous structure containing two proteins important in the pathogenesis of HSV-1 infection, alpha-trans inducing factor (alpha-TIF) (aka: VP16 and Vmw65) and virion host shut-off protein (VHS). VP16 is a transcriptional activator protein that forms a complex with host cell factor (HCF), which localize within the nucleus. These proteins, together with the host transcription factor, octamer-binding protein 1(OCT1), enable the recruitment of other host factors that stimulate transcription of immediate-early (IE) viral gene products (Wysika, 2003).

Viral glycoproteins: Alpha-TIF activates the viral replication cascade, and VHS shuts off host cell macromolecular synthesis (Kwong *et al*., 1988 and Wu *et al*., 1994). Approximately 1,000 molecules of alpha-TIF and 200 copies of VHS are present in the tegument and packaged in the virion (Riley, 1998 and

Strelow, 1995). Thus, these proteins can act early as virus particles enter the cells and uncoat. The viral glycoproteins embedded in the lipid envelope mediate attachment to host cell receptors, fusion of viral and host cell membranes, and envelopment and emergence of virus as newly formed nucleocapsids bud from host cell nuclei (Morrison, 1994). These glycoproteins are also the major targets of humoral and cellular immune responses and viral immune escape mechanisms by binding both complement and anti-HSV IgG molecules (Riley, 1998).

Viral replication: Viral replication takes approximately 24 hours and begins with the attachment of viral glycoproteins to cell-surface receptors, including glycosaminoglycan (GAG) heparan sulfate and the immunoglobulin-like cellular receptors (designated nectin1-alpha and delta and nectin2-beta) (Cocchi*, et al.,* 2000, Rakani, 1998). Glycoproteins gC and gB interact with GAG heparan sulfate, while gD provides a stable attachment to cellular receptors. Glycoprotein gC also binds complement protein C3b and inhibits complement activation (Spear, 2004). HSV strains with mutations in the gC gene are more susceptible to neutralization by human serum.

2.5 Epidemiology and Disease Manifestations

The epidemiology of herpes simplex infection is of substantial epidemiologic and public health interest. Worldwide, the rate of infection with herpes simplex virus—counting both HSV-1 and HSV-2—is around 90% (Wald and Corey, 2007). Although many people infected with HSV develop labial or

genital lesions (herpes simplex), the majority are either undiagnosed or display no physical symptoms—individuals with no symptoms are described as asymptomatic or as having subclinical herpes (Handsfield, 2000).

In many infections, the first symptom a person will have of their own infection is the horizontal transmission to a sexual partner or the vertical transmission of neonatal herpes to a newborn at term. Since most asymptomatic individuals are unaware of their infection, they are considered at high risk for spreading HSV. Many studies have been performed around the world to estimate the numbers of individuals infected with HSV-1 and HSV-2 by determining if they have developed antibodies against either viral species (Smith and Robinson, 2002). This information provides population prevalence of HSV viral infections in individuals with or without active disease. It has to be remembered that there are population subgroups that are more vulnerable for HSV infections, such as cancer chemotherapy patients (Elad *et al.,* 2010).

In Europe large differences in HSV-1 seroprevalence are seen in different European countries. HSV-1 seroprevalence is high in Bulgaria (83.9%) and The Czech Republic (80.6%), and lower in Belgium (67.4%), The Netherlands (56.7%), and Finland (52.4%) (Pebody *et al.,* 2004).

The typical age at which HSV-1 infection is acquired ranges from 5 to 9 years in Central and Eastern European countries like Bulgaria and the Czech Republic, to over 25 years of age in Northern European countries such as Finland, The Netherlands, Germany, and England and Wales. Young adults in

Northern European countries are less likely to be infected with HSV-1. European women are more likely to be HSV-1 seropositive than men (Pebody *et al.,* 2004).

HSV-2 seropositivity is widely distributed in Europeans older than 12, although there are large differences in the percentage of the population exposed to HSV-2. Bulgaria has a high (23.9%) HSV-2 seroprevalence relative to other European countries: Germany (13.9%), Finland (13.4%), Belgium (11.1%), The Netherlands (8.8%), the Czech Republic (6.0%), and England and Wales (4.2%) (Pebody *et al*., 2004).

Women are more likely to be seropositive than men, and likely acquire the virus at an earlier age. In each country of Europe, HSV-2 seropositivity becomes more common from adolescence onwards and increases in the population with age, with a decline in the older age groups in some countries (Pebody *et al.,* 2004). HSV-2 seroprevalence generally was greater among those with more lifetime sex partners (Xu *et al*., 2010).

The most recent data for HSV-2 was published in March 2010, based on a National Health and Nutrition Examination Survey study performed between 2005 and 2008 by CDC. In the United States about 1 in 5 Americans (16.2%) aged 14 to 49 is infected with HSV-2. HSV-2 prevalence was nearly twice as high among women (20.9%) than men (11.5%), and was more than three times higher among blacks (39.2%) than non-Hispanic whites (12.3%) (Xu *et al.,* 2010).

The most affected group was black women, with a prevalence rate of 48%. Prevalence increased with age and number of partners. Only 18.9% of those infected had previously been aware of their infection (Xu *et al*., 2010).

African Americans and immigrants from developing countries typically have an HSV-1 seroprevalance in their adolescent population that is two or three times higher than that of Caucasian Americans (Fatahzadeh and Schwartz, 2007). Many white Americans become sexually active while seronegative for HSV-1. The absence of antibodies from a prior oral HSV-1 infection leaves these individuals susceptible to herpes whitlow, herpes gladiatorium, and HSV-1 genital infection. Primary genital infection brings with it the risk of vertical transmission to the neonate, and is highest if the mother contracts a primary infection during the third trimester of pregnancy. In the U.S. the number of genital infections caused by HSV-1 is now thought to be about 50% of first episodes of genital infection (Mertz *et al*., 2003: Roberts *et al*., 2003).

In healthy adults, HSV-2 infection occurs more frequently in the United States than in Europe. Seroprevalence rates in the United States appeared to be increasing, rising from 16.4% in 1976 to 21.8% in 1994, (Malkin, 2004). However, this trend seems to have reversed itself in recent years, dropping to 17.2% in 2004 (Xu, 2006).

The current prevalence of genital herpes caused by HSV-2 in the U.S. is roughly one in four or five adults, with approximately 50 million people infected with genital herpes and an estimated 0.5 million new genital herpes

infections occurring each year (Fatahzadeh and Schwartz, 2007). African Americans appear more susceptible to HSV-2, although the presence of active genital symptoms is more likely in Caucasian Americans. The largest increase in HSV-2 acquisition during the past few years is in white adolescents. People with many lifetime sexual partners and those who are sexually active from a young age are also at higher-risk for the transmission of HSV-2 in the U.S (Retrieved, 2007, CDC, 2007, STD Facts, 2007).

Women are at higher risk than men for acquiring HSV-2 infection, and the chance of being infected increases with age (Fatahzadeh and Schwartz, 2007). The CDC reports that 48% of African American women in the United States are infected with the HSV-2 virus (CDC, 2011).

According to a study in Ontario in Canada of people between the ages of 15 to 16, 26.9% of men, 32% of non-pregnant women, and 55% of pregnant women tested positive for HSV-1 antibodies. Between the ages of 40 to 44, 54.7% of men, 88.7% of women, and 69.2% of pregnant women tested positive for HSV-1 antibodies. The overall age-gender standardized seroprevelance for HSV-1 antibodies was 51.1 % (Howard *et al,* 2003). Teenagers are less likely to be seropositive for HSV-2—antibodies against this virus are only found in 0–3.8% of 15- and 16-year-olds. However, 21% of individuals in their early forties have antibodies against HSV-2, reflecting the sexually transmitted nature of this virus. When standardizing for age, HSV-2 seroprevalence in Ontario for individuals between the ages of 15 to 44 was 9.1%. The rate at

which Canadian people are infected with HSV-2 is much lower than the estimated levels of HSV-2 seroprevalence in people of a similar age range in the United States (Howard et *al.,* 2003).

HSV-2 seroprevalence in pregnant women between the ages of 15–44 in British and Columbia is similar, with 57% having antibodies for HSV-1 and 13% having antibodies for HSV-2 (Smith and Robison, 2002). In British Columbia in 1999, the seroprevalence of HSV-2 antibody in leftover serum submitted for antenatal testing revealed a prevalence of 17.3%, ranging from 7.1% in women 15–19 years old to 28.2% in those 40–44 years (Patrick *et al.,* 2001). In attendees at an Alberta sexually transmitted infection (STI) clinic in 1994 and 1995, the seroprevalence of HSV-1 and -2 in leftover sera was 56% and 19%, respectively (Singh *et al.,* 2005). In Nova Scotia, 58.1% of 1,790 HSV isolates from genital lesion cultures in women were HSV-1; in men, 36.7% of 468 isolates were HSV-1 (Forward and Lee, 2003).

In Sub-Saharan Africa HSV-2 is more common than in Europe or the North America. Up to 82% of women and 53% of men in Sub-Saharan Africa are seropositive for HSV-2. These are the highest levels of HSV-2 infection in the world, although exact levels vary from country to country in this continent (Weiss, 2004).

In most African countries, HSV-2 prevalence increases with age. However, age-associated decreases in HSV-2 seroprevalence has been observed for

women in Uganda and Zambia, and in men in Ethiopia, Benin, and Uganda (Smith and Robinson, 2002).

Genital herpes appears less common in Northern Africa compared to Sub-Saharan Africa. For example, only 26% of middle-aged women have antibodies for HSV-2 in Morocco (Patneik, *et al.,* 2007). Women are more likely to be infected with HSV-2 once they are over the age of 40 (Patneik *et al.,* 2007).

Children in Egypt with acute lymphoblastic leukemia are often infected with HSV from a young age—HSV-1 or HSV-2 antibodies are present in an estimated 54% of children under the age of 5, and 77% in children over 10 years of age (Loutfy *et al,.* 2006). Algerian children are also likely to acquire HSV-1 infection at a young age (under 6) and 81.25% of the population has antibodies to HSV-1 by the age of 15 (Meguenni *et al,.* 1990)**.**

HSV-2 seroprevalency is high in Central and South America. Infection levels are estimated at 20% to 60%, relative to the rates in Europe and North America (Weiss, 2004). During the mid-1980s, HSV-2 prevalence was 33% in 25- to 29-year-old women and 45% in those aged 40 and over in Costa Rica. In the early 1990s HSV-2 prevalence was approximately 45% among women over 60 in Mexico.

The highest HSV-2 prevalence in Central or South America—60%—has been found in Colombian middle-aged women, although similar HSV-2 prevalence has been observed in younger women in Haiti (54%). HSV-2 infects about

30% of women over 30 years old in Colombia, Costa Rica, Mexico, and Panama. HSV-2 antibodies were found in more than 41% of women of childbearing age in Brazil (Smith and Robinson, 2002).

However, no increase in seroprevalence was associated with age in women over 40 years old in Brazil—HSV-2 prevalence was estimated at 50% among women aged 40–49, 33% among women 50–59, and 42% among women over 60. Women in Brazil are more likely to acquire an HSV-2 infection if their male partners had history of anal sex and had many sexual partners in his lifetime (Patneik *et al.,* 2007). In Peru, HSV-2 prevalence is also high among women in their 30s but is lower in men, (Smith and Robinson, 2002).

In the Eastern and South East Asia**,** HSV-1 seroprevalence in some Asian countries is low, relative to other countries worldwide, with only 51% women in Thailand, and between 50–60% in Japan possessing the antibodies (Smith and Robinson, 2002, Patneik *et al.,* 2007), HSV-2 seroprevalence in developing Asian countries is comparable (10–30%) to that observed in North America and Northern Europe (Weiss, 2004). However, estimates of HSV-2 infectivity in Thailand are higher than observed in other Eastern Asian countries; total HSV-2 seroprevalence is approximately 37% in this country (Patneik *et al.,* 2007). HSV-2 seroprevalence is low in women in the Philippines (9%), although commencing activity while young is associated with an increase risk of acquiring HSV-2 infection; woman starting sexual activity by the time they reach 17 are seven times more likely to be HSV-2

seropositive than those starting sexual activity when over 21 (Smith *et al.,* 2001).

In South Korea, incidence of HSV-2 infection in those under the age of 20 is low, only 2.7% in men and 3.0% in women (Shin *et al.,* 2007). Seroprevalence levels increase in older South Koreans however, such that the population over 20 that has antibodies against HSV-2 is 21.7% of men and 28% of women (Shin *et al.,* 2007).

In India 33.3% of individuals are seropositive for HSV-1 and 16.6% are seropositive for HSV-2. Those with both HSV-1 and HSV-2 antibodies are estimated at 13.3% of the population. Indian men are more likely to be infected with HSV-2 than women, and increasing seroprevalence of this virus is associated with an increasing age (Kaur *et al*., 2005).

In Middle East, Turkey a High levels of HSV-1 (97%) and HSV-2 (42%) were found amongst pregnant women in the city of Erzurum in Eastern Anatolia Region, Turkey (Smith and Robinson, 2002). In Istanbul however, lower HSV-2 seroprevalence was observed; HSV-2 antibodies were found in 4.8% of sexually active adults, while HSV-1 antibodies were found in 85.3% (Dolar *et al.,* 2006). Only 5% of pregnant women were infected with HSV-2, and 98% were infected with HSV-1. Prevalence of these viruses was higher in sex workers of Istanbul, reaching levels of 99% and 60% for HSV-1 and HSV-2 prevalence respectively (Dolar *et al.,* 2006).

The prevalence of HSV-2 in Jordan was found to be 52.8% for men and 41.5% for women (Abuharfeil and Meqdam, 2000).While in Israel the seroprevalence of HSV-1 was found to be 59.8% in the general population and it increases with age in both genders but the adolescent seroprevalence has been declining as in most industrialized nations (Davidovici *et al.,* 2006). An estimated 9.2% of Israeli adults are infected with HSV-2. Infection of either HSV-1 or HSV-2 is higher in females; HSV-2 seroprevalence reaches 20.5% in females in their 40s. These values are similar to levels in HSV infection in Europe (Davidovici *et al.,* 2006).

Antibodies for HSV-1 or HSV-2 are also more likely to be found individuals born outside of Israel, and individuals residing in Jerusalem and Southern Israel; people of Jewish origin living in Israel are less likely to possess antibodies against herpes (Davidovici *et al.,* 2006).

Among pregnant women in Israel a small scale cross sectional study found the prevalence of HSV-2 infection was 13.3% and that of HSV-1 was 94.9%. The HSV-2 infection rate was 3-fold higher among immigrants from the former Soviet Union (27.5%) than among Israeli-born Jewish and Arab women (9%) (Dan, *et al.,* 2003). Approximately 78% of HSV-2 infections in Israel are asymptomatic, (Feldman, *et al.,* 2003). HSV-1 causes 66.3% of genital herpes in the Tel Aviv area (Samva *et al.,* 2003).

Syria— Genital herpes infection from HSV-2 is predicted to be low in Syria although HSV-1 levels are high. HSV-1 infections is common (95%) among

healthy Syrians over the age of 30, while HSV-2 prevalence is low in healthy individuals (0.15%), and persons infected with other sexually transmitted diseases (9.5%). High risk groups for acquiring HSV-2 in Syria, include prostitutes and bar girls; they have 34% and 20% seroprevalence respectively (Ibrahim *et al*., 2000).

In Australia, a study using data from 1999 revealed the seroprevalence of HSV-1 was at 76.5%, with significant differences associated with age, gender and Indigenous status, which were not specified. An estimated 12% of Australian adults were seropositive for HSV-2, with higher prevalence in women (16%) than in men (8%), (Cunningham *et al.,* 2006). Lsarger cities had higher HSV-2 seroprevalence (13%) than rural populations (9%). Higher prevalence was found in Indigenous Australians (18%) than non-Indigenous Australians (12%) (Cunningham *et al.,* 2006).

As in the U.S., HSV-1 is increasingly identified as the cause of genital herpes in Australians; HSV-1 was identified in the anogenital area of only 3% of the population in 1980, but had risen to 41% in 2001 and this was most common in females and persons under 25 (Haddow *et al*,. 2006). The number of genital herpes infections appears to be rising in New Zealand with three times more cases in 1993 compared to 1977 (Lyttle *et al.,* 1994). In this country, HSV-2 affects 60% more women than men of similar age (Smith and Robinson, 2002).

The spectrum of disease caused by HSV infections includes a wide variety of primary and recurrent infections in mucous membranes, skin tissue and the central Nervous system (CNS). HSV-1 and HSV-2 are usually acquired by different routes and affect different areas of the body, but the signs and symptoms that they cause overlap (Roizman, 1996). While HSV-2 is more frequently associated with genital infections, HSV-1 infections are best known for causing herpes labialis. Intra-oral lesions are indicative for primary HSV-1 infection, lip lesions are more characteristic for recurrent infection. Although most genital HSV infections are caused by HSV-2, an increasing proportion is attributable to HSV-1 (Whitley, 2001).

