CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Herpes simplex virus type-2 is one of the most common sexually transmitted infection, It is highly prevalent in human populations in many parts of the worlds and the primary cause of genital ulcer disease (GUD) worldwide (Chen *et al*., 2000).

Herpes simplex virus-2 (HSV-2) is neurotropic virus that has a large linear, double stranded DNA genome protected by a capsid with icosahedral symmetry surrounded by an envelope consisting of a lipid bilayer with embedded glycol proteins, having yet a protein aceous region between the capsid and envelope called tegument, belong to alphaherpesvirinae subfamily, within the family herpesviridae (Grünewald *et al*., 2003).

Transmitted across mucosal membranes and non intact skin, that migrate to nerve tissues, where they persist in a latent state. The predominant symptom of the disease is genital lesions, but a majority of infected individuals experience no symptoms or mild ones that are often unrecognized. The high rate of asymptomatic cases enhances HSV-2 transmission because asymptomatic individuals shed the virus and transmit the disease (Corey *et al*., 2004).
A primary symptomatic genital herpes infection is usually the most severe, especially in women. It causes blistering and ulceration of the external genitalia and cervix leading to

vulval pain, dysuria, vaginal discharge and local lymphoadenopathy (Greenwood, 2002). Pregnant women are particularly vulnerable to the adverse sequelae of HSV-2 infection. Intra-uterine herpes simplex virus infection can cause significant morbidity and mortality in the developing fetus if the pregnant mother gets acute infection during pregnancy. The infection has been associated with abortion, premature delivery, low birth weight infants,

fetal malformations, and vertical transmission of the virus during childbirth. While neonatal herpes occurs in less than 1% of prevalent infections, the risk of transmission increases to 25–50% among asymptomatic women infected during pregnancy (Straface *et al*., 2012).

The postpartum period is a time when women are potentially more susceptible to STIs due to the traumatic nature of the vaginal delivery and subsequent lack of oestrogen during lactation (Albers *et al*., 2006).
HSV infection can easily spread in populations because of asymptomatic nature of disease, and is a suitable marker to evaluate the sexual behaviors (Corey and Handsfield, 2000).
Genital herpes are caused by two strains HSV-1 and HSV-2. In addition, it is estimated that prevalent HSV-2 infection is associated with a 2- to 4- fold increased risk of HIV-1 acquisition (Corey *et al*., 2004).

The seroprevalence of HSV-2 varies by region, country and population. Various studies have estimated that about 40% of pregnant women in sub-Saharan Africa, 30% in Latin America, 17–22% in North America, and 4 to 24% in Europe are HSV-2 infected (Anzivino *et al*., 2009).
Infection is a significant factor for increased risk of acquisition and transmission of human immunodeficiency virus (HIV). A meta-analysis of studies of HSV-2 found that infection with HSV-2 doubled the risk of becoming infected with HIV through transmission during sexual activity (Wald and Link 2002).
Because genital HSV-2 infection is generally recurrent and incurable viral disease, the presence of antibody to HSV-2 and a compatible clinical history would be strong presumptive evidence that the disease was recurrent genital herpes (Guerry *et al*., 2005).

1.2. Rationale

Genital herpes remains a major problem causing considerable abortion among pregnant women and a higher risk to their infants leading to serious complication including in intrauterine growth retardation, preterm labor, and congenital and neonatal herpes infections. More than 50% of affected infants have moderate or more severe neurological impairment, with a 20% overall mortality, approximately 90% of all neonatal herpes infections are transmitted during delivery and at least 5% transmitted in utero (Ciavattini *et al.*, 2007).

1.3. Objectives

1.3.1. General objective

To study the prevalence of Herpes simplex virus-2 (HSV-2) among pregnant women.

1.3.2. Specific objectives

1- To determine the prevalence of Herpes simplex virus II IgG antibodies in pregnant women in Khartoum State.

2- To demonstrate the role of genital herpes related to previous spontaneous abortion in women.

3- To determine the risk and influencing factors in pregnant women.

CHAPTER TWO

LITERATURE REVIEW

2.1. Background

Herpes has been known for almost 2,000 years. The term Herpes simplex only appeared for the first time in Richard Boulton's System of Rational and Practical Chirurgery in 1713. In the 1940s it was finally recognized as a virus. Since then several work has been done on the virus and a lot of information is known concerning the virus. From 1960 to 1970, HSV-2 infection was considered a possible causative agent of cervical cancer (Hawes and Kiviat, 2002).

