

CHAPTER ONE

INTRODUCTION

1.1 Introduction:

Herpes simplex virus (HSV) infection is one of most common viral sexually transmitted disease (STD) worldwide. HSV-2 is the cause of most genital herpes and is almost always sexually transmitted (Elena *et al.*, 2009). HSV-2 is the leading cause of genital ulcer disease in the developing countries. HSV-2 infection is highly prevalent world wide although most infected persons are either a symptomatic or have genital symptoms that remain unrecognized (Mnisi and Samie,2014).

Herpes viruses derived name from the Greek word herpein, which means to creep. They are double strand DNA virus that belong to the family Herpesviridae, subfamily Alphaherpesvirinae. Herpes virus cause persistant infection in human around the world (Carter,2007).

Herpes simplex virus (HSV) is a common pathogen that leads to lifelong latent infection and may be associated with transmission from mothers to their fetus and involve the fetoplacental unit (Giovanni *et al.*, 2011).

Primary genital HSV-2 infection in pregnant women can result in abortion, premature labor, congenital and neonatal herpes (Egbagba,2015).

Worldwide the number of individuals seropositive for HSV-2 increase with age (27-28) (Whitley, 2006). Risk of neonatal infection is in the range of 30%-50% when HSV infection is acquired in the last trimester, whereas risk is only 1% when it is acquired in early pregnancy (Pandey *et al.*, 2014).

HSV-2 seroprevalence in the US has increased by 30%, comparing the developing countries, substantially higher rates of HSV-2 has been observed in sub-saharan Africa, where prevalence in adult range from

30% to 80% in women. Prevalence in the general population of Asian countries shows lower values, from 10% to 30% (Cusini,2001).

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 intrauterine growth retardation, intrauterine fetal death, preterm labor, spontaneous abortion, congenital and neonatal herpes infections (Haider,2011).

More than 50% of affected infants have moderate or more severe neurological impairment, with a 20% overall mortality, approximately 90% of all neonates herpes infection are transmitted during delivery and at least 5% transmitted in utero (Ciavattini, 2007).

This study is done to conducted frequency of this virus among pregnant women in Wad-Madani(Algezira state) in Sudan by using ELISA technique.

1.3 Objective

1.3.1 General objective

To study the frequency of Herpes Simplex Virus type 2 (HSV-2) IgM and IgG among pregnant women in Al Gazira State.

1.3.2 Specific objective

1- To determine the frequency of Herpes Simplex Virus type 2 IgM/IgG antibodies in pregnant women using Enzyme Linked Immuno Sorbent Assay technique.

2- To detect the association between Herpes Simplex Virus infection and age, gestation trimester and history of abortion.

CHAPTER TWO

LITERATURE REVIEW

2. Literature review

2.1 Herpesviridae family

Herpesviridae is large family of DNA viruses that cause disease in human. The members of this family are also known as herpesviruses (Coulson., 2005). More than 100 herpes viruses had been isolated from arrange of hosts that include mammals, birds, fishes, reptiles, amphibians and mollusks (Carter and Saunders, 2007). The family name is derived from the Greek word herpein ("to creep"), referring to the latent, recurring infections typical of this group of viruses. Herpesviridae can cause latent or lytic infections (Kim *et al.*, 2004). In total, there are 8 Herpes virus types that infect humans (Herpes simplex viruses (HSV-1) and (HSV-2), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), human Herpes virus 6 (HHV-6), human Herpes virus 7 (HHV-7), and kaposi sarcoma-associated Herpes virus (HHV-8)) (Compel and Deluca., 2003).

2.2 Historical Background of Herpes Simplex Virus

HSV is said to have infected human populations even during the middle age (5th-16th century), the word Herpes is originally from the Greek word "herpein" which means "to creep" the creeping and spreading pattern along the neural tissues explains the distinctive characteristic of HSV well (Richard., 2009).

2.3 Taxonomy

The herpes virus was first isolated from the blue wildebeest in 1960 by veterinary scientist Walter Plowright. The genus Herpes virus was established in 1971 in the first report of the International Committee on Taxonomy of Virus (ICTV). This genus consisted of 23 viruses and 4 groups of viruses (Davison, 2010). In the second ICTV report in 1976 this genus was elevated to family level the Herpetoviridae. Because of possible confusion with viruses derived from reptiles this name was changed in the third report in 1979 to Herpesviridae. In this report the family Herpesviridae was divided into 3 subfamilies (Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae) and 5 unnamed genera: 21 viruses were listed. In 2009 the family Herpesviridae was elevated to the order Herpesvirales. This elevation was necessitated by the discovery that the herpes viruses of fish and molluscs were only distantly related to those of birds and mammals. Two new families were created the family Alphaherpesviridae which incorporates bony fish and frog viruses and the family Malacoherpesviridae which contains those of molluscs (Davison, 2010).

This order currently has 3 families, 3 subfamilies plus 1 unassigned, 17 genera, 90 species and plus 48 as yet unassigned viruses Alpha herpesvirinae contain Herpes Simplex Virus type 1 and 2 (Davison, 2010).

2.4 Classification

HSV belongs to the Order Herpesvirales, Family

Herpesviridae, Subfamily Alphaherpesvirinae, Genus Simplex virus, Species Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2) (Harvery simon., 2009).

Herpes simplex virus 1 and 2 also known as human herpes virus 1 and 2 (HHV-1 and HHV-2) are two members of the Herpes virus family, Herpesviridae, that infect human, both HSV-1 (which produce most cold

sores) and HSV-2 (which produce most genital herpes) are ubiquitous and contagious, they can be spread when an infected person is producing and shedding the virus. Lesions heal with scars characteristic of herpetic disease. Some times, the viruses cause very mild or atypical symptoms during outbreaks. However, as neurotropic and neuroinvasive viruses, HSV-1 and HSV-2 persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. After the initial or primary infection, some infected people experience sporadic episodes of viral reactivation or outbreaks. In an outbreak, the virus in a nerve cell becomes active and is transported via the neurons axon to the skin, where virus replication and shedding occur and cause new sores. It is one of the most common sexually transmitted infections (Wang *et al.*, 2004).

