Sudan University of Science and Technology College of Graduate Studies

Sero Detection of *Herpes Simplex Virus* type *1* (HSV-1) IgG Antibody among Pregnant Women in Ombada and Fedail Hospital, Khartoum State

الكشف المصلي عن فيروس الهربس البسيط من النوع الاول (HSV-1) IgG بين النساء الحوامل في مستشفى أمبدة و فضيل , بولاية الخرطوم

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

By:
Afnan Elhafiz Ali Hamza
(B.SC. Medical Laboratory Science, Microbiology, Omdurman Islamic University, 2017)

Supervised by:
Prof: Yousif Fadlalla Hamedelnil

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

((سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنْفُسِهِمْ حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ))

صدق الله العظيم

سورة فصلت (53)

Dedication

The entire work is dedicated to my parents special father's soul for their love, prayers, caring and sacrifices for educating and preparing us for my future, my close friend, and family members.

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First of all, I thank to **ALMIGTY ALLAH** for all the beneficent and most merciful. I am very thankful to my supervisor **Prof. Yousif Fadl Allah Hamad Alneel** for his help and guidance. I would like to express my special thanks to staff of Immunology Department in Fedail Hospital. In addition, thanks extended to Registrar obstetric and gynecology in Ombada Hospital.

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Abstract

Herpes simplex virus -1 is a viral infection that causes genital and oral herpes. Which can affect in pregnant and new born cause spontaneous miscarriage or skin rash.

This cross section study was aimed to detect anti-herpes simplex virus type 1 (HSV-1) IgG antibodies among pregnant women during the period from April to June 2022 in Ombada and Fedail Hospital, Khartoum State.

Out of 96 investigated specimens; there were 65(67.7%) were anti-HSV-1 IgG positive and 31(32.3%) were negative. In which 29(44.6%) had history of miscarriage and positive for anti-HSV-1 IgG and 35 (53.8%) had no previous miscarriage and positive for anti-HSV-1 IgG antibodies. Concerning gestational age; there were 33(50.7%) in second trimester, 17(26.1%) in first trimester and 15(23%) in third trimester were positive for anti-HSV-1 IgG antibodies. Regarding age groups; there were 31(47.6%) in age group 25-33, 21(32.3%) in (34-42) and 13(20%) in 16-24 were positive for anti-HSV-1 IgG antibodies.

This study concluded that; there is no significant statistical difference between history of abortion in pregnant women and HSV-1 infection and also no significant association between HSV-1 infection and Gestational stage.

المستخلص

فيروس الهربس البسيط -1 هو عدوى فيروسية تسبب الهربس التناسلي والفموي. و التي يمكن أن تصيب الحوامل وحديثي الولادة تسبب الإجهاض التلقائي أو الطفح الجلدي.

هدفت هذه الدراسة المقطعية إلى الكشف عن الأجسام المضادة IgG لفيروس الهربس البسيط من النوع 1 (HSV-1) بين النساء الحوامل خلال الفترة من أبريل إلى يونيو 2022 في مستشفى أمبدة ومستشفى فضيل بولاية الخرطوم.

من أصل 96 عينة تم فحصها , كان هنالك 65 (67.7%) من مضادات الهربس البسيط النوع الأول موجبة و 31 (32.3%) كانت سالبة. في 29(44.6%) لديهم تاريخ من الإجهاض سابق وكانت إيجابي لمضاد HSV-1 IgG و 35(53.8%) لم يكن لديهم إجهاض سابق و إيجابي للإجسام المضادة ل HSV-1 IgG. فيما يتعلق بعمر الحمل, كان هناك 33)(50.7%) في الأثلوث الثاني, 17(26.1%) في الأثلوث الأول و 15(23%) في الأثلوث الثالث كانت إيجابية للإجسام المضادة ل HSV-1 IgG.

خلصت هذه الدراسة إلى أن: لا توجد فروق ذات دلالة إحصائية بين تاريخ الإجهاض عند النساء الحوامل و عدوى HSV-1و أيضا لا توجد ارتباط مهم بين عدوى HSV-1 و مرحلة الحمل.