The role of HSV-2 in the development of cervical cancer has been questioned, since HSV-2 DNA was not found in cervical cancer biopsies. Herpes Simplex Virus Type-2 (HSV-2) is a sexually transmitted infection (STI) that is chronic widespread, an estimated 536 million people aged 15-49 are infected with herpes simplex type-2 (HSV-2) worldwide (Looker *et al*., 2008).

The infection occurs during pregnancy lead to the problem of neonatal herpes, a serious condition because of the high mortality and long-term neurological sequelae noted in about 20% of survivors (Kim *et al*., 2012).

HSV disease of the newborn can be acquired during one of the three time periods: in utero, perinatally, or postnatally. Pregnant women then become a target for surveillance of the infection in order to avoid the risk of neonatal transmission. Seroprevalence studies showed wide variations in infection rates by geographic location (Eskild *et al*., 2000).

Herpes simplex type-2 is gaining special attention as a significant risk factor for acquisition of human immunodeficiency virus (HIV). It is assumed that infection with HSV-2 disrupts the genital mucosa and provides a portal of entry for HIV, leading to increased susceptibility of HIV (Biswas *et al*., 2011).

2.2. Taxonomy

HSV-2 belongs to the family Herpesviridae. The family is divided into three subfamilies; α (alpha), β (beta) and γ (gamma) herpesviruses, based on biological properties. At present nine herpesviruses are recognized as natural human pathogens (Grünewald *et al*., 2003).

Herpes simplex virus types 1, 2 and Varicella-zoster virus (VZV) belong to the alphaherpesviruses *(*alphaherpesvirinae), which have a wide host range, a relatively short life cycle and establish latent infections preferentially in sensory ganglia (Efstathiou and Preston, 2005).

2.3. Structure and composition

All herpesviruses share some common properties, have a unique four layered structure; a core containing the large, double stranded DNA genome is enclosed by an icosahedron capsid which is composed of capsomers. The capsid is surrounded by an amorphous protein coat called the tegument. It is encased in a glycoprotein bearing lipid bilayer envelope, this complete particle known as a virion (Mettenleiter *et al*., 2006).

The HSV genome contains approximately 152-kbp (Rajcani *et al*., 2004).

HSV-2 contain at least 74 genes or (open reading frames ORFs) within their genomes The genomes of HSV-2 are complex and contain two unique regions called the long unique region (U_L) and the short unique region (Us). Of the 74 known ORFs, UL contain 56 viral gene , whereas Us contain only 12 (McGeoch *et al*., 2006).

The two unique region flanked by large inverted repeat sequences which are designated terminal and internal repeats of the long (TRL and IRL) and short (TRS and IRS) unique sequences (Rajcani *et al*., 2004).

The genes of the long and short unique sequences are designated U_L 1 to U_L 56 and Us 1 to Us 12, respectively (*McGeoch et al.,2006*).

These genes encode a variety of proteins involved in forming the capsid, tegument and envelope of the virus as well as controlling the replication and infectivity of the virus. On the outer side of the tegument is the envelope, which is a lipid bilayer derived from the host cell. HSV specifies at least 12 “gp” glycoproteins designated gB, gC, gD, gE, gG, gH, gI, gK,gL, gM and gJ and gN (Whitley and Roizman, 2001).

These glycoproteins function in several important roles, including pH-independent virus entry via fusion of the virion envelope with cellular membranes (Milne *et al*., 2005).

2.4. Replication

The double-stranded DNA of HSV-2 enters into the nucleus of the cell where replication takes part, a controlled program of viral gene expression is initiated. HSV-2 replicates by three rounds of transcription that yields; immediate-early (α) proteins that mainly regulate viral replication; early (β) proteins that synthesis and package DNA; and late (γ) proteins, most of which are virion proteins cells (Cunningham *et al*., 2006).

First, approximately 2 to 4 h after infection, the α-genes are expressed. These immediate early (IE) genes code for proteins which are mostly regulatory in function. They are essential for efficient production of the subsequent proteins. Second, the synthesis of β-genes (early genes) reaches a peak 5 to 7 h post infection. The proteins encoded by β-genes are involved in viral DNA synthesis and nucleic acid metabolism. The viral DNA polymerase is an example of a β-gene product. Third, the γ-genes (late genes) encode for structural components such as capsid and envelope proteins, Expression of late genes occurs last; this group of genes predominantly encode proteins that form the virion particle (*McGeoch et al., 2006).*

2.5. Transmission and acquisition

Human are the only natural resevior for transmission of HSV-2 (Kimberlin, 2004). Infection is acquired almost invariably via sexual contact with infected genital secretions. Of the partner and as such is decreased by condom use, congenitally the most common mode of transmission is via direct contact of the baby with infected vaginal secretions during delivery (Thompson and Whitley, 2011).