2.5 Viral structure

The structure of herpes viruses consists of a relatively large double-stranded, linear DNA genome encased within an icosahedral protein cage called the capsid, which is wrapped in a lipid bilayer called the envelope. The envelope is joined to the capsid by means of a tegument. This complete particle is known as the virion (Mettenleiter *et al.*, 2006). HSV-1 and HSV-2 each contain at least 74 genes (or open reading frames, ORFs) within their genomes. Although speculation over gene crowding allows as many as 84 unique protein coding genes by 94 putative ORFs. 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 (McGeoch *et al.*, 2006).

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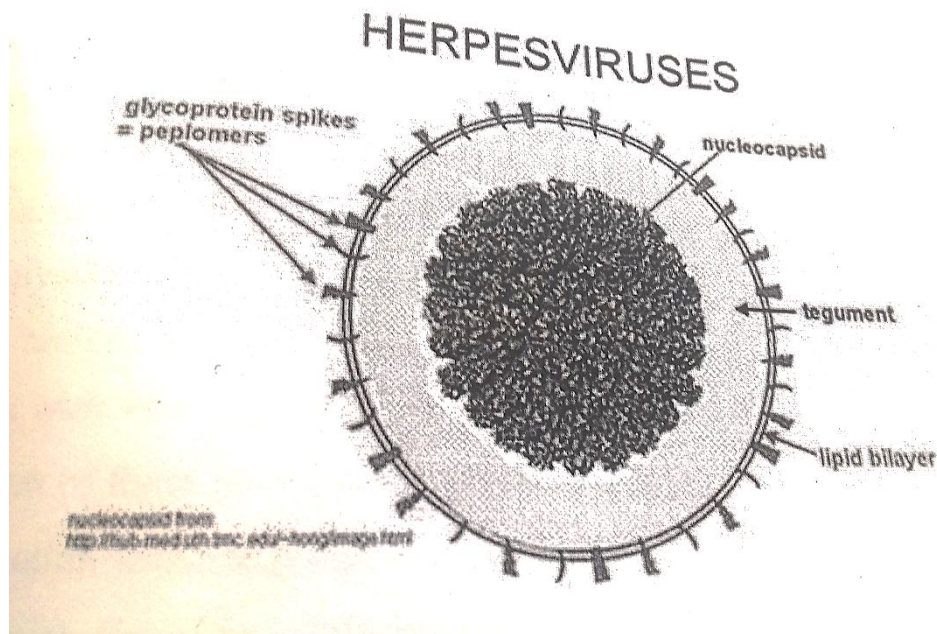


Figure 1: Structure of the HSV (Richard Hunt 2008)

2.6 Cellular entry

Entry of HSV into the host cell involves interactions of several glycoproteins on the surface of the enveloped virus, with receptors on the surface of the host cell, the envelope covering the virus particle, when bound to specific receptors on the cell surface, will fuse with the host cell membrane and create an opening, or pore, through which the virus enters the host cell (McGeoch *et al.*, 2006).

The sequential stages of HSV entry are analogous to those of other viruses. At first, complementary receptors on the virus and the cell surface bring the viral and cell membranes into proximity. In an intermediate state, the two membranes begin to merge, forming a hemifusion state. Finally, a stable entry pore is formed through which the viral envelope contents are introduced to the host cell. (Coulson., 2005).

The virus can also be endocytosed after binding to the receptors, and the fusion could occur at the endosome. (Richard., 2008). In the case of a

herpes virus, initial interactions occur when two viral envelope glycoprotein called glycoprotein C (g C) and glycoprotein B (g B) bind to a cell surface particle called heparan sulfate. Next, the major receptor binding protein, glycoprotein D (g D), binds specifically to at least one of three known entry receptors.(Eissa *et al.*,1990).These include Herpesvirus entry mediator (HVEM), nectin-1 and 3-O sulfated heparan sulfate. The receptor provides a strong, fixed attachment to the host cell.These interaction bring the membrane surfaces into mutual proximity and allow for other glycoprotein embedded in the viral envelope to interact with other cell surface molecules, once bound to the HVEM, gD changes its conformation and interacts with viral glycoproteins H(gH) and L (gL), which form a complex. The interaction of these membrane proteins results in the hemifusion state, afterward, gB interaction with the gH/gL complex creates an entry pore for the viral capsid gB interacts with glycosaminoglycans on the surface of the host cell. (Subramanian and Geraghty., 2007) .

2.7 Viral genome

The genomes of HSV-1 and HSV-2 are complex and contain two unique regions called the long unique region(UL) and the short unique region (US) . Of the 74 known ORFs, UL contains 56 viral genes, whereas US contains only 12. Transcription of HSV genes is catalyzed by RNAPolymerase II of the infected host(McGeoch *et al* ., 2006). Immediate early genes, which encode proteins that regulate the expression of early and late viral genes, are the first to be expressed following infection. Early gene expression follows, to allow the synthesis of enzymes involved in DNA replication and the production of certain envelope glycoproteins. Expression of late genes occurs last; these groups of genes predominantly encode proteins that form the virion particle (McGeoch *et al.*, 2006). Five proteins from (UL) form the viral capsid;

UL6,UL18,UL35,UL38 and the major capsid protein UL19 (Mettenleiter *et al.*, 2006).