Most individuals contract HSV infection during the first decades of life. The prevalence of HSV -1 antibody varies from 45% to 88% in adult populations and is influenced by sex, geographic location, socio-economic status and age (Whitley and Roizman, 2001). Antibodies to HSV -2 are rarely found before the age of onset of sexual activity, but HSV-2 seroprevalence increases to 35-60% by age 60 (Nohmias *et al*, and Fleming 1990). This prevalence has increased dramatically since the late 70's and is highest among prostitutes (75%) and male homosexuals (83%) (Nohmias *et al.,* 1990). Seroprevalence of HSV-1 antibodies increase with age starting in childhood and correlate with socioeconomic status, race, and cultural group. By age 30 years, 50% of individuals in a high socioeconomic status and 80% in a lower socioeconomic status are seropositive. Antibodies to HSV-2 begin to emerge at puberty,

correlating with the degree of sexual activity. The lifetime seroprevalence can be 20%-80% (Fleming *et al.,* 1997).

Herpes simplex viruses are among the most ubiquitous of human infections. The frequency of HSV infection has been measured by testing various populations for the presence of antibody, as both virus and the immune response are thought to persist after infection for the life of the host. Worldwide, 90% of people have one or both viruses. HSV-1 is the more prevalent virus, with 65% of persons in the United States having antibodies to HSV-1 (Xu *et al*., 2002). The epidemiology in Europe is similar, with at least half of the population seropositive for HSV-1. In the developing world, HSV-1 is almost universal, and usually acquired from intimate contact with family in early childhood (Whitley, *et al*., 1998). After childhood, the HSV-1 prevalence rates increase minimally with age. Rates of HSV-1 infection are similar for men and women. In the United States, African-Americans and Asians have higher rates of HSV-1 infection than whites. The majority of infections are oral, although most are asymptomatic. Some data suggest that in developed countries, acquisition of HSV-1 is delayed from early childhood to adolescence or young adulthood (Hashido *et al*., 1999 and Mertz *et al*., 2003).

HSV-2 infections are markedly less frequent than HSV-1 infections, with 15%–80% of people in various populations infected the virus (Corey and Wald, 1999). The rates of infection vary with country as well as levels of sexual activity. In some countries, such as Spain and the Philippines, the HSV-2

prevalence hovers around 10%, increasing to 20%–30% range for most European countries and the United States (Enders *et al*., 1998; Varela *et al*., 2001; Smith *et al*., 2001 and Malkin, *et al*., 2002). Developing countries bear a much higher burden of HSV-2 infection, with many populations in Africa having >50% prevalence in the general population (Weiss *et al*., 2001). Because HSV-2 infections are transmitted almost exclusively during sexual activity, the risk of HSV-2 reflects a person's level of sexual activity and the number of partners, and background prevalence of infection in the community. In communities with relatively low rates of infection, the risk of HSV-2 infection reflects more closely to sexual activity of the person. However, in communities with high prevalence of infection, demographic rather than behavioral factors reflect HSV-2 risk more accurately (Rosenthal *et al*., 1997; Austin, *et al*., 1999; Sucato *et al*., 2001). Women have a greater risk of HSV-2 acquisition, reflecting both increased biologic susceptibility and pattern of relationships with older men, who are more likely to be HSV-2 seropositive. HSV-2 prevalence in the United States is higher among African-Americans than among whites and Asians (Fleming *et al*., 1997). As a result, there is great disparity in infection rates according to both gender and race. For example, for white women, the risk of HSV-2 increases from about 18% among those with 2–4 lifetime partners to 35% for those with 10 to 49 lifetime partners (Fleming *et al*., 1997). In contrast, for African-American women the risk increases steeply even with fewer partners, and exceeds 60% for women with more than

4 lifetime partners. For white men, the risk is ~10% among those who report 2 to 9 lifetime partners, and reaches 40% in those with >50 lifetime partners. Among African-American men, the risk rises from 35% in those with 2–4 lifetime partners to ~60% in those reporting >50 The increase in the frequency of HSV-2 antibodies starts in adolescence, reflecting the initiation of sexual activity, and levels off in the 40s, probably reflecting cessation of new partner acquisition (Blower and Boe, 1993). In the United States, most people acquire HSV-2 in their 20s with a mean age at presentation of 24 years. In contrast, in South Africa, girls acquire HSV-2 infection in adolescence and >60% are infected by the age of 21 (Chen *et al*., 2000).

Apart from these most common orofacial and genital infections, HSV can also cause a variety of other diseases. Other cutaneous HSV -1 lesions generally manifest as eczema herpeticum, but can also be more disseminated. Viral infections of the eye are usually caused by HSV-1, most commonly presenting as conjunctivitis, blepharitis or herpetic epithelial keratitis (HEK) (Dorangar *et al.,* 1985; Leisong *et al.,* 1989). More severe cases of corneal HSV infections can also lead to potentially blinding diseases like herpetic stromal keratitis (HSK). A more detailed description of ocular HSV infections is given in the section below. Infection of the CNS can result in herpetic encephalitis, which is one of the most devastating manifestations of HSV infections. HSV encephalitis causes case fatality rates that exceed 70% among older children and adults if left untreated, and only a small minority regains normal

neurological function. HSV infections can also cause serious complications in neonates, frequently associated with encephalitis or disseminated infection and a lethal outcome. The incidence of neonatal HSV seems to be directly related to seroprevalence of HSV-2 and is most commonly acquired when the mother experiences a primary genital infection during child birth (Whitley, 2001). Finally, another group of individuals at risk for severe HSV infections are immune compromised patients like organ transplant recipients and AIDS patients (Whitley, 1990). Because of their inability to mount an adequate immune response to the virus, frequently reactivating HSV can cause severe local or systemic infections in these individuals.

The advent of the HIV epidemic initially eclipsed HSV-2 as a viral sexually transmitted disease of importance, but recent data have increasingly showed multiple interactions between the two viral infections (Corey *et al*., 2004). The development of molecular diagnostics has revealed that HSV-2 is the most common etiologic agent of genital ulcers in the developed and developing world (Chen *et al*., 2000; Serwadda *et al*., 2003). Even in regions in which syphilis and chancroid have been historically considered responsible for most genital ulcerations, the use of PCR-based techniques has clearly shown a predominance of HSV (Beyrer *et al*., 1998, Morse *et al*., 1997). In almost all studies, and in all populations, having HSV-2 infection increases the risk of HIV acquisition (Wald and Link, 2002, Freeman *et al*., 2006). The mechanism probably involves HSV-2 induced skin or mucosal ulcerations, as well as

influx of CD_4^+ cells into the herpetic lesions, cells that provide receptor for entry of HIV (Koelle *et al*., 1994). As transmission is more difficult to study than acquisition, the role of HSV-2 in the transmission of HIV is less well defined (Cameron, *et al*., 1989). However, the biology also suggests that HSV-2 infection may amplify HIV transmission, as HIV virion has been demonstrated in herpes ulcers (Schacker *et al*., 1998; Ballard, 2001). This topic of HSV and HIV interactions has been recently reviewed (Corey *et al*., 2000).

Nowadays, most HSV infections can be efficiently counteracted by treatment with aciclovir, a synthetic purine-nucleoside analogue that disrupts viral DNA replication. It is the most effective clinically used antiviral drug to date and has become the standard of therapy for HSV infections (Whitley, 2010).

2.6 Transmission of HSV

Transmission of HSV usually occurs by close contact via the mucosal surfaces. The type and severity of HSV infection that results depends on the host's immune status. Primary HSV infections are commonly subclinical. Most episodes of clinical disease are manifestations of recurrent HSV infections. Although many HSV infections are symptomatic, around one-third of the world's population has recurrent HSV infections, capable of transmitting HSV during episodes of productive viral replication. Viral shedding and thus transmission can however also occur without clinical symptoms, which particularly hold for genital infections.

Transmission of herpes viruses through organ transplantation poses a serious health problem. The most frequent cause of herpes virus transmission through organ transplantation involves CMV. Although probably rare, transmission of HSV through organ transplantation may also occur (Dumer *et al.,* 1987, Gottesdiener, 1989). HSV appears to be the most common infectious cause of blindness in developed countries (Leisong *et al.,* 1989) largely because of its recurrent nature. Ocular HSV infections are mainly confined to the anterior segment of the eye, including the conjunctiva and the eyelids. The overall prevalence of ocular HSV infections has been estimated at 149 cases per 100.000 person-years (Leisong *et al*., 1987). Ocular manifestations are only observed in about I% of exposed individuals, and only 5% of ocular HSV disease represent primary infections. Despite the relative rarity of ocular HSV disease, its outcome can have a rather profound morbidity. Primary ocular HSV infections usually result in mild-to-moderate diseases like conjunctivitis, blepharitis and HEK. However, recurrent manifestations of ocular infections can result in more serious disease like herpetic stromal keratitis (HSK). Recurrence rates of ocular HSV infections have been recorded in one-third up to 63% of patients and were more frequent in children adults (Shuster *et al.,* 1981; Leisegang *et al.,* 1987; Wishart, 1987).

2.7 Host Immune Responses to HSV

Many viruses and their hosts have coexisted for millennia during which both have been constantly adapting. Despite effective and rapid responses of the

immune system, herpes viruses are still common infectious agents in all groups of vertebrates. Acute and chronic viral infections are a constant battle between viral replication and the immune response of the host. If the immune system reacts too slow or in an inadequate manner, the host may suffer severe morbidity or die. On the other hand, an effective immune response will ultimately eradicate the virus or at least limit its replication. Despite the development of a usually highly effective immune system, some tissues of the host like the eye are poorly accessible to the immune system. These tissues are termed immune privileged sites.

Moreover, throughout evolution herpes viruses have adapted several mechanisms to escape from immune detection. Nevertheless, effective immune responses do develop upon viral infection of the eye following HSV infection; the host's immune system will basically combat the virus by two mechanisms. At first, a non-specific immune response (i.e. innate immunity) develops, Orchestrated by macrophages, natural killer (NK) cells and polymorphmononuclear cells (PMN) which are directed towards the site of infection. Innate responses start almost immediately after viral infection and provide a crucial first line of defense, especially in infection of naive hosts. Clearing of the virus however depends on a virus-specific immune response (i.e. adaptive immunity) in which both T- and B-lymphocytes are involved and takes several days to develop.

2.7.1 Innate Immunity

Leukocyte populations, excluding the classical T- and CD 8 T-lymphocytes are widely distributed in the body and are critical in controlling the overall extent of viral replication and to limit the spread of virus immediately after infection. Macro phages exert their antiviral function directly through phagocytosis of viral particles and subsequent degradation, and through the secretion of anti-viral cytokines like TNF-a and IFN-a. In an indirect manner, macrophages can mediate a number of important immune-regulatory effects through the secretion IL-12, TNF-a and IL-la (Ahmed, 1999). The importance of NK cells in early defense against HSV infection has been clearly established (Habu *et al.,* 1984). Severe herpes virus infections occur in individuals with genetic defects in NK function and in immunocompromised individuals (Biron *et al.,* 1989, Biron *et al.,* 1999). In HSV infection, NK cell-derived IFN-y exert an anti-viral effect through inhibition of viral replication (Lucin, 1994). Virus-infected cells can be recognized and actively killed by NK cells through opsonization by virus-specific antibodies following interaction with Fc receptors, and through a MHC-independent recognition of viral antigen (Fitzgerald-Bocarsly *et al.,* 1991) Infection with HSV also results in a down regulation of MHC class I expression (Hill *et al.,* 1995) making infected cells more susceptible for killing by NK cells (Lanier, 1997). Soluble factors like cytokines and chemokine are the non-cellular components of the innate immune system. All cell types of both the innate and adaptive immune system

contribute to the production of these factors, as well as some virus-infected or activated non immune cells such as fibroblasts. For example type I IFNs (IFN-a and IFN-b) are produced by the infected cell itself to induce protection against infection in neighboring cells. The functions of these cytokines in anti-viral immunity include inhibition of viral replication, activation of NK cell cytotoxic activity and induction of MHC class I expression (Ahmed, 1999, Biron, 2001). Besides cytokines, molecular chemotactic factors known as chemokine are produced upon viral infection by a variety of both immune and non-immune cells. These chemokine have important pro-inflammatory functions and contribute to innate immunity by attracting leukocytes to infected tissue (Schall, 1994, Ward, 1998). Overall, innate immunity functions not only to protect the host in the early stages of infection, but also to direct the qualitative and quantitative nature of adaptive immunity.

2.7.2 Adaptive Immunity

The humoral and cellular adaptive immune responses mediated by antibodies and cells, respectively, are the two main antigen-specific effector systems for resolving viral infections (Ahmed, 1999). Antibodies control virus replication by neutralising free viral particles or killing virus-infected cells through complement-mediated cytotoxicity or antibody-dependent cell-mediated cytotoxicity (ADCC). This mechanism of ADCC has been validated as a critical component of antiviral defense against HSV infections, especially in immunocompromised individuals and neonates (Kohl, 1991).

The major targets for antibodies are the surface glycoproteins and outer capsid proteins of HSV (Glorisco *et al.*, 1984, Para *et al.*, 1985). Binding of neutralising antibodies to glycoproteins prevents viral attachment and infection of host cells. Although the protective role of antibodies in HSV infection is not completely clear, they seem to exert a major effect during primary infection. Especially secretory immunoglobulins of the IgA isotype may be important in neutralising HSV particles that enter via the mucous membranes. However, high titres of neutralizing antibodies do not always prevent or diminish recurrent HSV infections. Antibodies serve as a major defence against free viral particles, while the main function of T cells is the recognition and elimination of virus-infected cells. T cells can be subdivided into two subsets, CD_4^+ and CD_8^+ T cells, which recognise viral antigen in association with host major histocompatibility complex (MHC) molecules II and I, respectively. In HSV -infected cells, viral proteins are degraded into short peptides that subsequently bind to MHC molecules. These MHC/peptide-complexes are then translocated to the cell surface for recognition by antigen-specific T cells (Ahmed, 1999). Both CD4' and CD_8^+T cells play a central role in antiviral immunity either directly by their cytolytic properties or indirectly by their secretion of cytokines upon activation or in case of CD_4^+ T cells by the induction of a specific humoral immune response. Remarkably, in HSV infections in humans, CD_4^+T cells have emerged as the predominant killer cell phenotype. Severe HSV infections are common in patients with impaired T

cell immunity (e.g. AIDS patients or transplant recipients), indicating that T cells play an essential role in controlling HSV infections (Schmid and Rouse 1992) For HSV infections, both clinical observations in humans and studies performed in experimental animal models have clearly indicated the importance of CD_8^+ cytotoxic T cells (CTL) in the resolution of HSV disease. Likewise, CD_4^+ T cells probably play an equally critical role in the resolution of HSV, given the importance of this cell type in mediating delayed type hypersensitivity responses and clearing local infections (Schmid, 1991). Similarly, in several mouse models of HSV infections, both CD_4^+ and CD_8^+ subsets of T lymphocytes have been shown to be important in protection against lethal infection and to enhance viral clearance (Lubinski, 1991).

2.7.3 Immune Evasive Mechanisms of HSV

The virus has developed various strategies to evade host immune surveillance and persist *in vivo.* This capability of this virus to circumvent defensive mechanisms of the immune system enables it to avoid detection and elimination by the immune system. One key adaptation of the virus to do this is to infect the Central Nervous System, a tissue that is not readily accessible to the immune system, and to establish latency within ganglion cells. The fact that during a latent infection no or very limited viral protein expression takes place and because of a lack of MHC class I expression on neural cells makes the virus impassive for T cell mediated recognition. During active viral replication however, HSV also has several "tricks" to evade the immune

system during lytic infection the HSV ICP47 protein, encoded by the US12 gene, interferes with the transporter associated with antigen presentation (TAP) (Hill *et al*., 1995). Through this action peptide translocation into the ER is blocked resulting in inhibition of viral antigen presentation through MHC class I and recognition by CD_4^+T cells. Additionally, HSV has recently also been shown to inhibit recognition of infected cells by CD_4^+T cells (Barcy *et al.,* 2001*)* showed that HSV -infected B cells exhibited a strongly reduced capacity to stimulate antigen-specific CD_4^+T cells (Barcy and Corey 2001) Although its mode of action is not yet known, this inhibitory effect on APC function is most likely mediated by the HSV ICP22 protein, encoded by the US 1 gene. Another strategy of HSV to evade immune responses involves interference with complement-mediated virus neutralization and lysis of infected cells through binding of complement component C3 by gC. (Lubiniski*, et al.,* 1998). When a host cell gets infected by a virus it will usually go into apoptosis. Alternatively, infected cells can be induced by T cells to enter the apoptotic phase. To be able to complete viral replication and produce progeny viral particles, HSV like many other viruses has adapted methods to inhibit this suicidal tendency of cells (Jerome *et al.,* 2001). On the other hand, HSV infection of T cells results in elimination of virus-specific T cells by inducing apoptosis of neighboring T cells in a F as-dependent fashion. This of immune evasion is referred to as fratricide (Raftery *et al.,* 1999).

2.8 Diagnosis

Primary genital herpes infection in pregnancy is particularly of primary concern as it can result in spontaneous abortion, low birth weight, premature delivery, and neonatal herpes, which itself can present with localized infection, encephalitis, and/or disseminated infection. Approximately 30% of HSV-1 and 50% of HSV-2 neonatal infections result in death or serious residual disability (Kesson, 2001). Antiviral chemotherapeutic agents against HSV are available (Wald, 2001; CDC, 2002,), therefore timely and accurate diagnostic methods are important.