Transmission can occur in the absence of lesional recurrence as a result of subclinical viral shedding. This risk of transmission and the severity of the neonatal disease has been reported to increase further if the woman is HIV-infected (Chen *et al*., 2005).

Congenital infection is very rare due to the acquisition of the virus in utero; it comes to the neonatal HSV infection when the appearances of the lesions are more than 48 hours after birth (Money and Steben, 2009).

2.6. Pathogenisis

HSV-2 enters the body through mucosal tissues or small lesions by fusion of the viral envelope with the plasma membrane. Epithelial cells and keratinocytes are the primary target cells for HSV-2, but it also infects neuronal and immune cells. While epithelial cells are destroyed during lytic HSV replication, neuronal cells are not destroyed and provide a reservoir for latent virus; Once travels via retrograde transport along nerve axons to the dorsal root ganglia (DRG), where latency is established (Cunningham *et al*., 2006).

The entry requires the combination of viral glycoprotein D (gD) with its receptors, including herpesvirus entry mediator (HVEM) is a member of the tumor necrosis factor receptor (TNFR) family, nectin-1 and -2 and specific sites in heparin sulfate proteoglycan (Jogger *et al*., 2004).

The interaction between gD and HVEM during acute infection with HSV decreases the subsequent CD8+ recall response at the genital mucosa (Kopp *et al*., 2012).

Once infected, the virus causes life-long infection of the host by establishing latency in the neurons of the sensitive ganglia. Reactivation occurs periodically, at times when the immune system is suboptimal, resulting in the formation of vesicles that break into ulcerations at the genital mucosa (Roizman *et al*., 2007).

2.7. Latency and Reactivation

The viral genome is maintained within ganglia for the life of a host in a quiescent condition termed latency, one of the major features of the HSV infection is the ability of the virus to remain latent in the sensory ganglia. Following an HSV infection viral

replication occurs in the oral or genital mucosa and the virus enters sensory nerve endings innervating the mucosal membranes. While epithelial cells are destroyed during lytic HSV replication, neuronal cells are not destroyed and provide a reservoir for latent virus. During reactivation, the virus travels from the dorsal root ganglia (DRG) back to the skin and causes detection of virus from epithelial surfaces (known as viral shedding). Viral nucleocapsids are transported to the nucleus of the sensory neurons (ganglia) by the retrograde axonal transportation system via the microtubules. At the nuclear membrane the content of viral DNA is released through the nuclear pores into the nucleus followed by a productive infection or the establishment of latency. In the latent infection the viral DNA is maintained as a circular episome and the expression of viral genes may be silenced. An 8.5 kb RNA is transcribed from within the long repeat region of the genome and spliced into a family of smaller species designated latency-associated transcript (LAT). The LAT gene promoter shows neuronal specificity and when neurons express LAT they establish latency, LAT is associated with the prevention of apoptosis of the neuronal cells. This process involves condensation of the chromatin on the lytic gene promotors followed by inactivation (Knipe and Cliffe, 2008).

2.8. Epidemiology

Herpes simplex virus type-2 (HSV-2) is a sexually transmitted pathogen that infects more than 500 million people worldwide and causes an estimated 23 million new infections each year (Looker *et al*., 2008).

HSV-2 seroprevalence ranges from 16% among 14–49 year olds in the United States (CDC, 2010), to greater than 80% in some areas of sub-Saharan Africa (Bailey *et al*., 2007).

Seroprevalence in women is up to twice as high as men, and increases with age, **i**n addition, ethnicity, poverty, cocaine abuse, earlier onset of sexual activity, sexual behavior, and bacterial vaginosis can facilitate the risk of infection in women before pregnancy (Cherpes *et al*., 2003).

HSV-2 is widespread even among people with low or moderate levels of sexual activity. For instance, 18.8% of American women with 2–4 lifetime sexual partners are HSV-2 seropositive (CDC, 2010).