2.8 Replication

According to Roizman and Mandel(2005).following infection of cell, a cascade of Herpes virus proteins, called immediate-early, early, and late, are produced. Research using flow cytometry on another member of the Herpes virus family, Kaposi s sarcoma-associated Herpes virus, indicates the possibility of an additional lytic stage, delayed-late. These stages of lytic infection, particularly late lytic, are distinct from the latency stage. The early proteins transcribed are used in the regulation of genetic replication of the virus. On entering the cell, an TIF protein joins the viral particle and aids in immediate-early transcription. The virion host shutoff protein (VHS or UL41) is very important to viral replication. This enzyme shuts off protein synthesis in the host, degrades host mRNA, helps in viral replication, and regulates gene expression of viral proteins. The viral genome immediately travels to the nucleus but the VHS protein remains in the cytoplasm. (Akhtar , and Shukla., 2009).

The late proteins form the capsid and the receptors on the surface of the virus. Packaging of the viral particles-including the genome,core and the capsid occurs in the nucleus of cell. Here, concatemers of the viral genome are separated by cleavage and are placed into pre-formed capsids. The primary envelope is acquired by budding into the inner nuclear membrane of the cell, then fuses with the outer nuclear membrane releasing a naked capsid into the cytoplasm. The virus acquired its final envelope by budding into cytoplasmic vesicles. (Taddeo, Roizman., 2006).

2.9 Latent infection

Herpes simplex virus may persist in a quiescent but persistent form known as latent infection, notably in neural ganglia. HSV-2 tend to reside in sacral ganglia, but these are tendencies only are not fixed behavior, during latent infection of a cell, HSVs express Latency Associated Transcript (LAT) RNA. LAT regulates the host cell genome and interferes with natural cell death mechanisms, by maintaining the host cells, LAT expression preserves a reservoir of the virus, which allows subsequent, usually symptomatic, periodic recurrences or "outbreaks" characteristic of non-latency. Whether or not recurrences are symptomatic, viral shedding occurs to infect anew host, a protein found in neurons may bind to herpes virus DNA and regulate latency (Akhtar and Shukla. , 2009).

2.10 Transmission

HSV-2 is cause of most genital Herpes and almost always sexually transmitted. HSV-1 is usually transmitted during childhood via non sexually contact. However HSV-1 has emerged as a principle causative agent of genital Herpes in some developing countries, it may also be sexual transmitted including contact with saliva such as kissing and mouth to genital contact(oral sex) (Xu *et al.*,2006; Paz-Bailey *et al.*,2007; Gupta *et al.*, 2007).HSV-2 transmitted across epithelial mucosal cells, as well as skin interruption, migrate to nervous tissue, where they persist latent state. HSV-2 is most commonly found in the lumbosacral ganglia (Kriebs, 2008). Pregnant women with acute infection during pregnancy are at risk of congenitally transmitting the infection to the fetus, congenital transmission as a result of primary infection during the pregnancy is higher if the infection acquired during the third trimester and congenital infection occurring during the first trimester may result in high risk of tragic outcomes, which may include abortion (Anowakowska

et al., 2006). Although there is small risk of vertical transmission, recurrent genital Herpes must be regarded as most common cause of neonatal infection and the passage through the infected birth canal is most probable route of transmission (Sauerbrei and Wutzler, 2007).

2.11 Epidemiology

In recent years, genital herpes has become an increasing common sexually transmitted infection. Comparing the developing countries, substantially higher rates of HSV-2 has been observed in sub-Saharan Africa, where prevalence in adult ranges from 30% to 80% in women and from 10% to 50% in men, finally more than 80% of female commercial sex workers are infected (Paz-Bailey *et al.*, 2007).

In South America available data are mainly for women in whom HSV-2 prevalence ranges from 20% to 40% . prevalence in the general population of Asian countries shows lower values, from 10 % to 30% (Paz-Bailey *et al.*,2007). HSV seroprevalence in patients attending STD clinics varies from 17% in Italy(6% in the general population) to 40% in Australia(14% in pregnant women). Age and sex are important risk factors associated with the acquisition of genital HSV-2 infection. In fact, the prevalence of HSV infection is very low in childhood and early adolescence but it rises with age, reaching the maximum around 40 years regarding pregnant population, there is a high prevalence of genital herpes (Elena, 2009).

In Baghdad with history of one or more unexplained abortion werescreened for the presence IgM and IgG antibodies against Toxoplasma gondii, Cytomegalovirus, Herpes simplex virus2, Rubella virus. Were found 10 (4.76%) were positive for HSV-2 IgM (Basim, 2014). In pregnant women from India a high seroprevalence of HSV was detected in the examined samples. It was much higher for IgG (64.9%) compared to IgM (2.1%) (Ammar *et al.*, 2015).

Evidence of TORCH infection was seen in 66.7% of women positive for serum IgM antibodies. In New Delhi, India maximum percentage was for HSV infection (30.10%) in study of TORCH IgM seroprevalence in women with abortions as adverse reproductive outcome in current pregnancy (Sana *et al.*, 2016).

2.12 Herpes Simplex Virus infections

Herpes simplex is a virus that can cause different types of infections in different age groups, in early childhood, it commonly causes blister-like sores in the mouth and around the lips and on tissues that are in contact with the mouth, such as a sucked thumb or finger. Genital Herpes can cause lesions on the male or female genital organs (Bruni and Roizman.,1998). Herpes simplex [sim-pleks] is caused by a virus called Herpes Simplex Virus (HSV). Herpes simplex is divided into two types; HSV-1 causes primarily mouth, throat, face, eye, and central nervous system infections, whereas HSV-2 causes primarily genital infections. However, each may cause infections in all areas (Chayavichitsilp *et al.*, 2009).

2.12.1 Signs and symptoms

HSV infection causes several distinct medical disorders. Common infection of the skin or mucosa may affect the face and mouth (orofacial herpes), genitalia (genital herpes), or hand (herpetic whitlow). More serious disorders occur when the virus infects and damages the eye (herpes keratitis), or invades the central nervous system, damaging the brain (herpes encephalitis). People with immature or suppressed immune systems, such as newborns, transplant recipients, or people with AIDS, are prone to severe complications from HSV infections (Dickerson *et al.*, 2004).