Unfortunately, the diagnosis of genital herpes based on clinical and physical examination is both insensitive and non-specific (Koutsk*et al.,* 1992 ; CDC, 2002). Most neonatal infections result from asymptomatic shedding from mothers who have acquired genital herpes in late pregnancy (Brown *et al.,* 1997, Eskild *et al.,* 2000). The challenge is to identify those mothers at risk and then apply interventions to reduce that risk. Recurrence rates for genital HSV-1 infections are also much lower than genital HSV-2 infections (Benedetti, *et al.,* 1999). It also appears that HSV-2 infection may be a significant risk factor for HIV acquisition (Wald *et al.,* 2002). For these reasons laboratory testing to not only confirm the diagnosis, but also differentiate between HSV-1 and HSV-2 infections, could play an increasingly important role in the proper management, prognosis, and counseling of patients.

Genital herpes can be diagnosed by clinicians if typical papular lesions progress to vesicles and ulcers are present. Although the features in patients can be highly variable, a definitive diagnosis should be confirmed with laboratory findings (Patel *et al.,* 2010). Laboratory-confirmed HSV diagnosis is important for appropriate treatment selection, and type identification gives important information about disease prognosis. Differentiating between HSV-1 and HSV-2 is possible only with laboratory diagnosis, and laboratory confirmation is also necessary to exclude other possible causes of ulcers in the genital area (Gupta *et al.,* 2007). With regard to different guidelines for laboratory diagnosis of genital herpes, for patients with symptoms, detection of viral DNA by nucleic acid amplification tests (NAAT) is most appropriate. Virus isolation on cell cultures can also be performed (Patel *et al.,* 2010).

In the absence of symptoms, swab testing for preventing HSV transmission is not appropriate because viral shedding is intermittent (Patel *et al.,* 2010, Domeika *et al.,* 2010, Aderson *et al.,* 2014).

2.8.1 Specimen Collection and Transportation

The sample of choice is a swab sample of active herpetic lesions. Before sampling, necrotic tissue should be removed. Typically swabs are taken from the base of lesion (Singh *et al.,* 2005, Belec, 2006). Vesicle fluid aspiration is taken and transported in a sterile syringe; if disseminated infection is suspected, blood for PCR should be taken with anticoagulant (EDTA). Other samples that may occasionally be tested are swabs of the cervix, urethra, or vagina (Belec,

2006). The blood sample for serological methods should be taken in a tube without anticoagulant.

2.8.2 Methods for direct Detection of the Virus

For direct HSV detection, available tests include antigen detection by direct immunofluorescence, nucleic acid amplification tests (NAATs) for viral DNA detection, and viral culture (Singh*, et al.,* 2005). NAATs are currently the most sensitive and specific methods. Among them, real-time PCR is recommended as a preferred diagnostic method (van Doornum, *et al.,* 2003). With high sensitivity and specificity, real-time PCR offers accurate diagnosis to a clinician. It allows both typing of HSV and quantification of viral load (in fluid samples). The risk of false positive results occurring due to sample contamination before amplification should be prevented with carefully planned procedures in the molecular laboratory (Singh *et al.,* 2005). Regarding transport and storage of samples, real-time PCR can tolerate less stringent conditions than samples for viral isolation (Belec, 2006). The important advantage of molecular methods is the possibility of detection of asymptomatic HSV shedding, but one should be aware that a negative PCR result does not exclude HSV infection because virus shedding is intermittent (Belec, 2006).

HSV antigen detection may be a suitable alternative for smaller laboratories as an alternative to viral culture or PCR when fresh lesions are present and the swab is of high quality (rich in cells). The appropriate transport conditions are

less stringent than for viral culture. Demonstration of antigen with direct immunofluorescence assay (DIF) is rapid, but with lower sensitivity in comparison to PCR, and is appropriate only for patients with fresh lesions (Singh *et al.,* 2005, Belec, 2006).Viral culture as a gold standard of laboratory HSV detection is possible only in highly specialized laboratories. Low-cost and well-established methodology supports the use of viral culture. This is a highly specific method, but it has low sensitivity, and appropriate sample handling with a continuous cold chain of sample transport to preserve virus viability is very important. Sensitivity is even lower for recurrences, which is why successful viral culture is mostly successful from fresh lesions in primary HSV infection (Belec, 2006).

2.8.3 Antibody detection methods

The detection of antibodies to HSV is recommended as an aid to diagnosing genital herpes and is particularly useful in identifying an asymptomatic carrier of HSV infection (Singh *et al.,* 2005). A defining characteristic of HSV infections is a slow antibody response. There are many tests available to detect HSV antibodies, and the majority of newer tests can now differentiate between types of HSV. Serological assays that are not type-specific are of no value in managing genital herpes (Wald, 2003). Type-specific HSV IgG antibodies usually become detectable within 2 weeks to 3 months after the initial infection (Belec, 2006, Patel *et al.,* 2010).

Serological testing is useful in the following ways;

- As an aid in diagnosing genital herpes infection, especially to differentiate between primary and recurrent infection;
- In patients with a history of recurrent or atypical lesions, or in patients with healing lesions with negative direct methods;
- For managing sexual partners of people with genital herpes when a risk of transmission exists;
- For identifying HSV infection in high-risk groups, although testing people in high-risk groups is not routinely recommended (Belec, 2006, Patel *et al.,* 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This research work was carried out in Kogi State, with the state capital at Lokoja. The state is a heterogeneous state with a population of 3,314,045 million people as of 2006 population census. It has many ethnic groups comprises Christians and Muslims majorly. It is a central state in Nigeria, to the North, it is bordered by Federal Capital (FCT), to the North – West by Kwara State and Niger State and to the South – West by Ondo State, to the South by Edo State and to the South – East by Anambra State and to the East by Enugu State and Benue State. It has three senatorial district, Kogi West, Kogi Central and Kogi East senatorial district. This study was conducted in selected referral hospitals in each of the three senatorial districts and one hospital at the state capital; they are General Hospital Idah, General Hospital Okene, General Hospital Kabba and Specialist Hospital Lokoja respectively.

3.1.1 Inclusion Criteria

This includes all consenting women of all categories within the age range of 15 – 49 years, who are attendees of the selected hospitals.

3.1.2 Exclusion Criteria

Women who were below 15 years and above 49 years of age and those within the stipulated age range, who did not give their consent were not included in the study.

3.2 Ethical Approval

The ethical approval was received from the State Ministry of Health, Kogi State with reference number MOH/KGS/1376/83.

3.3 Study Population

The study population includes women of child bearing/reproductive age that were between 15 – 49 years old, attending these selected hospitals irrespective of their educational status and place of residence.

3.4 Sample Size Determination

The sample size was determined by Naing equation (Naing *et al.,* 2006) and a reported 44.3% prevalence of HSV-2 infection among pregnant women attending a choice hospitals in Benin, Edo State, Nigeria (Kalu, 2013) at 95% confidence.

$$n = \frac{Z^2 Pq}{d^2}$$

Where n = number of samples

P = prevalence rate of previous study = 44.3%

Z = standard normal distribution at 95% confidence limit = 1.96

d = absolute desired precision of 5% = 0.05

q = 1 – p = 1 – 0.443 = 0.557

$$\text{n} = \frac{(1.96)^2 \times 0.443 \times 0.557}{(0.05)^2}$$

= 379.17

3.5 Sample Collection

A total of 5ml of blood sample were collected from 336 participants and 2ml was dispensed into a labeled sterile EDTA bottle for CD_4^+ count and 3ml of the blood was dispensed into a plain tube. The blood was allowed to clot and centrifuged and a clear serum was obtained using a separate sterile pasture pipette into another labeled sterile plain tube with cap. The CD_4^+ count was done the same day of sample collection while the serum was refrigerated at –2℃ until assay was done.

3.6 Methods

3.6.1. Detection of HSV-1 IgG by Elisa Technique

HSV-1 IgG was determined using ELISA TEST KIT (HSV-1 IgG ELISA BY CALBIOTECH. 1935, Cordel CT., El Cajon, CA92020 USA. www.calbiotech.com).

3.6.1.1 The Test Principle

Diluted client serum was added to wells coated with purified antigen. 1gG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody/antigen complex if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the colour generated is proportional to the amount of 1gG specific antibody in the sample.

3.6.1.2 Preparation of Reagent (1x wash buffer)

Wash buffer (25ml, 20x) was added to 475ml of deionized water and it is stored at room temperature.

3.6.1.3 Assay Procedure

All the reagents and specimens were removed from the refrigerator and brought to room temperature before use.

- Negative control, positive control and calibrator were ready to be used, the test sample was diluted to 1:21 dilution this was done by adding 10µµl of the sample to 200µl of the sample diluents and it was mixed well.
- One hundred micro liter of diluted sera, calibrator, controls were dispensed into appropriate well i.e. the reagent blank on the first well at 1A rows, then the calibrator into 3 wells, negative control into 2 wells and the positive control into the remaining 2 well on that row. After the sample were dispensed into the remaining wells appropriately using a disposable pipette tips. It was mixed well and then incubated for 20 minutes. After the expiration of incubation time the liquid was removed from the well and it was washed well with three changes of 300µl of 1x wash buffer. It was blotted on paper towel.
- One hundred micro liter of enzyme conjugate was added to each well and then incubated for 20 minutes at room temperature. The enzyme

conjugate was removed and washed well with three changes of 300µl of wash buffer and then blotted off on paper towel.

- One hundred micro liter of TMB substrate was added to the wells and it was incubated at room temperature for 10 minutes
- One hundred micro liter of stop solution was added
- Then OD of the plate was read at 450nm using Elisa reader machine.

The calibration factor for HSV-1is 0.55.

The results were obtained by calculating the cut-off value which is calculated by multiplying the OD of the calibrator and calibrator factor on the kit (CF).

Then to calculate the antibody index of each determination, the OD value of each sample was divided by the cut off value.

3.6.2 Detection of HSV-2 IgG by Elisa Technique

HSV-2 IgG was determined using ELISA TEST KIT (HSV-1 IgG ELISA BY CALBIOTECH. 1935, Cordel CT., El Cajon, CA92020 USA. www.calbiotech.com).

3.6.2.1 The Test Principle

Diluted client serum was added to wells coated with purified antigen. 1gG specific antibody, it present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody/antigen complex if present. Excess enzyme conjugate is off and substrate is added. The plate is incubated to allow the hydrolysis of the

substrate by the enzyme. The intensity of the colour generated is proportional to the amount of 1gG specific antibody in the sample.

3.6.2.2 Assay Procedure

All the reagents and specimen were removed from the refrigerator and brought to room temperature before use.

- Negative control, positive control and calibrator are ready to use, the test sample was diluted to 1:21 dilution this was done by adding 10µl of the sample to 200µl of the sample diluents and it was mixed well.
- One hundred micro liter of diluted sera, calibrator, and controls were dispensed into appropriate well i.e the reagent blank on the first well at 1A rows, then the calibrator into 3 wells, negative control into 2 wells and the positive control into the remaining 2 well on that row. After the sample were dispensed into the remaining wells appropriately using a disposable pipette tips. It was mixed well and then incubated for 20 minutes. After the expiration of incubation time the liquid was removed from the well and it was washed well with three changes of 300µl of 1x wash buffer. It was blotted on paper towel.
- One hundred micro liter of enzyme conjugate was added to each well and then incubated for 20 minutes at room temperature. The enzyme conjugate was removed and washed well with three changes of 300µl of washed buffer and then blotted of on paper towel.

- One hundred micro liter of TMB substrate was added to the well and it was incubated at room temperature for 10 minutes
- One hundred micro liter of stop solution was added
- Then OD of the plate was read at 450nm using Elisa reader machine.

The calibration factor for HSV-2 is 0.5.

The results were obtained by calculating the cut-off value which is calculated by multiplying the OD of the calibrator and calibrator factor on the kit (CF).

Then to calculate the antibody index of each determination, the OD value of each sample was divided by the cut off value.

3.6.3 Determination of CD_4^+Cell Count by Flowcytometry

CD_4^+ count was carried out using Flowcytometry technique by Sysmex Partec GmbH (Am Flugplatz 13, 02828 Görlitz, Germany).

3.6.3.1 Principle of the Test

When cells labeled by CD_4^+mAb PE are passed by a light, the fluorescent molecules are excited to a high energy state. Upon returning to their resting state, each with similar excitation wavelength and different emission wavelength are measured, sorted and counted.

3.6.3.2 Assay Procedure

The blood sample alone was placed on a roller mixer for 15minutes for proper mixing. Then 20µl of CD_4^+ easy count-CD_4^+mAb PE was pipetted into a partec tube and 20µl of blood sample was added to it in the test tube. The tube was incubated in the dark at room temperature for 15 minutes. 800µl of CD_4^+ easy

count was added to the tube and inserted into the sample suction port. This was then run immediately by clicking the start button on the monitor.

The result was read as cells/µl and displayed in digital form on the monitor (computer window) attached to the equipment and it was printed out.

3.7 Statistical Analysis

Descriptive data was expressed as mean ± standard deviation for continuous variables and percentages for categorical variables. Multinomial Logistic regression modeling was used to identify socio-demographic and behavioral risk factors that can independently predict HSV-1 and HSV-2 infections.Test of significance was set at $p < 0.05$. All statistics were done using SPSS/IBM software, version 20.

CHAPTER FOUR

RESULTS

4.1 Result Analysis

The seroprevalence of IgG-type specific antibody to HSV-1 and HSV- 2 infections among the 330 serum sample collected was 94.8% and 77% respectively. (Table 4.1).

For the age–related seroprevalence of IgG-type specific antibody to HSV-1 and HSV- 2 infections was 73.7% and 79.9% were obtained in the age group of 15 – 25 years respectively. In the age group of 26 – 35 years, the seroprevalence was 100% for HSV-1 and 78.4% for HSV-2 infections while, in the age group of 36 – 45 years the seroprevalence was 98.2% for HSV-1 and 82.5% for HSV-2 infections. (Table 4.2).

The seroprevalence according to marital status of IgG-type specific antibody of HSV-1 and HSV-2 infections was 99.2% for married women and 86.9% for single (unmarried women). (Table 4.3).

Table 4.1: Prevalence of HSV-1 and HSV-2 infection among apparently healthy women of childbearing age in Kogi State, Nigeria.

Type of infection	**No. screened**	**No. positive**	**% positive**
HSV-1	330	313	94.8
HSV-2	330	254	77.0
Total	**660**	**567**	**89.9**

Table 4.2: Age distribution for HSV-1 and HSV-2 infection among apparently healthy women of child bearing age in Kogi State.

Age group (years)	HSV-1		HSV-2	
	No. of sample	**No. (%) positive**	**No. of sample**	**No. (%) Positive**
15-25	176	165 (93.3)	176	13 (74.4)
26-35	97	97 (100)	97	76 (78.4)
36-45	57	56 (98.2)	57	47 (82.5)
Total	**330**	**318 (96.4)**	**330**	**254 (77)**

HSV-1 Vs Age Group P = 0.0216

HSV-2 Vs Age Group P = 0.4249

Table 4.3: Marital Status distribution for HSV-1 and HSV-2 infection among apparently health women of childbearing age in Kogi State, Nigeria.

	HSV-1		HSV-2	
Marital Status	**No. of sampled**	**No. of (%) positive**	**No. of sampled**	**No. of (%) positive**
Married	130	129(99.2)	130	113 (86.9)
Single	200	189 (94.5)	200	141 (70.5)
Total	**330**	**318(99.4)**	**330**	**254(77)**

HSV-1 Vs Marital Status P = 0.0521

HSV-2 Vs Marital Status P = 0.0449

The seropositivity of IgG-type specific antibody for HSV-1 and HSV-2 infections in correlation to educational status was 96.2% for HSV-1 and 79.9% for HSV-2. While among the uneducated subject the seroprevalence was 66.7% for HSV-1 and 75% for HSV-2 infections. (Table 4.4).

According to sexual behaviour, the seropositivity of IgG-type specific antibody to HSV-1 and HSV-2 was 100% for HSV-1 and 78.9% for HSV-2 infections among subject who had sexual intercourse before the age of 15 years, while 98.4% and 80.7% were recorded for HSV-1 and HSV-2 infections respectively, for subject who had sex above the age of 15 years of age. (Table 4.5).

According to number of sexual partners, the seropositivity IgG-type specific antibody to Hsv-1 and HSV-2 with regard to number of sexual partners before married was 100% for those who had four (4) partners for HSV-1 and HVS-2 infections. Those who had three (3) partners, the prevalence was 100% for HSV-1 and 83.3% for HSV-2 infections. For two (2) sexual partners before marriage the prevalence of 100% for HSV-1 and 75% for HSV-2 infection, while that of one (1) sexual partners before marriage, the prevalence of 99% for HSV-1 and 78.6% was obtained. (Table 4.6).

Table 4.4: Prevalence for HSV-1 and HSV-2 infection according to educational status among apparently health women of childbearing age in Kogi State, Nigeria.

Educational status	**HSV-1**		**HSV-2**	
	No. of sampled	**No. of (%) positive**	**No. of sampled**	**No. of (%) positive**
Educated	318	306 (96.2)	318	254 (79.9)
Uneducated	12	8 (66.7)	12	12 (75)
Total	**330**	**314(95.2)**	**330**	**266(80.6)**

HSV-1 Vs Educational Status P = 0.000

HSV-2 Vs Educational Status P = 0.9629

Table 4.5: Prevalence of HSV-1 and HSV-2 infection among subject that had sexual intercourse below and above 15years of age among apparently health women of childbearing age in Kogi State, Nigeria.