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List of abbreviations

BBR	Berberine
CSF	Cerebrospinal fluid
CPE	Cytopathic effect
DNA	Deoxyribonucleic acid
DFA	Direct fluorescent antibody
ELISA	Enzyme-linked immunosorbent assay
HSV-1	Herpes simplex virus -1
HSV-2	Herpes simplex virus -2
mRNA	Messenger ribonucleic acid
OCT	Optical coherence tomography
PCR	Polymerase chain reaction
SPSS	Statistical Package for The Social Sciences

CHAPTER ONE

INTRODUCTION

1.1. Introduction

Herpes simplex virus (HSV) belong to the human herpes viruses and are among the most ubiquitous viruses in adult population. There are two types, HSV 1 and 2 (Avgil and Ornoy, 2006).

HSV-1 and HSV-2 infect epithelial cells and establish latent infections in neurons. Type 1 is classically associated with oropharyngeal lesions and causes recurrent attacks of “fever blisters”. Both types 1 and 2 can cause neonatal infections that are often severe.(Jawetz *et al*., 2019).

Infection with HSV-1 is more often, associated with facial disease though it causes an increasing number of genital infections. It consists characteristically of grouped or single vesicular lesions that become pustular and coalesce to form single or multiple ulcers (Sherris, 2018)

HHVs were detected in various inflammatory diseases of female upper and lower genital tract (vaginitis and cervicitis), in extrauterine pregnancy (in fallopian tubes), in infertility (cervical channel, endometrium and ovaries). HSV, cytomegalovirus (CMV) and human herpesvirus 6 markedly increase the risk of spontaneous abortion, preterm birth and stillbirth (Kushch *et al*., 2021)

Among the female patients, the presence of IgG indicated previous exposure to CMV in 92% of cases and herpes simplex virus in 80.8% (Althaqafi *et al*., 2020).

Primary HSV infection may lead to illness in pregnancy and associated with transplacental virus transmission and fetal infection. In neonate, after intrauterine HSV infection may be cause skin lesion, diseases of the eye and neurologic damage (Sauerbrei and Wutzler, 2007).

1.2 Rationale

Recently, infection by HSV during prenancy is major cause of spontaneous abortion, premature birth and stillbirth in women. However it may be roles of the exposure many factors: social, genetic, endocrine, physiological and psychological factors as well as lifestyle habits that is reasons to infection (Farsimadan and Motamedifar, 2021).

The primary or secondry Infection by HSV may occur during pregnancy and may be dangerous to the neonate if infected during delivery, as it can cause a severe neonatal diseases (abortion or stillbirth) (Avgil and Ornoy, 2006).

HSV-1 epidemiology in the Middle East and North Africa (MENA) remains poorly understood. The seroprevalence of HSV-1 was estimated for 10 national population : 97% among Egyptian, 92.6% among Yemenis, 90.7% among Sudanese, 88.5% among Syrian, 86.5% among Jordanians, 82.3% among Qataris, 81.4% among Iranians, 81.4% among Lebanese, 80.5% among Palestinians and 77% among Pakistanis (Nasrallah *et al*., 2017).

1.3 Objectives

1.3.1. General objective

To detect of herpes anti-simplex virus type 1 IgG antibodies among pregnant women in Khartoum State.

1.3.2. specific objectives

A. To detect anti-HSV IgG antibodies among pregnant women by immunoblotting technique.

B. To determine the frequency of abortion associated with HSV among pregnant women.

CHAPTER TWO
LITERATURE REVIEW

2.1. Herpes Simplex Virus

Herpes simplex virus is a genus in the order Herpesvirales, which is large group of enveloped double-stranded DNA viruses. There are presently nine members that are known to infect man eight whose natural host is man and one transmitted as a zoonotic infection from monkeys that can cause fatal encephalomyelitis in humans (Arie *et al.*, 2009).

Their acute infections are manifested as diseases of various organs, predominantly the skin (herpes simplexvirus types 1 and 2 as well as varicella-zoster virus) or the lymphatic system (Epstein-Barr virus, human herpesviruses 6, 7 and 8). Also some of them can infect the pregnant women that are cause severe damage to the embryo (Susanne *et al*., 2013)

In the immunocompetent patient, HSV infection typically represents nothing more than a nuisance. While in in the immunocompromised patient, this infection is associated with increased morbidity and mortality. Recently introduced antiviral drugs regimens may reduce the morbidity and potential mortality of HSV especially in immunocompromised patients (Huber, 2003).