In all cases, HSV is never removed from the body by the immune system. Following a primary infection, the virus enters the nerves at the site of

primary infection, migrates to the cell body of the neuron, and becomes latent in the ganglion. As a result of primary infection, the body produces antibodies to the particular type of HSV involved, preventing a subsequent infection of that type at a different site (Gupta *et al.*, 2007).

2.12.2 Primary infection

Primary infection of HSV acquired by women during pregnancy accounts for half of the morbidity and mortality among neonates; while the remaining half result from the reactivation of an old infection. Primary genital HSV-2 infection in pregnant women can result in abortion, premature labor, congenital and neonatal herpes (Haider, 2011).

Perinatal and congenital herpes infection can occur predominately during delivery and infrequently in utero from mother with primary or recurrent herpes infection (Haider, 2011).

2.12.3 Latent infection

HSV may persist in a quiescent but persistent form known as latent infection, notably in neural ganglia (Ryan and Ray, 2004). HSV-2 tends to reside in the sacral ganglia, but note that these are tendencies only, not fixed behavior (Pinnoji *et al.*, 2007).

The exact mechanism of latency of the virus is unknown, it may either be: True latency- the virus is non replicative and is maintained within the cell either integration into the cellular chromosome or in an episomal form.

Virus persistence-this is best described as dynamic latency, whereby there is a tightly controlled low grade productive virus infection not leading to the lysis of the cell (Richard., 2008).

2.12.4 Reactivation

It is well known that many triggers can provoke a recurrence. These include: stress-physical or psychological, pneumococcal infection, meningococcal infection, fever, irradiation, including sunlight, fatigue or injury and menstruation (Richard., 2008).

2.12.5 Recurrent infection

Following primary infection, 60% of patients with genital Herpes will experience recurrences varies widely between individuals. The actual frequency decrease with age, increases with socioeconomic status and is related to race. Many individuals never experience any clinically apparent reactivation although more than half would be intermittently shedding virus in saliva, tears, semen or genital (cervical, urethral, prostatic) secretions(Richard., 2008).

2.12.6 Genital herpes

Genital herpes is an important public health disease and is the leading cause of genital ulcer disease worldwide is caused by infection with HSV, commonly by HSV-2. HSV-2 infections are acquired from contact with infectious secretions on oral, genital, or anal mucosal surfaces (Sapna *et al.*, 2008). Genital herpes can also be acquired from contact with lesions from other anatomical sites such as the eyes and non mucosal surfaces such as herpetic whitlow on fingers or from lesions on the buttocks and trunk (Sapna *et al.*, 2008). Genital HSV infection may be symptomatic or asymptomatic. Within women it causes blistering and ulceration of the external genitalia and cervix leading to vulval pain, dysuria, vaginal discharge and local lymphadenopathy. Vesicular and ulcerative lesions of the internal thigh, buttocks, perineum or in perianal skin are also been observed. In men the lesions typically develop on the glans, but also on the penis, internal thigh, buttocks or in perianal skin (Sauerbrei and Wutzler, 2007). Both in man and in women primary infection may be complicated by systemic symptoms such as fever, headache and myalgia (38% in men, 68% in women) and occasionally meningitis and by autonomic neuropathy resulting in urinary retention, mainly in women. Meningitis has been found in 42% of primary HSV-2. In particular, pregnant women with primary mucous membrane infection during the

third trimester have an increased risk for dissemination and they could transmit HSV to their babies during vaginal delivery (Sauerbrei and Wutzler,2007). Prodromal symptoms (itching, tingling and neuralgia) may occur hours or days before a recurrent herpes episode the great majority of recurrent genital herpes is due to HSV-2 because this virus reactivates more frequently than HSV-1. The majority of sexual HSV transmission occurs during asymptomatic periods because the patients are unaware of asymptomatic virus shedding (Dickson *et al.*, 2007).

In a study of sero frequency of Herpes among pregnant women in sudan; the sera of pregnant women attending Omdurman Maternity Hospital were examined for HSV IgG and IgM antibodies, they found that out of 90 pregnant ladies 91% and 10% were positive for IgG and IgM respectively and 8.9% were negative for both, their result also showed higher frequency for IgG among 20-30 years of age group, then they concluded that HSV can infect pregnant women and their neonates (Elsiddig *et al.*, 2015).

2.12.7 Neonatal Herpes

HSV infection of the newborn can be acquired inutero , intrapartum and postnatally. The mother is the most common source of infection for the first two routes of viral transmission. Intrauterine HSV infection is a rare disorder and accounts for 5% of HSV infections in neonates (Sauerbrei and Wutzler, 2007).The highest risk of intrauterine infection has been observed in pregnant about (50%) that develop disseminated HSV infections and 90% of those are related to HSV-2. Intrauterine viral transmission is highest during the first 20 weeks of gestation leading to abortion, stillbirth and congenital anomalies in infant who survive. The perinatal mortality is (50%). 70-85% of neonatal HSV infections are caused by HSV-2, congenital intrauterine infection, that usually is identified within the first 48 hours following birth, is characterized by

skin vesicles or scarring, eye lesions (chorioretinitis, microphthalmia, cataract), neurologic damage (intracranial calcifications, microcephaly, seizures, encephalomalacia), growth retardation and psychomotor development (Sauerbrei and Wutzler,2007).

A study on prevalence of Herpes simplex virus in maternal serum with recurrent spontaneous abortion was done on Egypt at 2007 and found that there was high frequency of HSV IgM antibodies 40% on sera of pregnant women, then concluded that viral infection with HSV was frequently associated with recurrent spontaneous abortion (Maysaa and Hossam, 2007).

Another study was performed to evaluate the obstetric outcomes of HSV-2 infection among pregnant women in Nigeria and found that 46% were HSV-2 seropositive, then concluded that first episode of HSV-2 infection is associated with an increased risk of occurrence of spontaneous abortion and preterm delivery (Kalu *et al.*, 2015).