The estimated incidence of neonatal herpes varies widely, from 4 to 31 in 100,000 live births. In populations with 80% seroprevalence, nearly 50% of HIV infections are attributable to prevalent HSV-2 (Wald and Link, 2002).

The mechanism of increased risk of HIV acquisition includes influx of HIV target cells.

In HIV-negative individual, genital ulcer might increase susceptibility to HIV infection by disrupting the mucosal barrier and by inflammatory changes, which increase recruitment of HIV target cells to the ulcer (Zhu *et al*., 2009).

2.9. Clinical significance

Herpes simplex type -2 viruses are common human pathogens that can cause primary and recurrent infections of mucous membranes. Primary HSV infections are usually symptomatic but may be sub clinical. symptomatic cases clinical presentation of genital herpes usually occurs after an incubation of a period of 2–20 days and lasts up to 21 days (Cusini and Ghislanzoni, 2001).

Herpes simplex type -2 virus is typically spread through sexual contact and results in a lifelong infection. The predominant symptom of the disease is genital lesions, but a majority of infected individuals experience no symptoms or mild ones that are often unrecognized (Corey and Handsfield, 2000).

Recurrent infections are generally less severe than the primary infection. A primary symptomatic genital herpes infection is usually the most severe, especially in women. The most commonly seen clinical manifestations include blistering and ulceration of the external genital and cervix leading to vulval pain, dysuria, vaginal discharge and local lymphoadenopathy. Intra-uterine HSV infection can cause significant morbidity and mortality in the developing fetus if the pregnant mother gets acute infection during pregnancy, the acquisition of genital herpes during pregnancy result in spontaneous abortion stillbirth, intrauterine growth retardation, preterm labour, congenital and neonatal herpes infections (Apurba *et al*., 2013).

Herpes simplex type-2 mucosal ulcerations are normally self-limited. However, systemic complications such as recurrent meningitis, hepatitis, and pneumonitis can occur during acquisition or reactivation of infection, particularly among patients with poor T-cell immunity due to AIDS, organ transplantation or chemotherapy. Meningitis is usually benign, but the HSV encephalitis has been associated with high mortality (Tyler, 2004).

Neonatal infection with HSV-2 is symptomatic in almost all cases and divided into localised, central nervous system (CNS) disease and disseminated disease. Localised congenital HSV infection is limited to the skin, eye or mouth, whereas CNS disease results in encephalitis, and disseminated disease leads to multiple organ damage (Remington *et al*., 2011).

2.10. Laboratory diagnosis

Diagnosis of genital herpes (HSV-2) must not be based solely on clinical presentation since it has a sensitivity of 40% and specificity of 99% with about 20% rate of false-positive (Sauerbrei and Wutzler, 2007).

Confirmation of the precence of HSV-2 infection by either serological test or viral detection technique include PCR and viral culture. Serological tests allows identification of silent carriers of HSV infection and provide useful information in symptomatic patients when virological tests such as culture, antigen detection and PCR are not helpful (Ashley, 2001).

2.10.1. Specimen

For active lesions, collection of vesicular fluid or exudates from small vesicles is the method of choice. After sampling, the specimens for viral culture, antigen or detection

of HSV DNA genome should be placed immediately into vials containing 1 ml of appropriate viral transport medium, or an universal transport medium because HSV is highly sensitive to desiccation and pH inactivation. Specimen should also be transferred quickly to a diagnostic virology laboratory on ice (+4°C) as the virus infectivity is heat labile (Legoff *et al*., 2014).

For sample lesions collection, a small cotton, cotton tipped, or Dacron swab on a wire shaft is used for viral culture as well as molecular biology, calcium alginate swabs are toxic to HSV and therefore should not be used for virus isolation in cell culture. Blood samples were collected from the subjects by vene puncture and serum samples were analyzed for HSV-2 IgG and IgM antibodies using type-specific serological test ,whole blood analyzed for HSV-2 using PCR technique (Walsh *et al*., 2008).

2.10.2. Viral isolation in cell culture

The standard laboratory method to confirm current HSV infection is virus isolation and typing in cell culture since HSV grows readily in tissue culture. A positive genital culture provides conclusive evidence of genital HSV infection; however, a negative result does not exclude the presence of infection. The virus may be isolated within 2–4 days from

swabs taken from herpetic skin and laryngeal or genital lesions however, the probability of obtaining a positive HSV culture in infected infants is greater with culture from the skin lesions (Kimberlin *et al.*, 2001).