HSV infection is very common worldwide with incidence of 22% of pregnant women are infected genitally with HSV. The most devastating consequence of maternal genital herpes is HSV disease in the newborn (Pinninti and Kimberlin, 2014).

HSV infection during pregnancy increased the risk of spontaneous abortion, premature birth and stillbirth (Shi *et al*., 2018)

2.1.1. Structure and composition

HSV have a unique four layered structure: a core containing the large, double-stranded DNA genome is enclosed by an icosapentahedral capsid which is composed of capsomers. The capsid is surrounded by an amorphous protein coat called the teguments. It is encased in a glycoprotein-bearing lipid bilayer envelope (Smith *et al*., 2014)

The glycoproteins, including gB, gH, gL and gD , are involved in cell attachment and penetration (Arii and Kawaguchi, 2018).

2.1.2. Replication

HSV-1 is characterized by a short (18–24 hour) replicative cycle that is cytolytic. Initial attachment and penetration of the host cell is mediated via the 11 types of glycoprotein spike found on the virus envelope. These spikes are responsible for the major antigenic differences between HSV-1 and HSV-2 and relate to the type-specific epitopes found on certain of the glycoproteins. Initial attachment of HSV-1appears to involve attachment to cell-surface glycosaminoglycans on heparin sulphate via glycoprotein C and glycoprotein B or on cells devoid of heparin sulphate, equivalent glycosaminoglycan moieties of other cell-surface proteoglycans, such as chondroitin sulphate (Zuckerman *et al*., 2009).

The parental capsid docks onto a host nuclear pore complex (NPC). The viral genome then translocates through the nuclear pore into the nucleoplasm, where it is transcribed and replicated to propagate infection. The viral and cellular proteins play roles in the process of capsid-nucleus attachment (Copeland *et al*., 2009).

HSV capsids are assembled around viral scaffolding proteins in the nucleus, and other VPs then interact with replicated viral DNA to allow DNA encapsidation (Zuckerman *et al*., 2009).

2.1.3. Transmission

Transmission of HSV occur via the contact of an infected person to an uninfected person. also can be transmitted through physical contact if exchange or exposure of saliva, oro-facial lesion, mucous membrane, genital fluids occurs or if corneal transplantation from n infected to health person occurs. The risk of transmission is significantly greater in women, infected by HSV during pregnancy then women who have longstanding infection (Moin *et al*., 2021)

Transmission of HSV infection to an infant during the first 3-4weaks of life can lead to devastating disease with the potential for poor outcome (Samies and James , 2020).

2.1.4. Epidemiology

The virus frequently infects human beings, causing a range of diseases from mild uncomplicated mucocutaneous infection to those that are life threatening (Whitley and Roizman, 2001).

Characterized by establishing lifelong infection with periods of latency interspersed with periodic episodes of reactivation. Acquisition of HSV by an infant during the peripartum or postpartum period results in neonatal HSV disease, a rare but significant infection that can be associated with severe morbidity and mortality, especially if there is dissemination or central nervous system involvement (James and Kimberlin, 2015).

Some study show the prevelance distribution of HSV-2 aginest HSV-1 in United States more than in European (Looker and Garnett, 2005).

Prevalence of herpes simplex viruses type 1 (HSV-1) varies widely across the world. the Seroprevalence of HSV-1 was 98% in pregnant women and 96% in blood donors in Turkey (Dolar *et al*., 2006).

The more prevalence of HSV infection is HSV-1 among female, those working and those who were married in Saudi Arabia was 88.8% that is effect by low education and increased with age. (Memish *et al*., 2015).

A high HSV-1 seroprevalence (82.4%) is reported from Nigeria (Reward *et al*., 2019).