2.12.8 Encephalitis

HSV encephalitis is one of the most devastating of all HSV infections and it has been estimated that it accounts for almost 20% of all cases of encephalitis worldwide (Dominique *et al.*, 2015).

2.13 Laboratory Diagnosis

2.13.1 Tzanck smear assay

Cytological and morphological examination methods were used for many years for diagnosis of various skin and mucocutaneous infections with HSV. Routinely, clinical smear sample either scraped from the base of vesicle, blister or postule or obtained directly from Herpes lesion without specific pre treatment were collected and transferred to glass slide which were stained with a various dyes such as Giemsa-wright, Hematoxylin, Eosin or the Papanicolaou stain and examined under light microscope in

order to identify the presence of cytopathic effects (CPE) associated with Herpes virus infection, such as the presence of multi nucleated giant cells, syncytium and ballooning cellular degeneration. Due to its ease of performance and low cost, this direct and however, the efficiency of Tzanck smear assay is limited by its low sensitivity, which is only the stage of lesion (Feinan *et al.*, 2014).

2.13.2 Direct fluorescent antibody

The direct fluorescent antibody (DFA) test an assay that can directly detect the presence of HSV antigen in specimens using pathogen specific fluoresce in tagged antibody without the need for secondary antibody reaction. The clinical sensitivity of diagnostic method is substantially greater than that of Tzanck smear test, although its sensitivity of HSV diagnosis is only 50-100% of that of viral culture. DFA testing has very high specificity 100% (Feinan *et al.*, 2014).

2.13.3 Molecular method

Molecular diagnosis of acute HSV can be accomplished via amplification and detection of specific viral genome targets. Early real time polymerase chain reaction (PCR) assays targeted highly conserved regions of the herpes virus DNA polymerase in order to amplify HSV-2. An early assay capable of molecular typing of HSV utilized a light cycler. PCR combined with melting point analysis and florescence resonance energy transfer (FRET). Sensitivity of PCR has been reported to be superior to that of viral culture through still depend on amount of viral DNA present as the site of infection (Neil, 2014).

2.13.4 Viral culture

Has long being considered the gold standard diagnostic modality of active infection, which is why it is often used to evaluate the sensitivity and specificity of other diagnostic methodologies. The ability of viral culture to isolate HSV-2 depends on executing prober sample collection,

transport and storage of the specimen as well as culturing with an appropriate permissive cell line. In routine use, clinical sample collected from skin or mucocutaneous lesion are usually transported in viral transport media and storage at 4⁰C until test is performed (Feinan *et al.*,2014).

Other sample types, such as CSF and body fluid do not require the use of viral transport media, but are simply placed into sterile container in order to avoid microbial contamination keeping the sample for 2-8⁰C until viral culture inoculation helps preserves viral infectivity and increase the virus recovering rate (Feinan *et al.*, 2014).

2.13.5 Serology

2.13.5.1 Whole antigen-based (non-g G specific) detection method:

Western blot (WB) assays have been described for serologic detection of HSV-2 (Neil, 2014). Whole antigen preparations from HSV-2 infective cell line and separated by Electrophoresis, adsorbed to a nitrocellulose gel, and exposed to patients serum. HSV status is determined by banding patterns specific to HSV-2 (Neil, 2014).

2.13.5.2 Glycoprotein G (g G)-based detection method

Newer serological methods incorporated type specific assay based on HSV glycoprotein g these type specific are easy differentiated based upon and additional HSV-2 specific domain. Commercial type specific Enzyme linked Immuno-sorbent Assay (ELISA) utilizes recombinant or native purified Gg-1 and Gg-2 for increase specificity. HSV-2 testing requires separate assays which adds to work load but also provides versatility (Neil, 2014).

2.13.5.3 Other serological detection methods

An important limitation to utility of HSV IgG - base serology is the challenge of result interpretation. Differentiating between chronic carriage and acute disease challenge. IgM- based serology assays have

been evaluated as a method to detect HSV infection at an early stage as well as to differentiate between chronic and acute infection (Neil, 2014).

2.14 Treatment

Several antiviral drugs are effective for treating herpes, including: acyclovir, valaciclovir (valtrex), famciclovir, and penciclovir. Acyclovir was the first discovered and is now available in generic. It is available in a topical cream, pills, and an intravenous. Valaciclovir (Valtrex): is also available in generic. Valaciclovir is a "pro-drug" of acyclovir and has been approved specifically for the treatment of herpes in HIV-positive people. Famciclovir (Famvir): is the pill form of a topical cream called penciclovir (Denavir). Usually, 500 mg of the drug is taken by mouth, twice daily, for five to ten days. Also used Trifluridine (Viroptic), drops are used to treat HSV infection of the eyes (Gianluca *et al.*, 2012).

2.15 Prevention and control

The high rates of undiagnosed or asymptomatic HSV infections complicate the prevention. A history of HSV infection in all pregnant women and their partner should be obtained at the first prenatal visit (Elena *et al.*, 2009). 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 (in particular during the third trimester of gestation). Moreover use of condoms throughout pregnancy should be recommended to minimize the risk of viral acquisition.

Prophylactic administration of acyclovir or valaciclovir in the third trimester of pregnancy should be provided to all pregnant with frequent genital herpes outbreaks and with active genital HSV infection near term or at the aim of delivery.

A careful examination of the vulva, vagina and cervix should be performed on any women who presents signs or symptoms of HSV

infection at the onset of labour. Artificial rupture of membranes should be avoided. All pregnant who have a suspected active genital HSV infection or prodromal symptoms of HSV infection should undergo caesarean section (Elena *et al.*, 2009). It is important to remember that fetal 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 transmission. 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, excretion, body fluids and immediately treated with intravenous acyclovir (Elena *et al.*, 2009). 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 (Elena *et al.*, 2009).