Age in years	**HSV-1**		**HSV-2**	
	No. of samples	**No. (%) positive**	**No. of samples**	**No. (%) positive**
< 15	19	19(100)	19	15(78.9)
>15	195	189(98.4)	192	153(80.7)
Total	**214**	**208(97.2)**	**211**	**168(79.6)**

Table 4.6: Prevalence of HSV-1and HSV-2 infection according to number of sexual partners before marriage among apparently health women of childbearing age in Kogi State Nigeria.

No of sexual partner	**No. of sample**	**No. of HSV-1 (%) positive**	**No. of HSV-2 (%) positive**
4	1	1(100)	1(100)
3	6	6(100)	5(83.3)
2	20	20(100)	15(75)
1	103	102(99)	81(78.6)
Total	**130**	**129(99.2)**	**102(78.5)**

The seropositivity of IgG-type specific antibody to HSV-1 and HSV-2 in respect to Sexual Transmitted Disease (STD) and Genital Ulcer (GU) acquisition was 69% and 75.9% respectively. While that of Genital Ulcer (GU) was 79.3% and 86.2% respectively (Table 4.7).

The overall means of $CD4^+$ counts for both infected and uninfected with these infections was (793.6± 210.9). The mean $CD4^+$ count for HSV-1 sero positive women was 793.9± 234.9, while the seronegative woman was 759.75±210.94. For HSV-2 seronegative women, the mean $CD4^+$ count was 793.20 ±234.96 and HSV-2 seronegative women was 795.13±235.86. Furthermore the mean $CD4^+$ count were classified into categories according to the WHO as normal, low and high (Table 4.8).

The comparison between $CD4^+$ cell count of HSV-1 and HSV-2 seropositivity subject with those of HSV-1and HSV-2 seronegative subject of the study population (Table 4.9). The data showed that there was no significant difference in the $CD4^+$ count between subjects tested positive and those who tested negative for both HSV-1 and HSV-2 infection respectively.

Table 4.7: Prevalence of HSV-1 and HSV-2 infection among subject who indicated that they had Sexual Transmitted Diseases (STD) and Genital Ulcer (GU) among apparently health women of child bearing age in Kogi State, Nigeria.

Type of Infections	**No. who indicated they had STD**	**No. who indicated they had Genital Ulcer**
Sample	29	29
HSV-1 No (%) positive	20(69)	23(79.3)
HSV-2 No (%) positive	22(75.9)	25(86.2)

Table 4.8: Mean $Cd4^{+}$ counts among apparently health women of childbearing age in Kogi State, Nigeria.

	CD_4^+ COUNT CATEGORIES							
	Normal		**Low**		**High**		**Overall**	
Subjects	N/%	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
All Subject	257	732.6±129.3	16	404.5±92.5	57	1178.1±185.4	330	793.6±210.9
HSV +ve	274	743.0±127.8	16	404.5±92.5	55	1182.0±181.2	318	794.9±235.8
HSV1 –ve	10	698.0±167.7	0	-	2	1068.5±86.9	12	759.7±210.9
HSV2 +ve	198	734.4±125.6	13	403.8±94.6	43	1181.7±195.4	254	793.2±234.9
HSV 2 –ve	59	724.6±142.1	3	407.6±102.6	14	1166.7±156.7	76	795.1±235.8

SD = Standard Deviation
N = Number Tested

Table 4.9: Mean $CD4^+$ count of subjects according to the status of their infection

	Mean CD_4^+ Cell Count		
Infections	**Positive**	**Negative**	**P-Value**
HSV-1	794.93 ± 235.87	759.75 ± 210.94	0.611
HSV-2	793.20 ± 234.96	795.13 ± 235.86	0.950

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The overall prevalence of HSV (HSV-1 and HSV-2) in the studied population is 76.7%. Genital HSV-1 infection is a common infection in man (Sizemore *et al*., 2006). Herpes simplex virus type-1 (HSV-1) can cause chronic ulcerative infection in immunosuppressed children leading to latency with subsequent reactivation in the conjunctiva resulting in scarring, thickening of the cornea and blindness. They are also a common cause of fatal sporadic encephalitis in 70% of paediatric patient (Shaibu *et al*., 2014). It is mostly acquired in childhood and is responsible for orolabial ulcers or lesions. Prevalence of oro-facial herpes simplex virus type-1 infection is a common worldwide problem (Anejo-Okopi *et al*., 2016). Infection with Herpes Simplex Virus Type-2 (HSV-2) is the primary cause of genital herpes and the most common cause of genital ulcer disease (GUD) worldwide (Agabi *et al.,* 2010). There is little information on the prevalence of HSV-2 in Nigeria.

In this study, the seroprevalence to HSV-1 IgG-specific antibodies in apparently healthy women of child bearing age in Kogi state was 94.8 % (313/330) (Table 4.1). This result agrees with a previous study done in Benin City Nigeria by (Kalu *et al*., 2015), where he reported prevalence of 96.3% for HSV-1 .Also in Kumasi Ghana a prevalence of 99.2% (Debrah *et al*., 2018). Similar work in the following countries reported prevalences as; Bulgaria

(83.9%), Czech Republic (80.6%). Also in agreement with this high prevalence rate are the works done in the Middle East (Smith *et al*., 2004). In Turkey they found the prevalence of HSV-1 infection to be 97% among pregnant women in Erzurum in Eastern Anatolia Region. However, in Istabul, 85.3% was found to be positive for HSV-1 infection among sexually active adults. Prevalence of these viruses was higher in sex workers of lstabul, reaching levels of 99% and 60% for HSV 1/HSV 2 respectively. Ibrahim *et al.*, (2000) reported 95% level of HSV-1 positivity among the Syrians, while Cunningham, *et al.,* reported 76.5% prevalence of HSV-1 in Australia. However, low prevalence of HSV-1 was reported in Belgium (67.4%), Netherlands (56.7%), and Finland (52.4%) respectively. However this result does not agree with the seroprevalence of 61% of HSV1 obtained for women in a Turkish study carried out by İğde and İğde (2011). Pereira *et al*., (2012) in a similar study among 261 sexually active women resident in the metropolitan area of Natal, Brazil and attending a public clinic for cervical screening reported a seroprevalence of 88.2% which is a little lower than the 94.2% obtained for women of child bearing age. The higher seroprevalence obtained in this study is likely due to subclinical or asymptomatic shedding by contacts, and this has important public health implications, particularly for younger women since the transmission of genital HSV-1 from mother to neonate may be highly efficient (Peña *et al.,* 2010). With regard to additional HSV epidemiology issues, it is also unknown how many of the HSV-positive

samples represent primary infections, reactivating clinical recurrences, or asymptomatic shedding. Previous analyses of the prevalence of HSV-1 may be limited by their focus on individuals with genital HSV disease or other symptoms. The significance of our study is that there may be a new silent epidemic under way, which is likely being driven by reduced sociological sensitivities regarding what constitutes sexual activity, particularly in the younger population.

The seroprevalence of HSV-2 obtained in this study was 77% (254/330) (Table 4.1). This result is in the same range with 78.4% seropositivity for HSV-2 observed in women in Ghana (Debrah *et al*., 2018), in a similar study by Pena *et al*., (2010) in the US. This observation represents a measurement of sub-clinical shedding. Individuals with genital HSV-2 infections display more frequent recurrences than those with genital HSV-1 infections. This result is much higher than11% global sero-prevalence rate for HSV-2 in an estimated 417 million people aged 15-49 worldwide (WHO, 2017). HSV-2 infection is widespread throughout the world and is almost exclusively sexually transmitted, causing genital herpes (WHO, 2017), thus the higher prevalent rate obtained in this study is likely a reflection of higher sexual transmission in the study area and lack of awareness. Recurrent symptoms of genital herpes may be painful and the infection can lead to social stigma and psychological distress, with an important impact on quality of life and sexual relationships. Neonatal herpes can occur when an infant is exposed to HSV in the genital

tract during delivery. This happens in an estimated 10 out of every 100,000 births globally, leading to lasting neurologic disability or death. The risk for neonatal herpes is greatest when a mother acquires HSV infection for the first time in late pregnancy. Women who have genital herpes before they become pregnant are at very low risk of transmitting HSV to their infants. HSV-2 and HIV have been shown to influence each other. HSV-2 infection increases the risk of acquiring a new HIV infection by approximately three-fold. In addition, people with both HIV and HSV-2 infection are more likely to spread HIV to others. HSV-2 is a common infections in 60-90% of people living with HIV (and other immunocompromised individuals), resulting in a more severe presentation and more frequent recurrences. This can lead to more serious, but rare, complications such as meningoencephalitis, esophagitis, hepatitis, pneumonitis, retinal necrosis, or disseminated infection (WHO, 2017).

The seroprevalence of HSV-1 obtained in this study was found to be associated with age ($p = 0.0216$) (Table 4.2). This finding is in agreement with the findings of Anejo-Okopi, *et al*., (2016) who reported that age had a significant association with HSV-1 infection in a similar study on herpes simplex virus in Jos, Plateau State, Nigeria. The prevalence of HSV-1 infection increases progressively from childhood (Bradley *et al.,* 2014). The acquisition of HSV-1 during childhood and adolescence especially in low-risk individuals increases sexual transmission of HSV-1 at older ages (15-20 years) (Anejo-Okopi *et al.,* 2016). In the age group 15-25years, the seroprevalence of

93.7% (167/176) for HSV-1 was observed. This observation was however higher than the 64% reported by Peña, *et al.,* (2010) in a similar study among women under 24 years of age in the United States, and concluded that age is an important determinant in genital HSV-1 positivity values. The significance of the higher value in this study implies that there may be a new silent epidemic under way, which is likely being driven by reduced sociological sensitivities regarding what constitutes sexual activity, particularly in the younger population (Peña, *et al*., (2010). Similarly, ages 26-35years and 36-45years were observed to have 100% (97/97) and 98.2% (56/57) HSV-1 seropositivity respectively. These results do not agree with the result obtained by Patton *et al.,* (2018) in a similar study among pregnant women in the United States, where they reported the seroprevalence of 58.5% for age group 20 to 29 years and 60.5% for ages 30 to 39 years respectively. Improved standard of living condition may account for this difference. The higher seroprevalence in this study indicates the presence of higher risks for nonsexual and sexual transmission. The result also showed that there is no significant difference in seroprevalence to HSV-1 between age groups 20 – 29 years and 30 – 39 years. This is a reflection of the fact that there are no differences in the sexual behaviors given that sexually transmitted HSV-1 infections are increasing as adolescents who lack HSV-1 antibodies at sexual debut become sexually active. Further investigation into the sexual transmission of HSV-1 may help elucidate why HSV-1 seroprevalence is so high among the women, there is

also an indication of a likely HSV-1 epidemic in this age groups in the study area. One possible explanation for increasing genital HSV-1 infection is that HSV-1 acquisition may be declining prior to sexual debut, rendering young people without HSV-1 antibodies susceptible to genital HSV-1 acquisition if exposed (Bradley, *et al.*, 2014). Poor living conditions and hygiene, over-crowding also explain these high rates (Bradley et *al*., 2014).

The result showed that the seroprevalence of HSV-2 in women age groups 15-25years, 26-35years and ages of 36-45years were 74.4% (131/176), 78.4% (76/97) and 82.5% (47/57) respectively (Table 4.2). There is no significant difference (p=0.4249) in seroprevalence of HSV-2 among the age groups studied. These results however, do not agree with the 5% seropositivity for HSV-2 obtained by Schulte, *et al*. (2014) in a similar study in asymptomatic women between the ages of 18 and 30 years unaware of any HSV infection in the United States. In a similar study by Gorfinkel *et al.,* (2013) in healthy women 18–30 years in Canada, a seroprevalence of 2.5% for HSV-2 was reported. Schulte *et al.,* (2014) also observed in their study that among participants screened for study participation and who were unaware of any HSV infection, progressively higher prevalence of the HSV-1 or HSV-2 antibody was observed in older subjects. The seroprevalence of 74.4% to 82.5% of HSV-2 obtained for the study area is very alarming and indicates an epidemic occurrence of the infection in the population .Neonatal infection rate,

although not part of this study is expected to be high, and also one of the exposure routes.

The prevalence of HSV-1 according to marital status was 99.2% for married subjects and 94.5% for singles/unmarried subjects (Table 4.3). There is no association between marital status and HSV-1 acquisition (p=0.0521). However, the prevalence rate among married women was higher than that of the single unmarried ladies. This observation in in agreement with the report of Malkin *et al.,* (2002) who did not record association between HSV-1 infection and marital status. However in a similar study by Malkin *et al.,* (2002) in the general French population, a lower prevalence of 66.8% was reported for married women and 54.0% was reported for unmarried women. This finding may be as a result of exposure to HSV-1 during childhood and risky sexual behaviors that promote the transmission of HSV-1 through the oral–genital route (Beydoun *et al*., 2010). Awareness campaign about the disease and changes in sexual behaviours is greatly lacking in the study area.

The prevalence of HSV-2 according to marital status in this study was 86.9% (113/130) for married women and 70.5% (141/200) for singles/unmarried women (Table 4.3). This findings showed that the distribution of HSV-2 in women of child bearing age is dependent on their marital status (p=0.0449). This finding is higher than the 49.1% obtained by Munjoma, *et al.,* (2010) in a similar study of HSV-2 among pregnant women in Zimbabwe. The characteristics that are associated with prevalent HSV-2 infection are

polygamous marriage, having multiple sexual partners, having ever used contraceptives, being infected with *Trichomonas vaginalis* and HIV-1. Other factors that are responsible for this high HSV-2 infection may be due to a combination of two factors; firstly, using condoms purely for contraceptive purposes and stopped during pregnancy and breastfeeding period and secondly, vaginal delivery which leaves the birth canal traumatized thereby making women more vulnerable to STIs if they have unprotected sex (Munjoma *et al*., 2010).

The prevalence of HSV-1 infection according to educational status was 96.2% (306/318) among the literate or educated women but lower 66.7% among the illiterate women (Table 4.4). This showed that HSV-1 infection is dependent on educational status (p=0.0000). This finding is in agreement with the findings of Korr *et al*., (2017) who reported association between educational status and HSV-1 seropositivity in a similar study to determine HSV-1 seroprevalence in Germany. However, the report by Korr, *et al.,* (2017) that women of lower level of education were associated with HSV-1 seropositivity did not agree with the finding in this study of higher HSV-1 positivity among educated women than those uneducated or illiterate. The higher prevalence among literate women in this study may be due to high sexual transmission routes, including decreased use of condoms in young women in schools where sexual transmission is high. There also seems to be a poor or lower coverage of sexual health education at schools.

The prevalence of HSV-2 was 79.9% (254/318), which was higher than 75% (9/12) seroprevalence among the illiterate women (Table 4.4). It was observed that HSV-2 seropositivity was not dependent on educational status (p=0.9629). This observation is not in agreement with the observation of Arama *et al.,* (2010) who reported association between HSV-2 seropositivity and education status in a similar study in Bucharest, Romania. They observed a notably higher HSV-2 seroprevalence among participants from Bucharest reporting lower rather than higher educational attainment. In their study, participants reporting a primary school level of education were more likely to report younger age at first intercourse (median 16 years for primary vs. 18.5 years for secondary) and less regular use of condoms with regular or occasional sexual partners (28% and 42% for primary, respectively vs. 53% and 82% for higher education, respectively). In contrast, participants with a primary school level education also reported fewer lifetime sexual partners than those with higher educational attainment (1 for primary vs. 2 for university).

The prevalence of HSV -1 was higher 100% (19/19) among those who had first sexual intercourse at age below 15years compared to 98.4% (189/192) for those who had first sexual intercourse at age (Table 4.5). There seems to be no significant difference ($p<0.05$) in the prevalence of HSV-1 among the women on the bases of age of sexual debut. Although the age of sexual debut varies significantly worldwide, there is a tendency towards a lower age in most countries. However, a younger age at first sexual contact was previously found

to be an important predictor of genital HSV-1 infection (Nieuwenhuis *et al.,* 2006). Nieuwenhuis *et al.,* (2006) in a similar study reported that age at first sexual contact in patients with "primary" genital HSV-1 did not differ significantly due to difference in the age at which genital HSV-1 infection was acquired. In a similar study by Pereira *et al.,* (2011), a prevalence of 23.90% was reported for people 17 years and below at first intercourse. The high and rising proportion of HSV-1 in genital lesions has been attributed to an increasing frequency of the oral-genital sex or perhaps to the reduced incidence of childhood HSV-1 infection which has been observed in industrialized countries as a result of improved standards of personal hygiene. The influence of the practice of oral-genital sex could not be evaluated in our study given that the questionnaires used for interviewing the patients did not contain any kind of questioning about the sexual practices of the participants. Considering that the practice of oral-genital sex as a substitute for vaginal intercourse is common among adolescents as a way to avoid unwanted pregnancies and also believing that this type of sexual practice does not transmit disease, it is very likely that this type of sexual behavior is the main reason for this reversal of roles between HSV-1 and HSV-2 in relation to genital tract infection (Pereira, *et al*., 2011).

As for the prevalence of HSV-2, a lower prevalence of 78.9% (15/19) was observed among women who indicated that they had first sexual intercourse at an age below 15years compared to the prevalence of 80.7% (155/192) in those

who had first sexual intercourse at an age above 15years (Table 4.5). However there seemed to be no significant difference ($p<0.05$) between the prevalence according to age of first sexual intercourse.