2.1.5. Pathogenesis

HSV-1, is a widespread human pathogen that replicates in epithelial cells of the body surface and then establishes latent infection in peripheral neurons. When HSV-1 replicates, viral progeny must be efficiently released to spread infection to new target cells. Viral spread occurs via two major routes. In cell-cell spread, progeny virions are delivered directly to cellular junctions, where they infect adjacent cells. In cell-free release, progeny virions are released into the extracellular milieu, potentially allowing the infection of distant cells (Rice, 2021)

The intrinsic, innate and adaptive immune responses are key to control HSV, and the virus has developed mechanisms to evade them.The immune response can

also contribute to pathogenesis, as observed in stromal keratitis and encephalitis.the interaction of HSV with the immune system and three of the best-studied pathologies: Herpes stromal keratitis, herpes simplex encephalitis and genital herpes (Zhu and Viejo-Borbolla, 2021).

The outcome of infection with HSV-1 and HSV-2 can be asymptomatic, mild or life-threatening. In most immunocompetent individuals HSV causes mild and self-resolving disease. However, HSV infection is also associated with high morbidity and mortality in certain individuals for reasons that are not completely understood. Several reports suggest also a link between HSV infection and neurodegenerative diseases. The interaction between HSV and the host, in particular with the immune system, determines the outcome of infection. Genetic defects in intrinsic and innate defense mechanisms in the CNS are linked to higher risk of suffering HSE (Shuyong and Abel, 2021).

2.1.5.1. Latency stage

Herpes simplex virus type 1 is a neurotropic herpesvirus that establishes latency within sensory neurones. Following primary infection, the virus replicates productively within mucosal epithelial cells and enters sensory neurones via nerve termini. The virus is then transported to neuronal cell bodies where latency can be established. Periodically, the virus can reactivate to resume its normal lytic cycle gene expression programme and result in the generation of new virus progeny that are transported axonally back to the periphery (Nicoll *et al*., 2012).

2.1.6. Clinical significance

2.1.6.1 Pregnancy and congenital infection

Genital herpes is a common sexually transmitted disease, affecting more than 400 million persons worldwide. It is caused by herpes simplex virus (HSV) and characterized by lifelong infection and periodic reactivation. A visible outbreak consists of single or clustered vesicles on the genitalia, perineum, buttocks, upper thighs, or perianal areas that ulcerate before resolving. Symptoms of primary infection may include malaise, fever, or localized adenopathy. Subsequent outbreaks, caused by reactivation of latent virus, are usually milder. Asymptomatic shedding of transmissible virus is common. Although HSV-1 and

HSV-2 are indistinguishable visually, they exhibit differences in behavior that may affect management (Groves, 2016).

Genital herpes in persons with HIV type 1 (HIV-1) infection is associated with more-severe and chronic lesions, as well as increased rates of asymptomatic genital shedding of HSV-2 (Strick *et al*., 2006).

The main risk of HSV transmission to the neonate is during vaginal delivery from infected asymptomatic mothers who acquire HSV genital infection late in pregnancy. Because the survival of HSV out of the oral-genital secretions is weak, indirect and/or nosocomial transmisions of HSV are very rare and should be controlled by common-sense precautions (Morand, 2002).

Neonates can be contaminated in utero via transplacental hematogenic transmission, at delivery (the most frequent route), or during the postnatal period (indirect transmission). The risk of neonatal contamination is greatest for primary infection (PI) or non-primary infection occurring the last month of pregnancy, but transmission is low for maternal recurrence during the week before delivery (Henrot, 2002).

2.1.6.2. Infection in Immunocompromised

Patients with immunodeficiency or treatment-related immunosuppression are at an increased risk of developing severe herpes simplex virus (HSV) infection. They present a fatal case of a generalized HSV-1 infection in a 22-year-old female afflicted by acute lymphoblastic leukemia who was treated with polychemotherapy. The terminal clinical course was characterized by abdominal pain, progressive hepatic failure, and disseminated intravascular coagulation (Herget *et al*., 2005).

2.1.6.3. HSV in Immunocompetent adults

Herpse infection can still be clinical significance in adult immunocompetent populations. However infection is typically asymptomatic and greatly prevalent viruses that can cause conjunctivitis, keratitis and other rarer ocular disorders such as acute retinal necrosis syndrome or neuroretinitis (Kleinschmidt and Gilden, 2001) .