2.15.1 Vaccination

No vaccine is currently available for HSV However, killed subunit, vaccinia hybrid and DNA vaccine are being developed to prevent acquisition of the virus or treat infected people. The glycoprotein D is being utilized in several subunit vaccine. Disabled infectious single cycle (DISC) vaccines are being developed that utilize live, defective mutant viruses lacking essential genes (Murray *et al.*, 2002). An ideal vaccine should induce immune responses adequate to prevent infection (Richard *et al.*, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This is descriptive cross sectional study, aimed to detect the frequency of HSV-2 IgM and IgG among pregnant women.

3.2 Study area and duration

This study was conducted in Algezira state at Wad-Madani maternity hospital and Dr. Altigani Sedeeg fertility center in sudan during the period from April to August 2018.

3.3 Study population

This study was done on pregnant women in two hospital in Algazira state.

3.4 Sample size

The sample size was ninety two pregnant women (n=92).

3.5 Sampling Technique

This study is based on non – probability convenience sampling technique . Samples were take from attended agreed pregnant women during refer.

3.6 Method of data collection

The data were collected through none self-administrated questionnaire from pregnant women.

3.7 Data analysis

The data was analyzed by statistical package for social sciences (SPSS) soft ware version18.

3.8 Ethical consideration

Permission to carry out the study was obtained from the College of Medical Laboratory Science, Sudan University of Science and Technology. Permission from two hospital was applied in the study.

3.9 Specimens collection

A volume of 3ml blood was collected from each pregnant woman in plain container for detection of IgM and IgG.

Blood specimens were centrifuged at 3000 rpm for 5 minutes to obtain serum then stored at -20⁰C until tested.

3.10 Enzyme Linked Immune Sorbent Assay (ELISA) detection of HSV-2 IgM and IgG

The technique was done according to instruction sheet (figure No7) and samples were allowed to reach room temperature for at least 30 minutes before use and then , mixed thoroughly by vortex (figure No3). Place the desired number of coated strips into the holder. Prepare 1:40 dilutions by adding 5 μ L of the sample, negative control, positive control and calibrator to 200 μ L of sample diluent. Mix well then dispense 100 μ L of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 μ L sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature, remove liquid from all wells. Repeat washing three times with washing buffer.100 μ L of enzyme conjugate was added to each well and incubate for 30 minutes at room temperature then remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.100 μ L of TMB chromogenic substrate was added to each well and incubate for 15 minutes at room temperature. 100 μ L of stop solution was added to stop reaction. Make sure there are no air bubbles in each well before reading. Read O.D. at 450 nm with a micro well reader (figure No4).

3.10.1 Calculation of results

To obtain cut off OD value: Multiply the OD of calibrator by Factor (f) printed on label of calibrator.

Calculate the IgM/IgG Index of each determination by dividing the OD values of each sample by cut-off O.D. value.

3.10.2 Interpretation of result

Negative: HSV-2 IgM/IgG Index less than 0.90 are negative for IgM/IgG antibody to HSV-2.

Equivocal: HSV-2 IgM/IgG Index between 0.91-0.99 is equivocal.

Sample should be retested.

Positive: HSV-2 IgM/IgG Index of 1.00 or greater are positive for IgM/IgG antibody to HSV-2.

CHAPTER FOUR

RESULTS

Out of ninety two pregnant women enrolled for the study,56 (60.86%) their age from 20-30 years,35 (38.04%) from 31-40 years and 1(1.08%) from 41-50 years.

Considering the gestation trimester of participant 75 (81.51%) were in first trimester,17 (18.48%) were in second trimester ,0 were in third trimester.

Out of ninety-two pregnant women , HSV-2 IgM antibodies were detected in 35 (38%)and57 (62 %)were negative, while HSV-2 IgG were detected in 90 (97.8%) and 2 (2.2 %) were negative.

Table (4.1) Frequency of HSV-2 IgM and IgG among pregnant women .

Frequency	Positive	Negative	Total
IgM	35 (38 %)	57 (62%)	92 (100%)
IgG	90(97.8%)	2(2.2%)	92(100%)

From total of 92 pregnant women, IgM sero positivity were found as 23 (41.07%) and 33(58.9%) were negative, among 20-30 years, 12 (34.3%) and 23(65.7%), among 31-40 years and 0 (0%) and 1(100%) were negative in age group 41-50 years as shown in table (4.2) .

Evaluation of HSV-2 IgM seropositivity with age according showed a statistically insignificant association (P=0.168) .

Table (4.2) .The association between HSV-2 IgM and age.

Age	IgM		Total	P. Value
	Positive	Negative		
20 - 30	23(41.07 %)	33(58.9%)	56 (60.86 %)	0.168
31- 40	12(34.3%)	23(65.7%)	35 (38.04%)	
41 - 50	0	1(100%)	1 (1.08%)	
Total	35(38.04 %)	57 (61.9%)	92 (100%)	

From total of 92 pregnant women IgG seropositivity were found as 55 (98.2%) and 1(1.78%) among 20-30 years , 34 (97.1%) and 1(2.8 %) were negative , among 31-40 years and 1 (100%) in age group 41-50 years as shown in table (4.3) .

Evaluation of HSV-2 IgG seropositivity with age showed a statistically insignificant association (P= 0.728).

Table (4.3) The association between HSV-2 IgG and age .

Age	IgG			P. Value
	Positive	Negative	Total	
20 - 30	55(98.2%)	1(1.78%)	56 (60.86 %)	0.728
31- 40	34(97.1%)	1(2.8%)	35 (38.04%)	

41 - 50	1 (100%)	0	1 (1.08%)	
Total	90 (97.8 %)	2 (2.2%)	92 (100%)	

The seropositivity of HSV-2 IgM were found maximum in the First 26 (34.6%) and 49 (65.3%) were negative followed by second 9 (52.9%) and 8 (47.05%) were negative , and third trimester 0 . as shown in table (4.4) .