The prevalence of HSV-1 according to number of sexual partners before marriage (table 4.6) showed that there is no significant difference ($p<0.05$) in the number of sexual partners before marriage and HSV-1 infection. This therefore means that there is no association between number of sexual partners before marriage and HSV-1 prevalence rate. This observation did not agree with the observation of a similar study by Bradley *et al.,* (2014), who reported that seroprevalence was higher among respondents who had ever had sex compared to those who had never had sex before marriage and among those with ≥3 lifetime sex partners compared to those with < 3 sex partners. The findings in this study showed that most of the teens and adolescents in the study area have been exposed in their childhood to herpes simplex virus type 1 (HSV-1). Antibodies produced against HSV-1 by these teens make them to be less susceptible to HSV-2 infection when they become sexually active as young adults (Zaideman, 2013).

From table 4.6, there seems to be an association between number of sexual partners before marriage and HSV-2 infection ($p<0.05$). This finding agrees with the findings of similar study by Frieden *et al.,* (2010) in America who reported that for all three racial/ethnic groups studied, HSV-2 seroprevalence was greater among those with more lifetime sex partners. Persons infected

with HSV-2 are at greater risk for HIV acquisition, even in the absence of HSV-2 symptoms. Also, increased susceptibility to HIV infection likely occurs because even HSV-2 ulcerations that are microscopic can provide a portal of entry for HIV, and HSV-2 reactivation recruits potential target cells for HIV to the genital skin and mucosa (Frieden *et al.,* 2010).

The prevalence of HSV-1 among the women who had STD in the study area was 64% (20/29) (Table 4.7). Ameh *et al*., (2016) in a similar study in Zaria, Kaduna State reported the prevalence of 78.3%. Human Papilloma Virus has been reported in women by Vahidnia *et al.,* (2013) in a similar study in Amsterdam, Netherland. Genital herpes is associated with other sexually transmitted infections (STIs) including HIV (Memish *et al.,* 2015).

The prevalence of HSV-2 among the women studied who had STD was 8.8% (29/330) (Table 4.7). This finding is not in agreement with the finding of Aebi-Popp *et al.,* (2016) in a similar study among women in Ukraine who observed the prevalence of 68 % of HIV-positive women with antibodies to HSV-2, 2.0 % (20/981) of HSV-2 seropositive women had positive serology for syphilis, 31 % (158/511) had a positive chlamydia test, 0.3% (2/759) were positive for gonorrhea. Women with HSV-2 antibodies were more likely to have a history of injection drug use (IDU), be positive for Hepatitis C Virus (HCV) antibodies and Hepatitis B antigen (HBsAg), and report an HIV-positive partner. HSV-2 seropositive women were more likely to report their

most recent pregnancy as having been unplanned, but reported better access to family planning than HSV-2 seronegative women (Aebi-Popp *et al.,* 2016).

The prevalence of HSV-1 infection obtained in this study among women who had genital ulcer was 79.3% (Table 4.7). Recently, the number of reported genital herpes cases caused by type 1 virus has increased (Peña *et al.,* 2010). Pereira *et al*. (2011) reported that there is an inverse relationship between genital HSV-1 infection and the age of patients which result to a high proportion of HSV-1 infection in genital lesions. This is attributable to an increasing frequency of the oral-genital sex or perhaps to the reduced incidence of childhood HSV-1 infection which has been observed in industrialized countries as a result of improved standards of personal hygiene.

In Table 4.7, the prevalence of HSV- 2 infection obtained among women studied who presented with genital ulcer was also 5.2% (17/330). The prevalence of those with genital ulcer was the same for those with HSV-1 and HSV-2 infection since those with genital ulcers were HSV-1 and HSV-2 coinfected. This figure is much lower than 75.0% (6/8) cases of clinical genital ulcer reported by Munjoma *et al.,* (2010) in a similar study among pregnant Zimbabwean women.

Genital HSV remains a highly prevalent infectious agent with a significant impact on sexual health and risk of HIV infection. We now understand that HSV-2 infection is not characterized by latency with occasional outbreaks but that it is a dynamic infection with frequent, often subclinical, shedding

throughout the genital tract that results in inflammation (Johnston and Corey, 2016).

Other authors have suggested that the prevalence of HSV-2 and genital ulcers in the female population is most likely due to the anatomical differences of male and female individuals, where the mucosal surface of the external genitalia of females could be more vulnerable (Pereira *et al*., 2011). HSV-2 targets the epithelial cells during the infectious process thus predisposing to HIV-1 infections which targets mainly CD_4^+ lymphocytes, which can mostly be reached via lesions in the mucosa.

The overall mean CD_4^+ count for the 330 women (both infected and uninfected) was 793.6 ± 210.9 cells. Van Benthem *et al.,* (2001) reported a CD_4^+ counts of less than 200 cells/ml in about 2% of HSV-2 infected women in a similar study carried out among women in Europe, although their study was among HIV infected women. However, this study did not involve the determination of the HIV status of the women, hence, a relationship cannot be drawn between the CD_4^+ counts and HSV/HIV coinfection. In this study the range of CD_4^+ counts was 404.5±92.5 to 793.2±234.9 cells/µl for both HSV-1 and HSV-2 infected individuals respectively. There was no significant difference ($p = 0.611$) between the CD_4^+ counts of HSV-1 and HSV-2 infected women and that of the uninfected women (Table 4.8), therefore, there seems to be no association between HSV-1 and 2 infection and CD_4^+ counts. Although, HSV-2 activity is associated with increased HIV viral load, definitive evidence linking HSV-2

seropositivity to accelerated HIV disease progression is lacking (Tan *et al*., 2013). Van Benthem *et al.,* (2001) reported that there was no evidence for an association between the recurrent genital ulcerations and markers of HIV disease progression. A decreasing CD_4^+ count was associated with an increase in the recurrence of genital ulcerations in HSV-2 positive women.

5.2 Conclusion

The high prevalence of HSV-1 and 2 among these study group highlight the potential public health impact of HSV infection, especially considering the risk of neonatal transmission and the attendant complications at birth. The significance of these observations is that there may be a serious and a silent epidemic under way, which is likely being driven by reduced sociological sensitivities regarding what constitutes sexual activity, particularly in the younger population. The risk factors found in this study responsible for this prevalence includes age, poor hygiene and living conditions, over-crowding , sexual behaviors and educational status, age at first sexual intercourse and number of sexual partners. The CD_4^+ count found in this study, for both HSV-1 and HSV-2 infection shows no significant difference irrespective of the infection with or both sub types of HSV. The variation in CD_4^+ counts may be due to the presence of other underlying infection especially HIV which utilizes CD_4^+ cells. Since HSV is known to infect only the epithelial cells of the skin and mucosal epithelium of the genitalia.

5.3 Recommendation

1. Government should organize an enlightenment campaign on the magnitude of the infection and the need for behavioral change and activity that will be geared towards reducing the prevalence.
2. Non-Governmental organization should also come to the aid of the State in areas of awareness campaign to bring about control and prevention of this infection.
3. Molecular epidemiological survey is required to throw more light on the molecular structure of the organism with the view to understanding the mode of infection and how to control it.
4. The infection control agency and the primary health staff should be informed with a view to deploying them to the study area in order to sensitize the public on ways to reduce the scourge, for example reduction of sexual activity and use of condom. Reduction of oral sex and unsafe sexual practices.

5.4 Contribution to Knowledge

The study has brought to light, the endemic nature and the high prevalence rate of HSV-1 and HSV-2 among women of child bearing age in Kogi State. The risk factor of the infection has also been revealed. The study also shows that the infection is an epidemic waiting to explode. The study further demonstrates that there is an urgent and dere need for the control of the infection in this area to reduce the level of the infection to the barest minimum.

REFERENCES

Abedi, G.M., Hamzehgardeshi, Z., Afshari, M. and Moosazadeh, M. (2016). The prevalence of herpes simplex virus type 1 and 2 infection in Iran: A meta-analysis. *International Journal of Reproductive Biomedicine (Yazd)*. 14(10): 615–624.

Abuharfeil, N. and Meqdam, M.M. (2000). "Seroepidemiologic study of herpes simplex virus type 2 and cytomegalovirus among young adults in northern Jordan". *New Microbiology*. 23(3): 235–239.

Ace, C.I., McKee, T.A., Ryan, J.M., Cameron, J.M. and Preston, C.M. (1989). Construction and characterization of a herpes simplex virus type 1 mutant unable to trans-induce immediate-early gene expression. *Journal of Virology*. 63: 2260-2269.

Aebi-Popp, K., Bailey, H., Malyuta, R., Volokha, A., Thorne, C. and The Ukraine European Collaborative Study in Euro Coord, (2016). High prevalence of herpes simplex virus (HSV)- type 2 co-infection among HIV- positive women in Ukraine, but no increased HIV mother-to-child transmission risk. *BMC Pregnancy and Childbirth*. 16: 94.

Agabi, Y.A., Banwat, E.B., Mawak, J.D., Lar, P.M., Dashe, N., Dashen, M.M., Adoga, M.P., Agabi, F.Y. and Zakari, H. (2010). Seroprevalence of herpes simplex virus type-2 among patients attending the Sexually Transmitted Infections Clinic in Jos, Nigeria. *Journal of Infections in Developing Countries*. 4(9): 572-575.

Ahmed, R. and Biron, C.A. (1999). Immunity to Viruses. In: Paul, WE, ed. Fundamental Immunology 4th ed. Philadelphia: Lippincott-Raven Publishers. Pp. 1295-1334.

Anderson, N.W., Buchan, B.W. and Ledeboer, N.A. (2014). Light microscopy, culture, molecular, and serologic methods for detection of herpes simplex virus. *Journal of Clinical Microbiology*. 52: 2-8. DOI: http://dx.doi.org/10.1128/JCM.01966.

Anejo-Okopi, J.A., Katnap, S.R., Audu, O., Hashimu, Z., Okojokwu, J.O., Zumbes, H.J., Audu, M., Okechalu, J. and Ejeliogu, E.U. (2016). The Seroprevalence of Herpes Simplex Virus Type-1 Among Children in Jos, Nigeria. *Central African Journal of Public Health.* 2(1): 18-21.

Ashley, R.L. (1998). Genital herpes; Type-specific antibodies for diagnosis and management. *Dermatology Clinics*. 16: 789-793.

Austin, H., Macaluso, M., Nahmias, A., Lee, F.K., Kelaghan, J., Fleenor, M. and Hook, E.W. (1999). Correlates of herpes simplex virus seroprevalence among women attending a sexually transmitted disease clinic. *Sexually Transmitted Disease*. 26: 329–334.

Barcy, S. and Corey, L. (2001). Herpes simplex inhibits the capacity of lymphoblastoid B cell lines to stimulate CD_4^+ T cells. *Journal of Immunology.* 166: 6242-6249.

Belec, A. (2006). Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. *Journal of Clinical Microbiology.* 44(2): 423-432. doi:http://dx.doi.org/10.1128/JCM.44.2.423-432.2006

Bello-Morales, R., Crespillo, A. J., Fraile-Ramos, A., Tabares, E., Alcina, A. and Lopez-Guerrero, J.A. (2012). Role of the small GTPase Rab27a during herpes simplex virus infection of oligodendrocytic cells. *BioMedical Central Microbiology.* 12: 265.

Benedetti, J.K., Zeh, J. and Corey, L. (1999). Clinical Reactivation of Genital Herpes Simplex Virus Infection Decreases in Frequency over Time. *Annals of Internal Medicine*. 131(1): 14-20.

Beydoun, H.A., Dail, J., Ugwu, B., Boueiz, A. and Beydoun, M.A. (2010). A retrospective study into the prevalence of herpes simplex virus 1&2 in female patients tested for Chlamydia trachomatis and/or *Neisseria gonorrhoeae* using vaginal swabs. *Clinical Microbiology Infections*. 19: E166–E168.

Beydoun, H.A., Dail, J., Ugwu, B., Boueiz, A. and Beydoun, M.A. (2010). Socio-demographic and behavioral correlates of herpes simplex virus type 1 and 2 infections and co-infections among adults in the USA. *International Journal of Infectious Disease*. 14S3: e154–e160. doi:10.1016/j.ijid.2009.12.007.

Beyrer, C., Jitwatcharanan, K., Natpratan, C., Kaewvichit, R., Nelson, K.E., Chen, C.Y., Weiss, J.B. and Morse, S.A. (1998). Molecular methods for the diagnosis of genital ulcer disease in a sexually transmitted disease clinic population in northern Thailand: predominance of herpes simplex virus infection. *Journal of Infectious Disease*. 178(1): 243–246

Biron, C.A., Byron, K.S. and Sullivan, J.L. (1989). Severe herpesvirus infections in an adolescent without natural killer cells. *New England Journal of*

Medicine. 320: 1731-1735.

Biron, C.A., Nguyen, K.B., Pien, G.C., Cousens, L.P. and Salazar-Mather, T.P. (1999). Natural killer cells in antiviral defense: Function and regulation by innate cytokines. *Annual Reviews Immunology*. 17: 189-220.

Biron, C.A. and Sen, G.C. (2001). Interferons and other cytokines. In: Knipe DM, Howley PM, eds. *Fundamental of Virology* 4th ed. Philadelphia: Lippincott-Raven Publishers: 321-351.

Blower, S. and Boe, C. (1993). Sex acts, sex partners, and sex budgets: implications for risk factor analysis and estimation of HIV transmission probabilities. *Journal of Acquired Immune Deficiency Syndrome.* 6: 1347–1352.

Bradley, H., Markowitz, L.E., Gibson, T. and McQuillan, G.M. (2014). Seroprevalence of Herpes Simplex Virus Types 1 and 2—United States, 1999–2010. *The Journal of Infectious Diseases*. 209(3,1): 325–333. https://doi.org/10.1093/infdis/jit458

Brown, Z.A., Selke, S., Zeh, J., Kopelman, J., Maslow, A., Ashley, R.L., Watts, D.H., Berry, S., Herd, M. and Corey, L. (1997). The Acquisition of Herpes Simplex Virus during Pregnancy. *New England Journal of Medicine*. 337(8): 509-515.

Brown, J.C. and Newcomb, W.W. (2011). Herpes virus capsid assembly: insights from structural analysis. *Current Opinion in Virology.* 1(2): 142-149.

Cates, W. (1999). Estimates of the Incidence and Prevalence of Sexually Transmitted Diseases in the United States. *Sexually Transmitted Diseases*. 26(4): S2-S7.

Centre for Disease Control (2002). Sexually Transmitted Diseases Treatment Guidelines. *Morbidity and Mortality Weekly Report*. 51(RR-6): 12-17.

Centre for Disease Control (2011). Genital Herpes Rates Still High". Centers for Disease Control and Prevention. Retrieved 2011-02-03.

Centers for disease Control and Prevention (2013). National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Division of STD Prevention.

Sexually transmitted diseases: Genital Herpes- CDC fact sheet. Available: http://www.cdc.gov/std/Herpes/STD Fact-Herpes.

Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M. and Moore, P.S. (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science*. 266: 1865.

Chen, C. Y., Ballard, R.C., Beck-Sague, C. M., Dangor, Y., Radebe, F., Schmid, S., Weiss, J.B., Tshabalala, V., Fehler, G., Htun, Y. and Morse, S.A. (2000). Human immunodeficiency virus infection and genital ulcer disease in South Africa: the herpetic connection. *Sexually Transmitted Disease*. 27(1): 21–29.

Chowdhury, S., Chouljenko, V. N., Nadheri, M. and Soulas, K.G. (2013) The amino terminus of herpes simplex virus type-1 (HSV-1) glycoprotein K (gK) is required for virion entry via the paired immunoglobulin-like type-2 receptor alpha (PIL- R- alpha). *Journal of Virology.* 87: 3305-3331

Cocchi, F., Menotti, L., Dubreuil, P., Lopez, M. and Campadelli-Fiume, G. (2000). Cell-to-cell spread of wild-type herpes simplex virus type 1, but not of syncytial strains, is mediated by the immunoglobulin-like receptors that mediate virion entry, nectin1 (PRR1/HveC/HIgR) and nectin2 (PRR2/HveB). *Journal of Virology*. 74(18): 3909-3917.

Corey, L. and Spear, P.G. (1986). Infections with herpes simplex viruses (1). *New England Journal of Medicine*. 314: 686-691.

Corey, L. (1994). The current trend in genital herpes. Progress in prevention. *Sexually Transmitted Diseases*. 21(2): S38-44.

Corey, L. and Handsfield, H.H. (2000). Genital Herpes and public health: addressing a global problem. *Journal of American Medical Association*. 283: 791-794.

Corey, L., Wald, A., Celum, C.L. and Quinn, T.C. (2004). The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. *Journal of Acquired Immune Deficiency Syndrome*. 35: 435–445

Cunningham, A.L., Taylor, R., Taylor, J., Marks, C., Shaw, J. and Mindel, A. (2006). "Prevalence of infection with herpes simplex virus types 1 and 2 in

Australia: a nationwide population based survey". *Sexually Transmitted Infections*. 82(2): 164–168.

Debrah, O., Agyemang-Yeboah, F., Asmah, R.H., Timmy-Donkoh, E., Seini, M. M., Fondjo, L.A., Sight, N. and Dabo, E.O. (2018). Sero-prevalence of herpes simplex virus type-1 and type-2 among women attending routine Cervicare clinic in Ghana. *BMC Infectious Diseases*. 18: 378-185.

Dan, M., Sadan, O., Glezerman, M., Raveh, D. and Samra, Z. (2003). "Prevalence and risk factors for herpes simplex virus type 2 infection among pregnant women in Israel". *Sexually Transmitted Disease.* 30(11): 835–838.