In this study, report a case of an isolated unilateral neuroretinitis with primary HSV infection in an immunocompetent adult without other related clinical features (Lázaro *et al*., 2022).

2.1.7. Laboratory diagnosis

The most frequently used tests for the diagnosis of HSV-1 infection are detection of antigen (by ELISA) and DNA, or mRNA. The use of quantitative DNA detection techniques has been increasing in recent years because they are highly sensitive and provide viral load measurements that can give important prognostic information (Pollack *et al*., 2011).

Accepted diagnostic method to demonestrated HSV-1 disease by using the "In-Cell WesternTM" Assay (ICW) from LI-COR, a quantitative immunofluorescence assay that exploits laser-based scanning of near infrared (NIR) (Fabiani *et al*., 2017)

2.1.7.1. Specimens collection

HSV-1 detection may be done on a variety of samples including vesicle swabs, blood, sputem CSF, and tissue . Some samples may require a special procedure to collect such as CSF is the most sensitive and others body fluid (Jawetz *et al*., 2019)

2.1.7.2. Antigen testing

Antigen detection by Direct fluorescent antibody (DFA), May be helpful to identify acute HSV infection in active lesions. DFA uses specific targeting moieties, such as antibodies, tagged with fluorophores to stain the virus These antibodies typically target the glycoproteins present on the surface of the viral particles. Samples were collected by swabbing and scraping the base of a skin lesion of the infected patients. The sample obtained was then rubbed onto glass microscope slides, air-dried, fixed, and made to react with the commercially available monoclonal antibodies labeled with fluorescein isothiocyanate (a fluorescent probe specific to HSV type 1 and 2) to form an immunocomplex. The emission of green fluorescence from the cells indicated the presence of HSV infection, as observed under a fluorescence microscope (Peuli *et al*., 2021).

2.1.7.3. Shell via assay

The technique involves inoculation of the clinical specimen on to cell monolayer grown on a coverslip in a shell vial culture tube, followed by low-speed centrifugation and incubation.The infected cell monolayer is then stained for the presence of viral antigens by direct fluorescent antibody .In this way, viruses that normally take days to weeks to produce a cytopathic effect (CPE) can be detected within 1 to 2 days by shell vial cell culture (Athmanathan *et al*., 2002).

2.1.7.4. Nucleic acid detection

Polymerase chain reaction (PCR) is used to detect HSV-1 in vesicle swabs, blood, sputem CSF, and tissue. PCR depends on the multiplication of primers specific and probes targeted at HSV-1 DNA polymerase gene. A major advance for PCR diagnosis of HSV infection is to use real-time PCR for detection and quantification. Amplification of the target DNA, and hybridization to the sub-typing specific fluorescent probes are conducted in a single PCR and therefore the chances of possible contamination are minimized. Real-time PCR has also been proved to be more sensitive in detecting asymptomatic shedding or shedding episodes in the absence of clinically obvious lesions (Junlian *et al*., 2015).

2.1.7.5. Serological tests

The enzyme-linked immunosorbent assay (ELISA) is the most commonly available serologic test for measuring antibody to HSV-1. Also fluorescence assays, indirect hemagglutination and latex agglutination tests are available. Serelogical test to detect antibodies to HSV glycoproteins G-1 and G-2, which evoke a type-specific antibody response. Focus technologies produces the HerpeSelect-1 enzyme-linked immunosorbent assay tests and the HSV-1 HerpeSelect1 Immunoblot. These tests can be used to confirm a genital herpes diagnosis, establish diagnosis of HSV infection in patients with atypical complaints, identify asymptomatic carriers, and identify persons at risk for acquiring HSV (Wald and Ashley-Morrow, 2002).

2.1.8. Prevention of HSV infection

Effective preventive strategies are badly needed. Vaccines are not available to provide protection pre-exposure. Present strategies include education of the public to increase awareness about genital herpes; education concerning the substantial protection of consistent condom use in both sexes; education of health-care professionals and patients concerning recognition of vague clinical signs for increased detection; testing of asymptomatic partners; and counseling the seronegative. Abstaining from sex during clinically-overt outbreaks and episodic treatment may be of some protective value, although incomplete, as silent excretion of virus is the major transmission factor. Suppressive therapy of the infected partner (e.g. aciclovir or valaciclovir 500 mg once daily) reduces, but does not eliminate, transmission to the noninfected partner. Consistent use of condoms significantly reduces transmission of HSV (Zuckermanb *et al*,.2009).