Evaluation of HSV-2 IgM antibodies according to trimester showed a statistically insignificant association with IgM seropositivity (P= 0.610)

Table (4.4) The association between HSV-2 IgM and gestation trimester.

Trimester	IgM			P. Value
	Positive	Negative	Total	
First	26(34.6%)	39(65.3%)	75 (81.52 %)	0.610
Second	9(52.9%)	8(47.05%)	17 (18.48%)	
Third	0	0	0	
Total	35(38.04 %)	57 (61.9%)	92 (100%)	

The seropositivity of HSV-2 IgG were found maximum in the first trimester 75 (100%) , followed by second trimester 15 (88.2%) and 2 (11.76%) were negative, and third trimester 0 as shown in table (4.5).

Evaluation of HSV-2 IgG antibodies according to gestation trimester showed a statistically insignificant association with IgG seropositivity (P= 0.223)

Table (4.5) The association between HSV-2 IgG and gestation trimester .

Trimester	IgG			P .Value
	Positive	Negative	Total	
First	75(100%)	0	75 (81.52 %)	0.223
Second	15(88.2%)	2(11.76%)	17 (18.48%)	
Third	0	0	0	
Total	90(97.8%)	2 (2.17%)	92(100%)	

Out of 92 pregnant women with history of previous abortion 43 (46.8%) , 19 (54.29%) of them were positive for HSV-2 IgM and 24 (55.8%) were negative for IgM. In the present study women with no history of abortion were 49 (53.26%) , out of them. 16 (32.6%) had IgM antibodies to HVS-2 and 33(67.3%) had no IgM antibodies to HSV-2. In this study there was no significant difference between HSV-2 IgM and history of abortion (P= 0.260)

Table (4.6) The association between HSV-2 IgM and history of abortion .

History of abortion	IgM			P. Value
	Positive	Negative	Total	

Yes	19(44.2%)	24(55.8%)	43 (46.74 %)	0.260
No	16(32.6%)	33(67.3%)	49 (53.26%)	
Total	35(38.04 %)	57(61.9%)	92 (100%)	

Out of 92 pregnant women had a previous history of abortion 43 (46.74%) all of them were positive for HSV-2 IgG, while 49 (53.26%) who had no previous abortion ,47(96%) showed IgG antibodies to HSV-2 and only 2(4%) had no IgG antibodies to HSV-2. In this study there was no significant difference between HSV-2 IgG and history of abortion (P= 0.184)

Table (4.7) The association between HSV-2 IgG and history of abortion.

History of abortion	IgG			P. Value
	Positive	Negative	Total	
Yes	43(100%)	0	43 (46.74 %)	0.184
No	47(96%)	2(4%)	49(53.26%)	
Total	90(97.8%)	2 (2.17%)	92(100%)	

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Herpes simplex virus (HSV) is a common sexually transmitted infection (STI) and the prevalence of this infection has increased significantly over the last two decades in many developed and developing countries, especially at reproductive age group. It is an important cause of intra-uterine fetal and neonatal infection when transmitted from mother to fetus (Paz – Bailey, 2007). The present study revealed that HSV-2 among pregnant women was (38%) for IgM and (97.8%) for IgG. This result is higher to that obtained by Basim Mosa (2014) in Baghdad (Basim, 2014), who reported that prevalence of HSV-2 among pregnant women was (4.76%). The frequency of HSV was observed also in sub-Saharan Africa, in which adult ranges from 30% to 80% in women (Paz-Bailey *et al.*, 2007). The result was conducted by (Maysaa and Hossam , 2007) in Egypt (40%) IgM positive on sera of pregnant women this result slightly similar to present study.

The seropositivity of HSV in this study was more among pregnant women with age group ranged from 20-30 (65.7%), similar result was found in a study of Sana Tiwari in New Delhi, India (Sana *et al.*, 2016).

A high frequency of HSV was detected in pregnant women (97.8%) for IgG. Slightly similar result was found in a study conducted among pregnant women at Omdurman Maternity Hospital where prevalence (91.1%) were positive for IgG, their result also showed higher frequency for IgG among 20-30 years 41(50%) of age group, then they concluded that HSV can infect pregnant women and their neonates (Elsiddig, 2015).

According to gestation trimester, seropositive of HSV IgM was the highest for first trimester (74.3%) followed by second (25.7%) and third (0), differ and higher result seropositivity was obtained in India in which the seropositivity of (3.3%) in the first, were (1.1%) in second and (2.6%) in third respectively (Amar, 2015) and Ibrahim Malik Teaching Hospital (1.7%) for first ,(3.7%) in second , (0)in third trimester respectively (Hadeel, 2015).

5.2 Conclusion

The frequency rate of HSV-2 among pregnant women in Wad-Madani Maternity Hospital and Dr. Altigani Sedeeg fertility center was (38%) IgM and (97.8%) IgG. Higher frequency of IgM and IgG showed in pregnant women with age group 20-30 year, and pregnant women in first trimester. The higher frequency in women who had previous abortion for IgM only. In these study the association between HSV-2 IgM and IgG Abs with age, gestation trimester and previous abortion is insignificant .

5.3 Recommendations

Routine screening of the TORCH for women during pregnancy.

Preferable when using IgM antibody for detection HSV antigen using IgG antibody with it.

Caesarean section should be considered for all women infected with herpes, particularly those developing symptom within 6 week of delivery, as the risk of viral shedding in labour is very high.

More investigation is needed to validate these results by using larger sample size to explain the result and to study the role of HSV in the abortion.