Darougar, S., Vishart, M.S. and Viswalingam, D. (1985). Epidemiological and clinical features of primary herpes simplex virus ocular infection. *British Journal of Ophthalmology.* 69: 1.

Davidovici, B., Green, M., Marouni, M.J., Bassal, R., Pimenta, JM. And Cohen, D. (2006). "Seroprevalence of herpes simplex virus 1 and 2 and correlates of infection in Israel". *Journal of Infections*. 52(5): 367–373.

Davidovici, B., Grotto, I., Balicer R.D., Robinson, N.J. and Cohen, D. (2006). "Decline in the prevalence of antibodies to herpes simplex virus types 1 and 2 among Israeli young adults between 1984 and 2002" (PDF*). Sexually Transmitted Disease.* 33(11): 641–645.

Dolar, N., Serdaroglu, S., Yilmaz, G. and Ergin, S. (2006). "Seroprevalence of herpes simplex virus type 1 and type 2 in Turkey". *Journal of the European Academy of Dermatology and Venereology*. 20(10): 1232–1236.

Domeika, M., Bashmakova, M., Savicheva, A., Kolomiec, N., Sokolovskiy, E., Hallen, A., Unemo, M. and Ballard, R.C. (2010). Guidelines for the laboratory diagnosis of genital herpes in eastern European countries. *Euro Surveill*. 15(14): 19703.

Dordevic, H. (2006). Serological response to herpes simplex virus type 1 and 2 infection among women of reproductive age*. Medicinski Pregled*. 159: 591-597.

Dummer, J.S., Armstrong, J., Somers, J., Kusne, S., Carpenter, B.J., Rosenthal, J.T. and Ho, M. (1987). Transmission of infection with herpes simplex virus by renal transplantation. *Journal of infectious Disease.* 155: 202-206.

Edathodu, J., Ali, B. and Alrajhi, A. (2009). CD4 validation for the World Health Organization classification and clinical staging of HIV/AIDS in a

developing country. *International Journal of Infectious Diseases.* 13: 243 - 246.

Eisenberg, R.J., Lambris, J.D. and Friedman, H.M. (1998). Herpes simplex virus type 1 glycoprotein gC mediates immune evasion invivo. *Journal of Virology.* 72(10): 8257-8263.

Elad, S., Zadik, Y., Hewson, I., Hovan, A., Correa, M.E., Logan, R., Elting, L.S., Spijkervet, F.K. and Brennan, M.T. (2010). "A systematic review of viral infections associated with oral involvement in cancer patients: a spotlight on Herpesviridea". *Support Care Cancer*. 18(8): 993–1006. doi:10.1007/s00 520-010-0900-3. PMID 20544 224.

Enders, G., Risse, B., Zauke, M., Bolley, I. and Knotek, F. (1998). Seroprevalence study of herpes simplex virus type 2 among pregnant women in Germany using a type-specific enzyme immunoassay. *European Journal of Clinical Microbiology and Infectious Disease*. 17: 870–872.

Eskild, A., Jeansson, S., Hagen, J.A., Jenum, P.A. and Skrondal, A. (2000). Herpes Simplex Virus Type-2 in Pregnant Women: The Impact of the Stage of Pregnancy. *Epidemiology and Infections*. 125(3): 685-692.

Fatahzadeh, M. and Schwartz, R.A. (2007). "Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management". *Journal of the American Academy of Dermatology*. 57(5): 737–763; quiz 764–766..

Feldman, P.A., Steinberg, J., Madeb, R., Bar, G., Nativ, O., Tal, J. and Srugo, I. (2003). "Herpes simplex virus type 2 seropositivity in a sexually transmitted disease clinic in Israel" *Isreali Medical Association Journal.* 5(9): 626–628.

Filipescu, A., Rafila, A., Arama, S., Hristeav, A., Malkin, J.E. and Pimenta, J.M. (2010). Type-specific herpes simplex virus-1 and herpes simplex virus-2 seroprevalence in Romania: comparison of prevalence and risk factors in women and men. *International Journal of Infectious Diseases*. 14(3): 25-31.

Fitzgerald-Bocarsly, P., Howell, D.M., Pettera, L., Tehrani, S. and Lopez, C. (1991). Immediate-early gene expression is sufficient for induction of natural killer cell-mediated lysis of herpes simplex virus type 1-infected fioblasts. *Journal of Virology*. 65: 3151-3160.

Fleming, D.T., McQuillan, G.M., Johnson, R.E., Nahmias, A.J., Aral, S.O., Lee, F.K. and Louis, M.E. (1997). Herpes simplex virus type 2 in the United

States, 1976 to 1994. *New England Journal of Medicine*. 337(16): 1105-1111.

Fleming, D.T. and Wasserheit, J.N. (1999). From epidemiological synergy to public health policy and practice: The contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sexually Transmitted Infections*. 75: 3-17.

Forward, K.R. and Lee, S.H. (2003). "Predominance of herpes simplex virus type 1 from patients with genital herpes in Nova Scotia". *Canadian Journal of Infectious Disease*. 14(2): 94–96.

Freeman, E.E., Weiss, H.A., Glynn, J.R., Cross, P.L., Whitworth, J.A. and Hayes, R.J. (2006). Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *Acquired Immune Deficiency Syndrome*. 20(1): 73–83.

Frieden, T.R., Briss, P.A., Stephens, J.W. and Thacker, S.B. (2010). Seroprevalence of Herpes Simplex Virus Type 2 among Persons Aged 14–49 Years — United States, 2005–2008. *Morbidity and Mortality Weekly Report*. 59(15): 456 – 459.

Garland, S.M. and Steben, M. (2014). Genital herpes. *Best Practice and Research: Clinical Obstetrics and Gynaecology*. 28: 1098-110. DOI: http://dx.doi.org/10.1016/j.bpobgyn.2014.07. 015

Gibson, W. and Roizman, B. (1972). Proteins specified by herpes simplex virus. 8. Characterization and composition of multiple capsid forms of subtypes 1 and 2. *Journal of Virology.* 10: 1044-1052.

Glorioso, J., Schroder, C.H., Kumel, G., Szczesiul, M. and Levine, M. (1984). Immunogenicity of herpes simplex virus glycoproteins gC and gB and their role in protective immunity. *Journal of Virology.* 50: 805-812.

Goodman, J.L. (1989). Possible transmission of herpes simplex vims by organ transplantation. *Transplantation*. 4H09-613.

Gorfinkel, I. S., Aoki, F., McNeil, S., Dionne, M., Shafran, S.D., Zickler, P., Halperin, M., Lagley, J., Bellamy, A., Schulte, J., Heineman, T. and Belshe,

R. (2013). Seroprevalence of HSV-1 and HSV-2 antibodies in Canadian women screened for enrolment in a herpes simplex virus vaccine trial. *International Journal of STD & AIDS*. 24(5): 345–349.

Gottesdiener, K. (1989). Transplanted infections: donor-to-host transmission with the allograft. *Annals of Internal Medicine*. 110 (12): 1001-1016

Gottlieb, S., Douglas, J.M., Schmid, D.S., Bolan, G., Iatesta, M., Malotte, C.K., Zenilman, J., Foster, M., Barón, A.E., Steiner, J.F., Peterman, T.A. and Kamb, M.L. (2002). Seroprevalence and Correlates of Herpes Simplex Virus Type 2 Infection in Five Sexually Transmitted Disease Clinics. *Journal of Infectious Disease*. 186(10): 1381-1389.

Grunewald, K., Desai, P., Winkler, D.C., Heymann, J.B., Belnap, D.M., Baumeister, W. and Steven, A.C. (2003). Three-dimensional structure of herpes simplex virus from cryoelectron tomography. *Science*. 302: 1396-1398.

Gupta, R., Warren, T. and Wald, A. (2002). Genital herpes. *Lancet*. 370 (9605): 2127-2137.

Gupta, R., Warren, T. and Wald, A. (2007). Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *Lancet*. 370: 2127-2137.

Habu, S., Akamatsu, K., Tamaoki, N. and Okumura, K. (1984). In vivo significance of NK cells on resistance against virus (HSV-1) infections in mice. *Journal of Immunology.* 133: 2743-2747.

Haddow, L.J., Dave, B., Mindel, A., McPhie, K.A., Chung, C., Marks, C. and Dwyer, D.E. (2006). "Increase in rates of herpes simplex virus type 1 as a cause of anogenital herpes in western Sydney, Australia, between 1979 and 2003". *Sexually Transmitted Infections*. 82(3): 255–259.

Handsfield, H.H. (2000). "Public Health Strategies to Prevent Genital Herpes: Where Do We Stand?". *Current Infectious Disease Report*. 2(1): 25–30. doi:10.1007/s11908-000-0084-y. PMID 11095834.

Hercberg, S. (2002). Seroprevalence of HSV-1 and HSV-2 infection in the general French population. *Sexually Transmitted Infections*. 78: 201–203

Herpes Simplex". University of Maryland Medical Center. Retrieved 2007-09-03.

Herpes". Stanford University Sexual Health Peer Resource Center. Retrieved 2007-09-03.

Herpetic Eye Disease Study Group. (2001). Predictors of recurrent herpes simplex virus keratitis. *Cornea.* 20: 123-128.

Hill, A., Jussgovic, P., York, I., Russ, G., Bennink, J., Yewdell, J., Ploegh, H. and Johnson, D. (1995). Herpes simplex virus turns off the TAP to evade host immunity. *Journal of l!atur*; 375: 411-415.

Howard, M., Sellors, J.W., Jang, D., Robinson, N.J., Fearon, M., Kaczorowski, J. and Chernesky, M. (2003). "Regional distribution of antibodies to herpes simplex virus type 1 (HSV-1) and HSV-2 in men and women in Ontario, Canada". *Journal of Clinical Microbiology.* 41(1): 84–89.

Ibrahim, A.I., Kouwatli, K.M. and Obeid, M.T. (2000). "Frequency of herpes simplex virus in Syria based on type-specific serological assay*". Saudi Medical Journal*. 21(4): 355–360.

İğde, F.A. and İğde, M. (2011). Seroepidemiological Study of Herpes Simplex Virus Type 1 in the Black Sea Region of Turkey. *Balkan Medical Journal*. 28: 37–42.

Jerome, K.R., Chen, Z., Lang, R., Torres, M.R., Hofmeister, J., Smith, S., Fox, R., Froelich, C.J. and Core Y.L. (2001). HSV and glycoprotein J inhibit caspase activation and apoptosis induced by granzyme B or Fas. *Journal of Immunology.* 167: 3928-3935.

Johnston, C. and Corey, L. (2016). Current Concepts for Genital Herpes Simplex Virus Infection Diagnostics and Pathogenesis of Genital Tract Shedding. *Clinical Microbiology Reviews*. 29(1): 149-161.

Jovasevic, V., Liang, L. and Roizman, B. (2008) roteolytic cleavage of VP1-2 is required for release of herpessimplex virus 1 DNA into the nucleus. *Journal of Virology.* 82: 3311-3319.

Kalu, E.I., Ojide, C.K., Fowotade, A. and Nwadike, V.U. (2014). HSV-2 prevalence and sexual life of pregnant women. *Journal of Infections in Developing Countries*. 8(8): 1006-1012.

Kalu, E.I., Ojide, C.K., Chuku, A., Chukwuonye, I.I., Agwu, F.E., Nwadike, V.U., Korie, F.C. and Okafor, G. (2015). Obstetric outcomes of human herpes virus-2 infection among pregnant women in Benin, Nigeria. *Nigerian Journal of Clinical Practice*. 18: 453-461

Kaur, R., Gupta, N. and Baveja, U.K. (2005). "Seroprevalence of HSV1 and HSV2 infections in family planning clinic attendees". *Journal of Communicable Disease*. 37(4): 307–309.

Kawana, T., Matsunaga, Y. and Inouye, S. (1999). Changes in prevalence of herpes simplex virus type 1 and 2 antibodies from 1973 to 1993 in the rural districts of Japan. *Microbiology and Immunology*. 43: 177–180.

Kesson, A.M. (2001). Management of Neonatal Herpes Simplex Virus Infection. *Paediatrics Drugs*. 3: 90-91.

Koelle, D.M., Abbo, H., Peck, A., Ziegweld, K. and Corey, L. (1994). Direct recovery of herpes simplex virus (HSV) – specific T lymphocyte clones from recurrent genital HSV-2 lesions. *Journal of Infectious Disease*. 169: 956–961

Kohl, S. (1991). Role of antibody-dependent cellular cytotoxicity in defense against herpes simplex virus infections. *Reviews of Infectious Disease*. 13: 108-114.

Kolawole, O.M., Adu, F.D., Agbede, O.O., Oni, A.A. and Bakare, R.A. (2008). Epidemiological Patterns of Human Immunodeficiency Virus and Herpes Simplex Virus Co-Infection in Ibadan, Nigeria. *African Journal of Biomedical Research*. 11: 23-26.

Korr, G, Thamm, M, Czogiel, I ,Poethko-Mueller, C, Bremer, V. and Jansen, K. (2017). Decreasing seroprevalence of herpes simplex virus type 1 and type 2 in Germany leaves many people susceptible to genital infection: time to raise awareness and enhance control. *BMC Infectious Diseases*. 17: 471. DOI 10.1186/s12879-017-2527-1

Koutsky, L.A., Stevens, C.E., Holmes, K.K., Ashley, R., Kiviat, N.B., Critchlow, C.W. and Corey, L. (1992). Underdiagnosed of Genital Herpes by Current Clinical and Viral Isolation Procedures. *New England Journal of Medicine*. 326: 1533-1539.

Kwong, A.D. and Kruper, J.A. (1988). Frenkel N. Herpes simplex virus virion host shutoff function. *Journal of Virology*. 62: 912.

Lanier, L.L. (1997). Natural killer cell receptors and MHC class I interactions. *Current Opinion of Immunology*. 9: 126-131.

LEARN ABOUT HERPES > Fast Facts". ASHA Herpes Resource Center. Retrieved 2007-09-03.

LeGoff, J., Pere, H. and Belec, L. (2014). Diagnosis of genital herpes simplex virus infection in the clinical laboratory. *Virology Journal*. 11: 83-99.

Lennette, E.H., Johnson, D.P. and Green, R.H. (1999). Laboratory Diagnosis of Viral Infections, Third Ed. Marcel Dekker. New York, NY. 305–332.

Liesegang T.J., Melton, L.J., Daly, P.J. and Llstrup D. (1989). Epidemiology of ocular herpes simplex Incidence in Rochester. Minn, 1950 through 1982. *Archives of Ophthalmology*. 107: 1155-1159.

Looker, K.J., Garnett, G.P. and Schmid, G.P. (2008). An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. *Bulletin of World Health Organization*. 86(10): 737-816. .

Lowenstein, A. (1919). Aetiologische untersuchungen uber den fieberhaften, herpes. *Munchener Medizinische Wochenschrift*. 66: 769-770.

Lubinski, J.M., Wang, L., Soulika, A.M., Burger, R., Wetsel, R.A., Colten, H., Cohen, G.H., Eisenberg, leukemia in Egypt Kundi″. *Saudi Medical Journal.* 27(8): 1139–1145. PMID 16883441.

Lucin, P., Jonjic, S., Messerle, M., Polic, B., Hengel, H. and Koszinowski, U.H. (1994). Late phase inhibition of murine cytomegalovirus replication by synergistic action of interferon-gamma and tumour necrosis actor. *Journal of General Virology.* 75: 101-110.

Lyttle, P.H. (1994). "Surveillance report: disease trends at New Zealand sexually transmitted disease clinics 1977–1993". *Genitourinary Medicine.* 70(5): 329–335.

Malkin, J.E. (2004). "Epidemiology of genital herpes simplex virus infection in developed countries. *Herpes.* 11(1): 2A–23A.

Meguenni, S., Djenaoui, T., Bendib, A., Bouhadjar, H., Lalliam, N., Allouache, A. and Bouguermouh, A. (1989). "Herpes simplex virus infections in Algiers". *Archives de l'Institut Pasteur d'Algérie*. 57: 61–72.

Memish, Z.A., Almasri, M., Chentoufi, A.A., Al-Tawfiq, J.A., Al-Shangiti, A.M., Al-Kabbani, K.M., Otaibi, B., Assirri, A. and Yezli, S. (2015). Seroprevalence of Herpes Simplex Virus Type 1 and Type 2 and Coinfection with HIV and Syphilis: The First National Seroprevalence Survey in Saudi Arabia. *Sexually Transmitted Disease.* 42(9): 526-532. doi: 10.1097/OLQ 00000000000336.

Mertz, G.J., Benedetti, J., Ashley, R., Selke, S.A. and Corey, L. (1992). Risk factors for the sexual transmission of genital herpes. *Annals of Internal Medicine.* 116: 197-202.

Mertz, G.J., Rosenthal, S.L. and Stanberry, L.R. (2003). "Is herpes simplex virus type 1 (HSV-1) now more common than HSV-2 in first episodes of genital herpes?". *Sexually Transmitted Disease*. 30(10): 801–802.

Mester, J.C. and Rouse, B.T. (1991). The mouse model and understanding immunity to herpes simplex virus. *Reviews of Infectious Disease.* 13(11): S935-945

Morrison, L. and Knipe, D.M. (1994). Immunization with replication-defective mutants of herpes simplex virus type 1: sites of immune intervention in pathogenesis of challenge virus infection. *Journal of Virology*. 68: 689.