This method, however, has its own limitation where the sensitivity of the test can be compromised by poor sampling and transportation of the specimen and in cases where lesions are healed, they may result in negative culture (Berardi *et al.*, 2011).

2.10.3. Antigen detection

Viral antigen can be easily detected by direct or indirect immunofluorescence (IF) assay using fluorescein-labelled type-specific monoclonal antibodies on smears, or by enzyme immunoassay (EIA) on swabs. For detecting HSV in lesions, the sensitivity of antigen detection tests may be the same as that of culture assay but is lower than nucleic acid amplification test sensibility (Burrows *et al*., 2002).

As indirect IF assay and EIA perform satisfactorily in symptomatic patients, these direct methods may offer a rapid diagnostic alternative in settings where laboratory facilities are limited and where specimen handling and transportation conditions could inactivate the virus. This is true for remote locations where prolonged specimen transport time under inappropriate conditions may occur before delivery to the microbiology laboratory.

Under a fluorescence microscope, infected cells will be recognized by the presence of a characteristic pattern of apple-green fluorescence in the nucleus and cytoplasm of the basal and parabasal cells.

2.10.4. Nucleic acid detection

Molecular biology has emerged for the last ten years as an attractive potent method to to detect and possibly quantify HSV-2 DNA. Polymerase chain reaction techniques involve the amplification of particular sequences of DNA. and can thus detect evidence of viral DNA at low concentrations. In one very large study, PCR results were three to five times more likely to be positive than were cultures; while cultures were more likely

to be positive at increasing concentrations of virus. PCR provides increased sensitivity over culture and may ultimately replace culture as the standard of care for diagnosis (Domeika *et al*., 2010).

Several procedures have been proposed to detect and quantify HSV genomes in clinical samples, including competitive PCR, PCR detection followed by DNA enzyme immunoassay hybridization , real time PCR assay (Legoff *et al*., 2006).

The majority of commercial PCR targeting the HSV genome are currently based on real-time PCR which allows both the detection and the quantification of HSV-2 in clinical specimen. has improved the probability of obtaining a speedy diagnosis, nonetheless, negative results does not rule out the presence of an infection (Kimberlin, 2007).

2.10.5. **Serological tests**

Detection of HSV-specific IgG antibodies can be done sensitively by several immunological methods. Accurate type-specific HSV-2 serologic assays are based on the detection of HSV-2 specific gG2 (HSV-2) antibodies using native, purified or recombinant gG2 as antigens. Type-specific IgG antibodies are negative in early presentations of herpes disease, and become detectable two weeks to three months after the onset of symptoms and persist indefinitely. The presence of HSV-2 antibodies especially IgG in pregnant women is an indication of recurrent infection if vesicles are found in the genital tract (Arvin *et al.*, 2006).

Consequently, primary HSV infections can be documented by using any serologic methods to show seroconversion with paired sera. HSV IgM testing substantially increased the ability to detect early infection in patients who lack detectable IgG, but may be negative during primary disease. IgM testing can also be positive during reactivation of disease and cannot be used to distinguish primary from recurrent infection. Because of these limitations, HSV IgM testing has limited availability in routine diagnostic settings and cannot be recommended in routine clinical practice. The sensitivities of these gG type-specific tests for the detection of HSV- 2 antibody vary from 80–98%, and false-negative results might be more frequent at early stages of infection (LeGoff *et al*., 2008).

2.11. Prophylaxis of HSV-2 infection

The high rate of undiagnosed or asymptomatic HSV-2 infections complicate the prevention. In order to avoid the majority of neonatal herpes cases, identification of the infection at risk mother is the goal. The first and most important step is the determination of the pregnant women serostatus to establish their susceptibility to the infection during early pregnancy (Baker, 2007).

The most effective measure to prevent perinatal herpes infections is to avoid viral exposure to the neonate when primary genital herpes develops in late pregnancy whereas the risk of severe neonatal infection is small in recurrent episodes (Sauerbrei and Wutzler, 2007).

A history of HSV infection in all pregnant women and their partner should be obtained at the first prenatal visit, Women with a negative personal history of HSV and especially those with a positive history in the male partner, should be strongly advised to have no oral and sexual intercourse at the time of recurrence in order to avoid infection particular during the third trimester of gestation , use of condoms throughout pregnancy should be recommended to minimize the risk of viral acquisition although the male partner has no active lesions (Sauerbrei and Wutzler, 2007).