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Appendix



Fig2 Washer

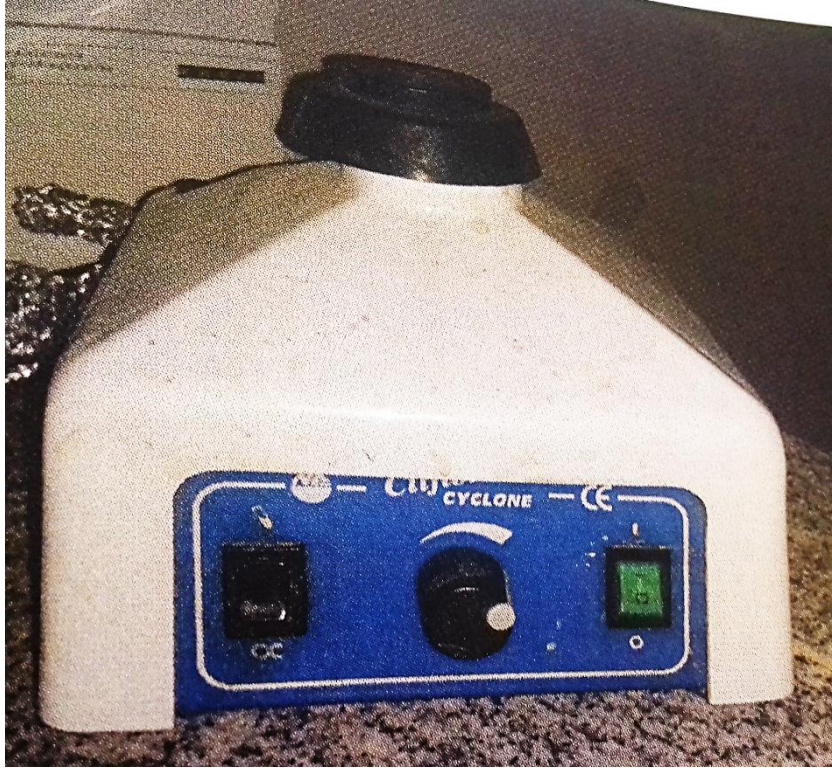


Fig 3 Vortex



Fig4 Reader of ELISA

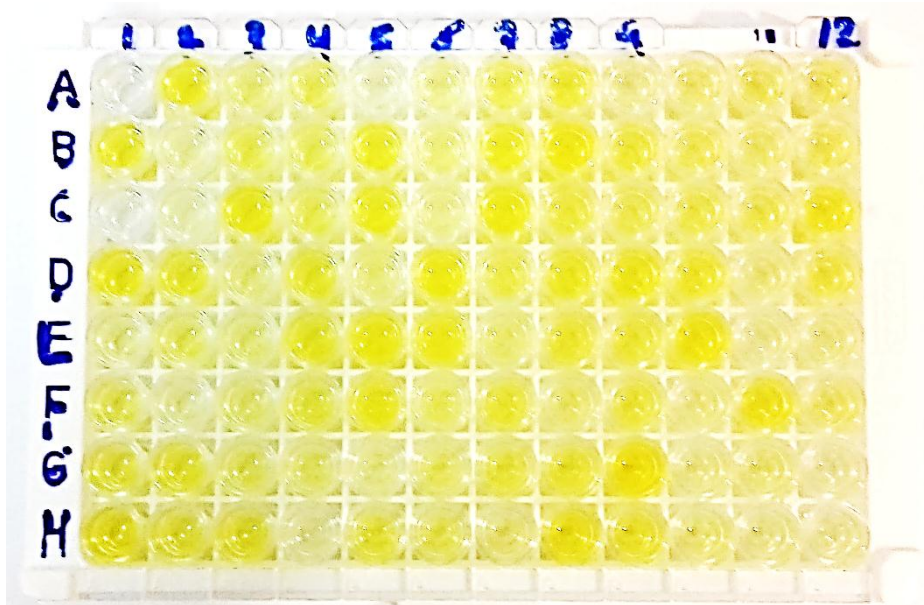


Fig5 ELISA Plate IgM

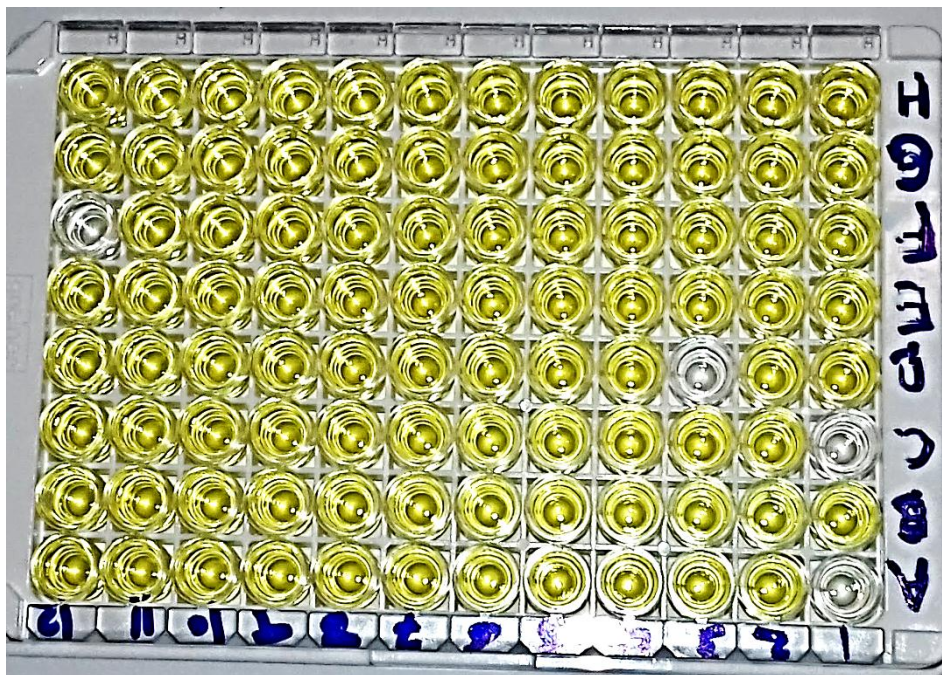


Fig6 ELISA Plate IgG