Munjoma, M.W., Kurewa, E.N., Mapingure, M.P., Mashavave, G.V., Chirenje, M.Z., Rusakaniko, S., Hussain, A. and Stray-Pedersen, B. (2010). The prevalence, incidence and risk factors of herpes simplex virus type 2 infection among pregnant Zimbabwean women followed up nine months after childbirth. *BMC Women's Health*. 10(2): 1 – 8. http://www.biomedcentral.com/1472-6874/10/2

Nahmias, A.J., Lee, F.K. and Beckman-Jahmias, S. (1990). Sera-epidemiological and -sociological patterns of herpes simplex virus infection in the world. *Scandinavian Journal of Infectious Disease.* 69: 19-36.

Naing, L., Winn, T. and Rusli, B.N. (2006). Practical issues in calculating the sample size for prevalence study. *Archives of Orofacial Science*. 1: 9-14.

Nieuwenhuis, R.F., Gerard, J., van Doornum, J., Paul, G.H., Mulder, H.A., Neumann, M. and van der Meijden, W. (2006). Importance of Herpes Simplex Virus Type-1 (HSV-1) in Primary Genital Herpes. *Acta Dermato-Venereologica.* 86: 129–134

Olantuji, M.K., Oluwatomi, O.A., Nzurumike, C., Mohammed, M.S. and Ogah, J.I. (2016). Seroprevalence and co-infection of Human Immunodeficiency Virus (HIV) and Herpes Siplex Virus (HSV) among pregnant women in Lokoja Kogi State Nigeria. *Iranian Red Crescent Medical Journal*. 18(10): e25284. Doi:10.5812/ircmj.25284

Oni, A.A., Adu, F.D., Ekweozor, C.C. and Bakare, R.A. (1996). Genital herpes simplex virus infection in females in Ibadan Nigeria. *West African Journal of Medicine*. 15: 107-110.

Para, M.F., Parish, M.L., Noble, A.G. and Spear, P.G. (1985). Potent neutralizing activity associated with antiglycoprotein 0 specificity among monoclonal antibodies selected for binding to herpes simplex virions. *Journal of Virology.* 55: 483-488.

Patel, R, Alderson, S, Geretti, A, Nilsen, A, Foley, E, Lautenschlager, S., Green J, van der Meijden W., Gomberg, M. and Moi, H. (2011). Guidelines for the laboratory diagnosis of genital herpes in eastern European countries. *International Journal of Sexually Transmitted Disease and Acquired Immune Deficiency Syndrome.* 22(1):1-10. http//dx doi org /10 1258/ijsa 2010,010278.

Patnaik, P., Herrero, R., Morrow, R.A., Munoz, N., Bosch, F.X., Bayo, S., El Gueddari, B., Caceres, E., Chichareon, S.B., Castellsague, X., Meijer, C.J., Snijders, P.J. and Smith, J.S. (2007). "Type-specific seroprevalence of herpes simplex virus type 2 and associated risk factors in middle-aged women from 6 countries: the IARC multicentric study". *Sexually Transmitted Disease*. 34 (12): 1019–1024. .

Patrick, D.M., Dawar, M., Cook, D.A., Krajden, M. and Rekart, M.L. (2001). "Antenatal seroprevalence of herpes simplex virus type 2 (HSV-2) in Canadian women: HSV-2 prevalence increases throughout the reproductive years". *Sexually Transmitted Disease.* 28(7): 424–428.

Patton, M.E., Bernstein, K., Liu, G., Zaidi, A. and Markowitz, L.E. (2018). Herpes Seroprevalence Among US Women Seroprevalence of Herpes Simplex Virus Types 1 and 2 Among Pregnant Women and Sexually

Active, Non pregnant Women in the United States. *Clinical Infectious Diseases*. 20(00): 1–8.

Pebody, R.G., Andrews, N. and Brown, D. (2004). "The sero-epidemiology of herpes simplex virus type 1 and 2 in Europe". *Sexually Transmitted Infections.* 80(3): 185–191.

Peña, K.C., Adelson, M.E., Mordechai, E. and Blaho, J.A. (2010). Genital Herpes Simplex Virus Type 1 in Women: Detection in Cervicovaginal Specimens from Gynecological Practices in the United States. *Journal of Clinical Microbiology.* 48(1): 150-153.

Pereira, V.S.S., Moizeis R.N.C., Fernandes, T.A.A., Araujo, J.M.G., Meissner, R.V. and Fernandes, J.V. (2012). Herpes simplex virus type 1 is the main cause of genital herpes in women of Natal, Brazil. *European Journal of Obstetrics and Gynecology and Reproductive Biology*. 161: 190–193.

Prober, C.G., Corey, L., Brown, Z.A., Hensleigh, P.A., Frenkel, L.M., Bryson, Y.J., Whitley, R.J. and Arvin, A.M. (1992). The management of pregnancies complicated by genital infections with herpes simplex virus. *Clinical Infectious Disease*. 15(6): 1031-1038.

Radtke, K., Kieneke, D., Wolfstein, A., Michael, K.Steffen, W., Scholz, T., Karger, A., and Sodeik, B. (2010). Plus-and-minus-end directed microtubule motors bind simultaneously to herpes simplex virus capsids using different inner tegument structures. *PLoS Pathogen.* 6: e1000991.

Raftery, M.J., Behrens, C.K., Muller, A., Krammer, P.H., Valczak, H. and Schonrich, L. (1999). Herpes simplex virus type I infection of activated cytotoxic T cells: Induction of fratricide as a mechanism of viral immune evasion. *Journal of Experimental Medicine.* 190: 1103-1114

Rajagopal, S., Magaret, A., Mugo, N. and Wald, A. (2014). Incidence of Herpes Simplex Virus Type 2 Infections in Africa: A Systematic Review. *Open Forum Infectious Disease.* 1(2): ofu043.

Rajcáni, J. and Vojvodová, A. (1998). The role of herpes simplex virus glycoproteins in the virus replication cycle. *Acta Virology* . 42: 103.

Riley, L.E. (1998). Herpes simplex virus. *Seminar in Perinatology*. 22: 284.

Roberts, C.M., Pfister, J.R. and Spear, S.J. (2003). "Increasing proportion of herpes simplex virus type 1 as a cause of genital herpes infection in college students". *Sexually Transmitted Disease.* 30(10): 797–800.

Roizman, B. (1996). Herpes simplex viruses and their replication. In: Fields BN *et al* editors. *Fields Virology* 3rd ed. Philadelphia: Lippincott-Raven Publishers. Pp. 2297-2342.

Samra, Z., Scherf, E. and Dan, M. (2003). "Herpes simplex virus type 1 is the prevailing cause of genital herpes in the Tel Aviv area, Israel". *Sexually Transmitted Disease.* 30(10): 794–796. .

Schall, T.J. and Bacon, K.B. (1994). Chemokines, leukocyte trafficking, and inflammation. *Current Opinion in Immunology*. 6(6): 865-873.

Schmid, D.S. and Iawle, A.C. (1991). T cell responses to herpes simplex viruses in humans. *Review of Infectious Disease.* 3: 946-949.

Schmid, D.S. and Rouse, B.T. (1992). The role of T cell immunity in control of herpes simplex virus. *Current Topics in Microbial Immunology.* 179: 57-74.

Schneeiss, K.E. (1962). Untersuchungen zur typendifferenzierung des herpesvirus hominis. *Z Immuno-Forsch*. 124: 24-28.

Schulte, J.M., Bellamy, A.R., Hook, E.W., Bernstein, D.I., Levin, M.J., Leone, P.A., Sokol-Anderson, M.L., Ewell, M.G., Wolff, P.A., Heineman, T.C. and Belshe, R.B. (2014). HSV-1 and HSV-2 seroprevalence in the united states among asymptomatic women unaware of any herpes simplex virus infection (Herpevac Trial for Women). *Southern Medical Journal.* 107(2): 79-84. doi: 10.1097/SMJ.0000000000000062

Schuppe, H.C., Meinhardt, A., Allam, J.P., Bergmann, M., Weidner, W. and Haidl, G. (2008). Chronic orchitis: a neglected cause of male infertility? *Andrologia*. 40: 84-91

Serwadda, D., Gray, R. H., Sewankambo, N. K., Wabwire-Mangen, F., Chen, M.Z. and Quinn, T.C. (2003). Human immunodeficiency virus acquisition associated with genital ulcer disease and herpes simplex virus type 2 infection: a nested case-control study in Rakai, Uganda. *Journal of Infectious Disease*. 188(10): 1492–1497

Shaibu, A.M., Aminu, M., Musa, B.O.P. and Bugaje, M.A. (2014). Seroprevalence of IgG Antibodies to Herpes Simplex Virus Type-1 in Nigerian Children. *Nigerian Journal of Medicine*. 23(1): 40-45.

Sheaffer, A.K., Newcomb, W.W., Gao, M., Yu, D., Weller, S.K., Brown, J.C. and Tenney, D.J. (2001). Herpes simplex virus DNA cleavage and packaging proteins associate with the procapsid prior to its maturation, *Journal of Virology.* 75: 687-698.

Shin, H.S., Park, J.J., Chu, C., Song, H.J., Cho, K.S. and Lee, J.S. (2007). "Herpes simplex virus type 2 seroprevalence in Korea: rapid increase of HSV-2 seroprevalence in the 30s in the southern part". *Journal of Korean Medical Science*. 22(6): 957–962.

Singh, A.E., Romanowski, B., Wong, T., Gourishankar, S., Myziuk, L. and Fenton, J. (2005). "Herpes simplex virus seroprevalence and risk factors in 2 Canadian sexually transmitted disease clinics". *Sexually Transmitted Disease*. 32(2): 95–100.

Singh, A., Preiksaittis, J., Ferencry, A. and Romanowski, B. (2005). The Laboratory diagnosis of herpes simplex virus infection. *Canadian Journal of Infectious Disease and Medical Microbiology*. 16: 92-98.

Sizemore, J.M., Lakeman. F., Richard, W.A. and Hook, E.W. (2006). The Spectrum of Genital Herpes Simplex Virus Infection in Men Attending a Sexually Transmitted Disease Clinic. *The Journal of Infectious Diseases*. 193(7): 905–911.

Smith, J.S. and Robinson, N.J. (2002). "Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review". *Journal of Infectious Disease*. 18(1): S3–28. doi:10.1086/343739. PMID 1235318 3.

Smith, J.S., Herrero, R., Muñoz, N., Eluf-Neto, J., Ngelangel, C., Bosch, F.X. and Ashley, R.L. (2001). "Prevalence and risk factors for herpes simplex virus type 2 infection among middle-age women in Brazil and the Philippines". *Sexually Transmitted Disease*. 28(4): 187–194.

Smith, J.S. and Robinson, N.J. (2002). "Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review". *Journal of Infectious Disease*. 186(1): S3–28. doi:10.1086/343739. PMID 12353 183.

Spear, P.G. (2004). Herpes simplex virus: receptors and ligands for cell entry. *Cell Microbiology.* 6: 401.

STD Facts – “Genital Herpes". Centers for Disease Control and Prevention. Retrieved 2007-09-03.

Storch, G.A. (2000). Essentials of Diagnostic Virology. Harcourt Brace & Co. New York, NY.

Strelow, L.I. and Leib, D.A. (1995). Role of the virion host shutoff (vhs) of herpes simplex virus type 1 in latency and pathogenesis. *Journal of Virology*. 69: 6779.

Sucato, G., Celum, C., Dithmer, D., Ashley, R. and Wald, A. (2001). Demographic rather than behavioral risk factors predict herpes simplex virus type 2 infection in sexually active adolescents. *Pediatrics Infectious Disease Journal*. 20: 422–426.

Tan, D.S.H., Murphy, K., Shah, P. and Walmsley, S.L. (2013). Herpes simplex virus type 2 and HIV disease progression: a systematic review of observational studies. *BMC Infectious Disease*. 13: 502.

Torok, E., Moran, E. and Cooke, F. (2009). Congenital infections. In: Oxford Handbook of Infectious Diseases and Microbiology. 1st edition. New York: Oxford University Press. 8245 – 8269.

UPDATE: CDC Stands Behind Report That Half of Black Women Have Herpes". News One. Retrieved 2011-02-03.

Van Benthem, B.H.B., Spaargaren, J., Van den Hoek, J., Merks, J., Coutinho, R.A., Prins, M. and the European Study on the Natural History of HIV Infection in Women (2001). Prevalence and risk factors of HSV-1 and HSV-2 antibodies in European HIV infected women. *Sexually Transmitted Infections* . 77: 120–124.

Van Doornum, G.J., Guldemeester, J., Osterhaus, A.D. and Niesters, H.G. (2003). Diagnosing herpesvirus infections by real-time amplification and rapid culture. *Journal of Clinical Microbiology*. 41: 576-580.

Varela, J. A., Garcia-Corbeira, P. and Aguanell, M.V. (2001). Herpes simplex virus type -2 seroepidemiology in Spain: prevalence and sero conversion rate among sexually transmitted disease clinic attendees. *Sexually Transmitted Disease.* 28: 47–50.

Wald, A., Langenberg, A.G., Link, K., Izu, A.E., Ashley, R., Warren, T., Tyring, S., Douglas, J.M. and Corey, L. (2001) Antiviral chemotherapeutic agent against HSV. *Journal of American Medical Association*. 285: 3100-3106.

Wald, A. and Link, K. (2002). Risk of Human Immunodeficiency Virus Infection in Herpes Simplex Virus Type-2Seropositive Person: A meta-Analysis. *Journal of Infectious Disease*. 185(1): 45-51.

Wald, A. and Ashley-Morrow, R. (2002). Serological testing for herpes simplex virus (HSV)-1 and HSV-2 infection. *Clinical Infectious Disease*. 35(2): S173-182.

Wald, A., Celum, C.L. and Quinn, T.C. (2004). The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. *Journal of Acquired Immune Deficiency Syndrome*. 35: 435–445.

Wald, A. (2006). Genital HSV-1 infections. *Sexually Transmitted Infections*. 82: 189-190.

Wald, A. and Corey, L. (2007). "Chapter 36: Persistence in the population: epidemiology, transmission". Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge University Press. *ISBN* 978-0-521-0.

Wald, A. and Corey, L. (2007). Persistence in the population: epidemiology, transmission". Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge University Press. Chapter 36: *ISBN 978-0-521-82714-0.*

Wald, A. (2011). Genital shedding of herpes simplex virus among symptomatic and asymptomatic person with HSV-2 infection. *Journal of American Medical Association*. 305: 1441-1449

Ward, S.G. and Westwick, J. (1998). Chemokines: understanding their role in T-lymphocyte biology. *Biochemical Journal.* 333: 457-470.

Weiss, H., Buve, A. and Robinson N. (2001). The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations. *Acquired Immune Deficiency Syndrome*. 15(4): S97–S108.

Weiss, H. (2004). "Epidemiology of herpes simplex virus type 2 infection in the developing world″. *Herpes*. 11(1): 24A–35A.

Whitley, R.J. (1990). Herpes Simplex Viruses. In: Fields BN *et al*., editors. *Fields Virology* 2nd ed. Philadelphia: Lippincott-Raven Publishers pp:1843-1887.

Whitley, R.J., Corey, L., Arvin, A., Lakeman, F.D., Sumaya, C.V., Wright, P.F., Dunkle, L.M., Steele, R.W., Soong, S.J. and Nahmias, A.J. (1998). Changing presentation of herpes simplex virus infection in neonates. *Journal of Infectious Disease.* 158:109–116.

Whitley, R.W. and Roizman, B. (2001). Herpes simplex virus infections. *Lancet*. 357: 1513.

Roizman, B. (1996). Herpes simplex viruses and their replication. In: Fields BN et al editors. *Fields Virology* 3rd ed. Philadelphia: Lippincott-Raven Publishers. Pp. 2297-2342.

World Health Organization (2017). Herpes simplex virus. January 31, 2017. Accessed: June 30, 2018 http://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus.

Wilhelmus, K.R., Coster, D.J., Donovan, H.C., Falcon M.G. and Jones B.R. (1981). Prognosis indicators of herpetic keratitis. Analysis of a five-year observation period after comeal ulceration. *Archives of Ophthalmology*. 99: 1578-1582.

Wishart, M.S., Darougar, S. and Viswalingam, N.D. (1987). Recurrent herpes simplex virus ocular infection:epidemiological and clinical features. *British Journal of Ophthalmology*. 71: 669-672.

Wu, T.J., Monokian, G., Mark, D.F. and Wobbe, C.R. (1994). Transcriptional activation by herpes simplex virus type 1 VP16 in vitro and its inhibition by oligopeptides. *Molecular and Cellular Biology*. 14: 3484.

Wysocka, J. and Herr, W. (2003). The herpes simplex virus VP16-induced complex: the makings of a regulatory switch. *Trends in Biochemical Science.* 28: 294.

Xu, F., Schillinger, J. A., Sternberg, M.R., Johnson, R.E., Lee, F.K., Nahmias, A.J. and Markowitz, L.E. (2002). Seroprevalence and coinfection with herpes simplex virus type 1 and type 2 in the United States, 1988–1994. *Journal of Infectious Disease.* 185(8): 1019–1024.

Xu, F., Sternberg, M.R., Kottiri, B.J., McQuillan, G.M., Lee, F.K., Nahmias, A.J., Berman, S.M. and Markowitz, L.E. (2006). "Trends in Herpes Simplex Virus Type 1 and Type 2 Seroprevalence in the United States". *Journal of American Medical Association.* 296(8): 964–973.