The risk of infection to the infant appears to be higher when the first infection occurs during the third trimester of pregnancy. In this case there may not be sufficient time for the development of maternal IgG and their passage to the fetus, and the risk of neonatal infection is 30 to 50% (Gardella and Brown, 2011).

A careful examination of the vulva, vagina and cervix should be performed on any woman who presents signs or symptoms of HSV infection at the onset of labour. All pregnants who have a suspected active genital HSV infection or prodromal symptoms of HSV infection should undergo caesarean section, although membranes are intact, when genital herpes lesions are not present, caesarean delivery is not required but lesions should be covered with an occlusive dressing before vaginal delivery (Rudnick and Hoekzema, 2002).

It is important to remember that foetal scalp electrodes monitoring during labour and vacuum or forceps delivery should be used only if necessary, since these practices appear to increase the risk of HSV-2 transmission (Baker, 2007).

Neonates, born to women with active genital lesions, with a confirmed or suspected HSV infection should be isolated, managed with contact precautions to avoid direct contact with skin and mucosal lesions, excretions, body fluids and immediately treated with intravenous acyclovir, since neonatal herpes can also be acquired postnatally, postpartum women, family members and nursery personnel with active herpetic lesions of the mouth, skin or breast should take necessary precautionary measures to prevent direct contact with the neonate and/or should be excluded from the neonatal unit until the lesions are fully healed (Sauerbrei and Wutzler, 2007).

2.12. Vaccination

About prophylactic vaccines, partially effective prophylactic vaccines may still be useful if they shift the threshold of infection, or if they prevent or improve disease. They could reduce HSV2 incidence by preventing infection or by reducing the shedding or clinical recurrences in a HSV2-infected individual. On the other hand, these vaccines could increase HSV2 incidence reducing symptomatic signs of disease without effect on viral shedding. In terms of vaccine components and mechanisms, HSV-2 vaccines are divided as follows: inactivated vaccine, live attenuated vaccine, replication defective vaccine, subunit vaccine, peptide vaccine, live vector vaccine and DNA vaccine have been used in developing both prophylactic and therapeutic vaccines, since several antiviral therapies are available to control disease and spread, but these are not completely effective and do not affect latent virus (Ramachandran and Kinchington, 2007).

Widely used routes of delivery for HSV-2 vaccines include subcutaneous, intramuscular, intraperitoneal, intranasal, intravaginal (IVAG), intradermal and intravenous delivery, the route of immunization has an impact on the efficacy of HSV-2 vaccine via different immune responses at different sites (Awasthi *et al.,* 2012).

2.13. Therapy

Treatment with acyclovir and valacyclovir by 36 weeks of pregnancy to term reduces the frequency of clinical manifestations, vertical transmission, elimination of the virus during birth by reducing the percentage of caesarean. Since acyclovir and valacyclovir are not officially approved for treatment of pregnant women, patients should be informed to give consent before the administration. However, no increase of foetal abnormalities was ascribed to these treatments, although long term outcomes were not evaluated (CDC, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a cross- sectional study.

3.1.2. Study area

The study was conducted in two hospitals and one Diagnostic Centre in Khartoum State. These were Omdurman Friendship Hospital, Al Saudi Obstetrics and Gynecology Hospital, Ultra Laboratory Diagnostic Centre. The practical part of this study was done in Research Laboratory, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

This study was conducted during the period from February to May 2016.

3.1.4. Study population

Pregnant women with different trimesters of pregnancy stage. They aged between (16-40) years old. Some of them had a history of miscarriage.

3.1.5. Data collection

The Sociodemographic data were collected by structured questionnaire (appendix 1) from each pregnant woman included different questions which asked to the pregnant women; age, gestational stage and the history of miscarriage if they had an abortion or not.

3.2. Sampling size

A total of ninety two (n=92) pregnant women were participated in this study.

3.3. Ethical consideration

This study was approved by the College of Medical Laboratory Science Ethical Committee, SUST and the permission was taken from every hospital the study was conducted in. Verbal consent were obtained from each pregnant woman before the samples were taken.

3.4. Laboratory methods

3.4.1. Collection of blood specimens

A volume of 3 ml blood were collected from each pregnant woman through vain puncture technique under aseptic technique then displaced into an EDTA container.

3.4.2. Specimen processing

Blood specimens were centrifuged at 3500 rpm for 5 minutes to obtain plasma. The later was gently collected into plane container and stored at – 20°C until the serological analysis.

3.4.3. Analysis of specimens

The specimens were analyzed for the presence of HSV-2 IgG antibody by a commercially available enzyme -linked immunosorbent assay “HSV-2 IgG ELISA” kit (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, BT41 1QS United Kingdom). The assay were performed following the instructions of the manufacturer. Positive and Negative controls were included in each assay. According to the information included in the kit’s insert, the immunoassay used has a clinical specificity 90.0% and the clinical sensitivity is 92.1 %.

3.3.4. Principle of HSV-2 IgG ELISA

The ELISA test kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative detection of HSV-2 IgG antibodies to HSV-2 in human serum or plasma. The microwell plate is coated with HSV-2 recombinant antigens. During testing, the specimen diluent and the specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain IgG antibodies to HSV-2, it will bind to the antigens coated on microwell plate to form immobilized antigens-HSV-2 IgG antibody complexes. If the specimen do not contain IgG antibodies to HSV-2, the complexes will

not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme conjugated anti- human IgG antibodies is added to microwell plate and then incubated. The enzyme conjugated anti human IgG antibodies will bind to immobilized to antigen-HSV-2 IgG antibody complexes present. After the 2nd incubation, the microwell plate is washed again to remove unbound materials. Chromogenic solution contains Tetra Methyle Benzendine (TMB) and hydrogen peroxide are added and then incubated to produce a blue color indicating the amount of HSV-2 IgG antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity which corresponds to the amount of HSV-2 IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

3.4.5. Procedure

The reagents and specimens were allowed to reach room temperature. 100 ul of positive control, negative control and the cut-off calibrator were added to their respective wells, then 100 ul of specimen diluent was added to each well except the blank and 5 ul of each specimen was added. The plate was covered with the cover sealer and incubated for 30 minutes at 37°C. At the end of incubation period each well was washed for 5 min with 350 ul of working wash buffer , 100 ul of conjugate was added to each well except the blank, the plate was covered and incubated again, for 30 minutes at 37°C. By the end of the incubation period, each well was washed 5 times with working wash buffer. Finally 50 ul of substrate A and substrate B solutions were added to each well included the blank, then the plate was incubated at 37°C for 10 minutes. At the end of incubation period, the stop solution was added.

3.4.6. Quality control and calculation of the results

Reagents, Standard and control were checked for storage, stability and preparation before starting work. Each microwell plate was considered separately when the results were

calculated and interrelated; the result were calculated by related each specimen absorbance (A) to the cut-off (C.O) of the plate.

Calculation of the cut-off value (C.O) = mean absorbance of cut-off calibrator – blank absorbance.

The optical density (OD) value of the blank well must be less than 0.050 at 450 nm.

The OD value of the positive control must be more than 0.800 at 450 nm.

The OD value of the negative control must be less than 0.100 at 450 nm.

3.4.7. Interpretation of results

Positive more than cut-off value.

Negative less than cut-off value.

Borderline equal to the cut-off value.

3.5 Data analysis

The data that collected from questionnaire and laboratory results were analyzed by

SPSS version 15 computerized program.

CHAPTER FOUR

RESULTS

A total of ninety two blood specimens (n=92) were obtained from pregnant women attending two hospitals and one laboratory Diagnostic Centre in Khartoum State. These were Omdurman Friendship Hospital 56 (60.9%), Al Saudi Obstetrics and Gynecology Hospital 23 (25%) and Ultra Laboratory Diagnostic Centre 13 (14.1%) (Table 1). All specimens were examined for the presence of HSV-2 IgG antibodies using ELISA kit.

The result showed that out of 92 blood specimens investigated, 7 (7.6%) were positive for HSV-2 IgG antibodies. The rest 85 (92.4%) were negative (Table 2). From the positive blood specimens 1 (5.9%) was in the first trimester, 1 (4.8%) in second trimester and 5 (9.3%) in the third trimester (Table 3). Out of 41 specimens of the pregnant women with history of abortion examined, 2 (4.8%) were positive for HSV-2 IgG antibodies, while the rest 39 (95.2%) were negative. Moreover out of 51 specimens of pregnant women without any history of abortion examined, 5(9.8%) were positive for HSV-2 IgG antibodies, while the rest 46 (90.2%) were negative (Table 4). According to the age of each pregnant woman, out of 32 women in the age group (16−25), 4 (12.5%) were positive, and out of 48 women in age group (26−35), 3 (6.3%) were positive and out of 12 women in age group (36−45) were negative (Table 5).