Xu, F., Sternberg, M.R., Gottlieb, S.L., Berman, S.M., Markowitz, L.E. and Forhan, S.E. (2010). "Seroprevalence of Herpes Simplex Virus Type 2 Among Persons Aged 14–49 Years—United States, 2005–2008". *Morbidity and Mortality Weekly Report* (*MMWR*). 59 (15): 456–459.

Yudovin-Farber, I., Gurt, I., Hope, R., Domb, A.J. and Katz, E. (2009). Inhibition of herpes simplex virus bypolyamines. *Antiviral Chemistry and Chemotherapy.* 20: 87-98.

Zaideman, E. (2013). More U.S. Teens Susceptible to HSV-1 Infection, A Growing Cause of Genital Herpes. *Journal of Infectious Diseases*. 312: 557-770.

APPENDIX I

MATERIALS

Materials needed for the research are listed below:

- Vacutainers syringe/needle 400 pieces
- Questionnaires – 400 copies
- EDTA container 400 pieces
- NON EDTA/plain blood containers 3000 pieces
- Hand gloves – 1 packet
- Tunicate – 2 pieces
- Cotton wool – 2 rolls
- Methylated spirit – 5 litres
- Equipment:

(a) Elisa washer and reader machine and calbiotech HSV-1 and HSV-2 1gG Elisa Kits

(b) CD4 count machine and count check beads reagent.

Components of the Kit

Microwells coated with HSV-1 antigen

Sample Diluent:1 vial(ready to use)

Calibrator: 1 vial (ready to use)

Positive Control: 1 vial (ready to use)

Negative Control: 1 vial (ready to use)

Enzyme Conjugate 1 bottle (ready to use)

TBM Substrate: 1 Bottle (ready to use)

Stop Solution:1 bottle (ready to use)

Wash Solution, 20× 1 bottle.

Interpretation of result:

Antibody Index Interpretation

< 0.9 – No detectable Ab to HSV-1 or 2

0.9 – 1.1 – Border line positive. Follow up testing is recommended

>1.1 – Detectable 1gG antibody to HSV-1 or 2 by Elisa of one which to convent antibody index to 1μ/ml, Ab Index is multiply by 100.

< 901μ/ml No detectable 1gG antibody

90 – 110 1μ/ml Borderline

>1101μ/ml Detectable 1gG antibody

Components of the Kit

Microwells coated with HSV-1 antigen

Sample Diluent:1 vial (ready to use)

Calibrator: 1 vial (ready to use)

Positive Control: 1 vial (ready to use)

Negative Control: 1 vial (ready to use)

Enzyme Conjugate 1 bottle (ready to use)

TBM Substrate: 1 Bottle (ready to use)

Stop Solution:1 bottle (ready to use)

Wash Solution, 20×: 1 bottle.

Reagent Preparation (1x wash buffer)

Wash buffer (25ml, 20x) was added to 475ml of deionized water and it is stored at room temperature.

Interpretation of Result

Antibody Index Interpretation

< 0.9 – No detectable Ab to HSV-1 or 2

0.9 – 1.1 – Border line positive. Follow up testing is recommended

>1.1 – Detectable 1gG antibody to HSV-1 or 2 by Elisa of one which to convent antibody intex to 1μ/ml, Ab Index is multiply by 100.

< 901μ/ml No detectable 1gG antibody

90 – 110 1μ/ml Borderline

>1101μ/ml Detectable 1gG antibody

Components of the Kit

1 Vial of CD_4^+ mAb PE

1 Bottle of no lyse buffer

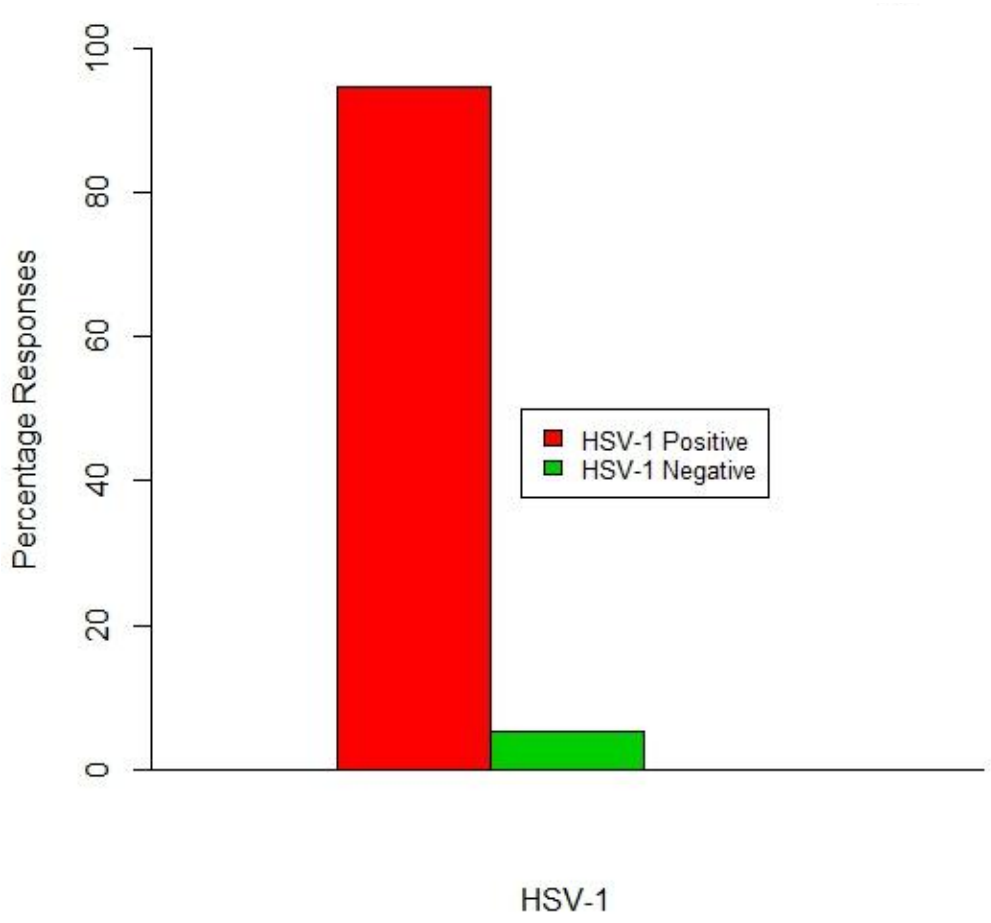
Standardization of the Equipment (Sysmex)

The equipment was rinsed by wash fluid, and Count Check Bead standard was used to adjust the equipment to the normal range. The Count Check Bead was then rinsed by wash fluid immediately.

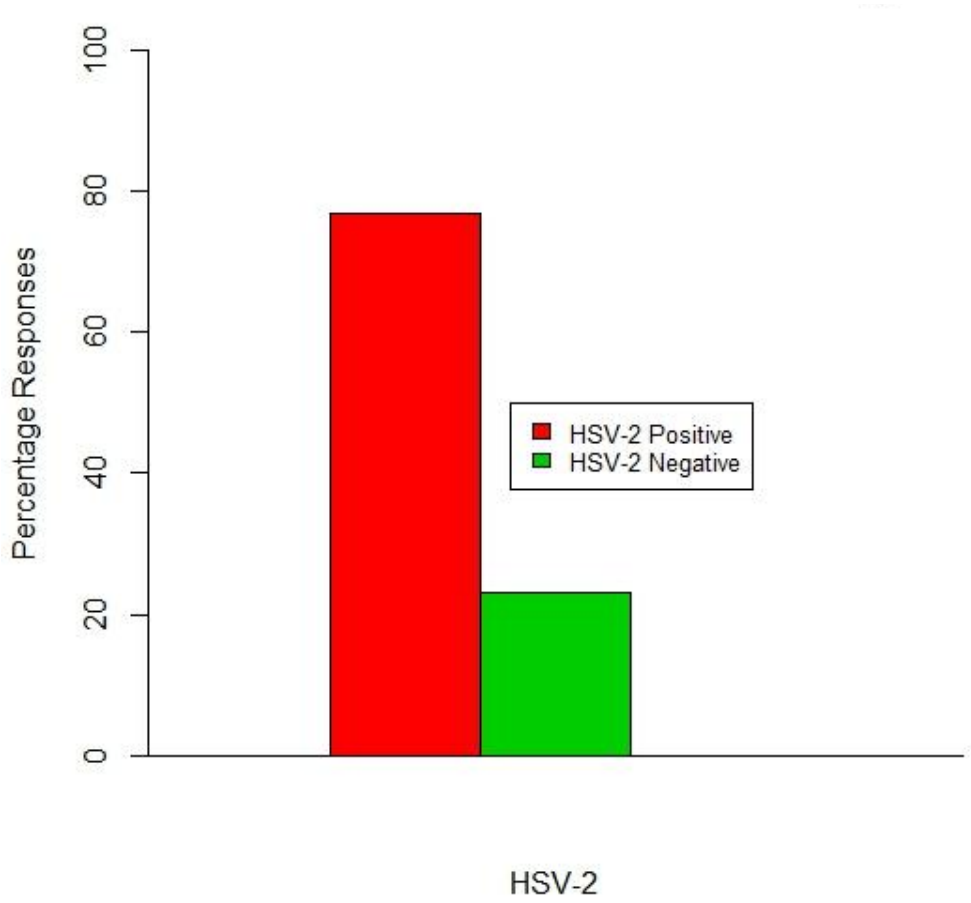
Interpretation of result

The results obtained were compared with the normal range and it is interpreted as low, normal or high.

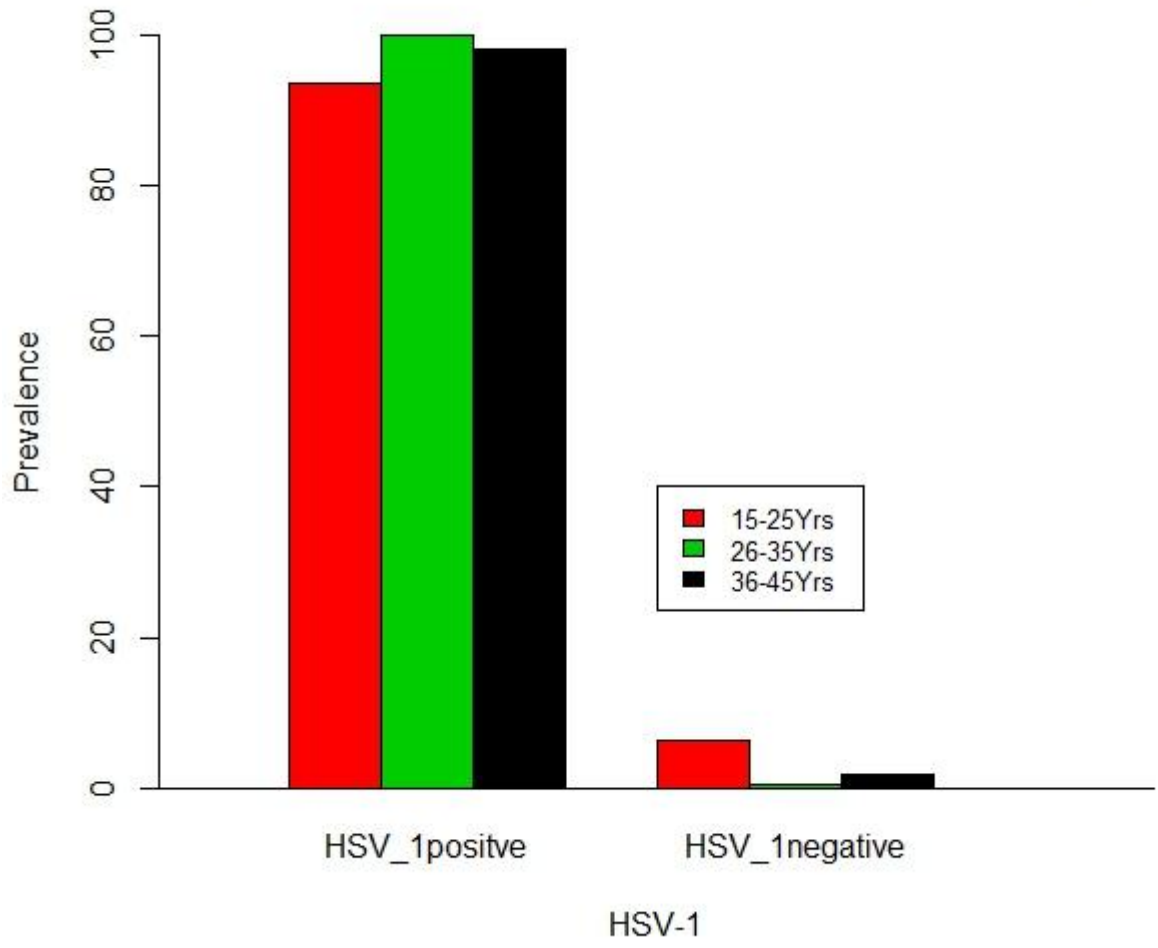
APPENDIX II



Bar Plot for HSV-1 Prevalence of Chile Bearing Women



Bar Plot for HSV-2 Prevalence of Chile Bearing Women



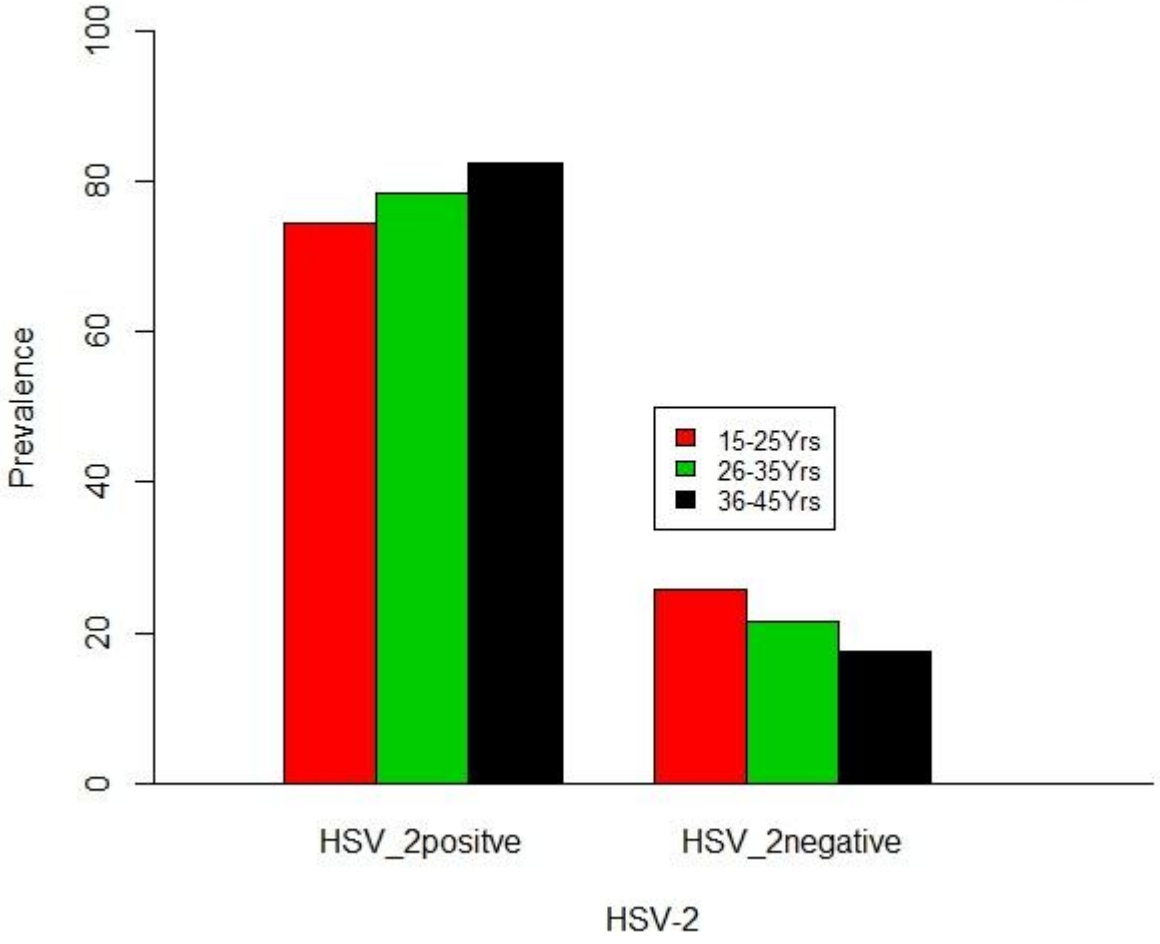
Age Distribution for HSV-1 Prevalence of Chile Bearing Women

HYPOTHESIS

H_0: The distribution of HSV – *1 in women of child bearing age is independent on their age*

H_1: The distribution of HSV – *1 in women of child bearing age is dependent on their age*

Conclusion: Since P-value for the test statistics is obtained as p=0.0216, we reject H_o and conclude that the distribut[ion of HSV-1 in women of child bearing age is dependent on their age.



Age Distribution for HSV-2 Prevalence of Chile Bearing Women

HYPOTHESIS

H_0: *The distribution of HSV – 2 in women of child bearing age is independent on their age*

H_1: *The distribution of HSV – 2 in women of child bearing age is dependent on their age*

Conclusion: Since P-value for the test statistics is obtained as p=0.4249, we accept H_o and conclude that the distribution of HSV-2 in women of child bearing age is independent on their age.