Table 1. Distribution of pregnant women according to the hospital

Hospital	**Pregnant women**	
	N	**%**
Omdurman Friendship Hospital	56	60.9
Al Saudi Obstetrics and Gynecology Hospital	23	25.0

Ultra Laboratory Diagnostic Centre	13	14.1
Total	92	100

n= Number

Table 2. Frequency of HSV-2 among all participated

Results	**Participates**	
	N	**%**
Positive	7	7.6
Negative	85	92.4
Total	92	100

Table 3. Frequency of HSV-2 according to the gestational stage

Gestational stage	**Participates n.**	**Positive**	**%**
First trimester	17	1	5.9
Second trimester	21	1	4.8

Third trimester	54	5	9.3
Total	92	7	20

Table 4. Frequency of HSV-2 according to the history of abortion

Abortion	**Result**	**n**	**%**
Yes (n=41)	Positive	2	4.8
	Negative	39	95.2
No (n=51)	Positive	5	9.8
	Negative	46	90.2

Table 5. Frequency of HSV-2 according to the age group

Age group	**N**	**Positive**	**%**
16 — 25	32	4	12.5
26 — 35	48	3	6.3
36 — 45	12	0	0

Total	92	7	18.8

CHAPTER FIVE

DISCUSSION

5.1. Discussion

It has been shown the prevalence of genital tract infection by HSV-2 varies substantially in the different geographic regions, including those within the same country and other once. When a primary infection is contracted during pregnancy, the fetus is at high risk of acquiring the infection either intrauterine or at delivery. In the present study of pregnant

women were enrolled in Khartoum State, out of 92 blood specimens investigated, the results showed only 7(7.6%) were positive. This results is higher than that obtained in Iraq by Abul-Razak *et al.*, (2013) who reported that (2.2%) out of 91 samples of pregnant women were positive for HSV-2 IgG and more similar to results that obtained in India by Rathore *et al.*, (2010) were found (7.5%) of Indian pregnant women were positive for HSV-2 IgG antibodies; and another study in northeast India by Biswas *et al*., (2011) who found that the seropositivity rate of HSV-2 IgG antibodies among pregnant women was (8.7%). In Italy (8.4%) were positive for HSV-2 IgG among Italian pregnant women done by Jindal *et al.*, (2012). This study is lower than other study done by El-Amin *et al* (2013) in Sudan who found 45 (34.6%) tested positive for the HSV-2 IgG out of 130 pregnant women, and another study conducted in Cote D'ivoire by Boni *et al*., (2015) were found (96.5%) out of 170 pregnant women. In Saudi Arabia out of 459 samples (6.5%) of the samples have detectable level of HSV-2 IgG by Alzahrani *et al*., (2007). Another study was highest in Ghana by Frederick *et al.*, (2015) who founded (68.13%) out of 91 blood samples were positive for HSV-2. Compared to another study carried out by Hadeel Elsir and Wafa Ibrahim, (2015) out of 90 samples of HSV-2 infected pregnant women attending Ibrahim Malik Teaching Hospital in Sudan (63.3%) of HSV-2 IgG antibodies were detected. The variation in the results that obtained may be due to sample size technique used for analysis, sexual activities of each pregnant woman was enrolled, the geographic regions that the studies were conducted and the limited period within which those study were carried out.

5.2. Conclusion

1. The current study revealed low seroprevalence rate of HSV-2 among pregnant women in Khartoum State.

2. Pregnant women in the third trimester of gestational stage had the highest frequency of infection than other stage.

3. The level of infection in the pregnant women without history of abortion was highest than pregnant women with a history of abortion.

4. The highest prevalence was in the age group (16-25, years).

5.3. Recommendation

1. Further studies with larger number of samples by more advanced technique are required include real-time quantitative PCR to determine the proportion of acute HSV2 infection in the population of pregnant women and estimate the rate of transmission to the newborn to validate the results of present study.
2. Elimination of neonatal HSV-2 requires the development of an effective HSV-2 vaccine that will protect against genital HSV2 infection.
3. The significant proportion of pregnant women infected by HSV-2 that presented morphological alterations in the uterine cervix suggests the colposcopy or cytology exams as part of routine prenatal care.
4. This study should be replicated in other parts of Sudan so as to determine the overall susceptibility levels in the entire country.

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