2.1.9. Treatment

HSV-1 infection should be treated with antiviral agents such as acyclovir, valacyclovir and famciclovir. Metabolic of these nucleoside derivatives interfere with the synthesis of viral DNA by inhibiting viral DNA polymerase (Saleh *et al*., 2021).

Also It can be used herbal medecine, pharmacologically active agents. The naturally occurring plant alkaloid berberine (BBR) is one of the phytochemicals with a broad range of biological activity, including anticancer, anti-inflammatory and antiviral activity. It has been shown that BBR reduces virus replication and targets specific interactions between the virus and its host. BBR intercalates into DNA and inhibits DNA synthesis and reverse transcriptase activity. It inhibits replication of herpes simplex virus (HSV) (Warowicka *et al*., 2020).

2.2. Previous studies

A study conducted to detect the prevalence of HSV-1 among women with infertility problems, recurrent abortion or exhibiting intrauterine growth restriction during pregnancy in Saudi Arabia, shows that out of 761, 80.8 % were (IgG) seropositive (Althaqafi *et al*., 2020).

Another study conducted to estimate Seroprevalence of HSV-1 among women with first-trimester spontaneous abortions in Chongqing, China, show that out of 100 pregnany women , (92.0%) were HSV-1/2 IgG positive (Gao *et al*., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This is a cross- sectional study descriptive.

3.2. Study area

The study was conducted in two hospitals in Khartoum State. These were Ombada Hospital and Fedail Hospital . The practical part of this study was done in the Immunology Laboratory, Fedail Hospital.

3.3. Study duration

This study was conducted during the period from April 2022 to September 2022.

3.4. Inclusion criteria : pregnant women.

3.5. Exclusion criteria : non-pregnant women.

3.6. Study population

Pregnant women with and without history of miscarriage were included.

3.7. Sample size

A total of ninety one blood samples (n=96) were obtained from pregnant women.

3.8 sampling techniques

3 ml of blood samples were collected.

3.9. Method collection

Data were collected through direct contact with participant using standard questionnaire.

3.10. Ethical considerations

This study was approved by scientific research committee college of Medical Laboratory Science, Sudan University of Science and Technology (SUST). Permission from hospital was taken and verbal consent was taken from each candidate.

3.11. Laboratory methods

3.11.1. Collection of specimens

A volume of 3 ml blood were collected from each pregnant women through venipunctures technique then displaced into plan container. Each blood sample was centrifuged at 3000 g for 5 minutes, then serum was gently collected into plain container and stored at -20 °C until the serological analysis.

3.11.2. Immunoblot

3.11.2.1. Procedure

All reagents and sera were settled to reach room temperature. Firstly, sera were diluted 1:51 with working-strength universal buffer using a clean pipette tip and mix well by vortexing. Then removed the required amount of test strips with tweezers from the packaging and placed directly in an incubation channel filled with buffer. Incubate for 15 minutes at room temperature on rocking shaker. Then completely removed the liquid from the trays. Each channel was filled with 1.5ml of the diluted serum samples using a clean pipette tip and incubate for 30 minutes at room temperature on a rocking shaker. The liquid was aspirated of each channel and washed 3 × 5 minutes each with 1.5ml of working strength universal buffer on a rocking shaker. Pipetted 1.5ml working strength enzyme conjugate (alkaline phosphatase labelled anti-human IgG) into each channel. Then incubated for 30 minutes at room temperature on rocking shaker. Aspirated off liquid and pipette 1.5ml substrate solution into the channels of the incubation tray. Incubated for 10 minutes at room temperature on rocking shaker. Aspirated off the liquid from each channel and wash each strip 3× 1 minute with deionised or distilled water. Finally they test strips placed on the evaluation protocol air dry and evaluated through (EUROLineScan) .

3.11.2.2. Quality control

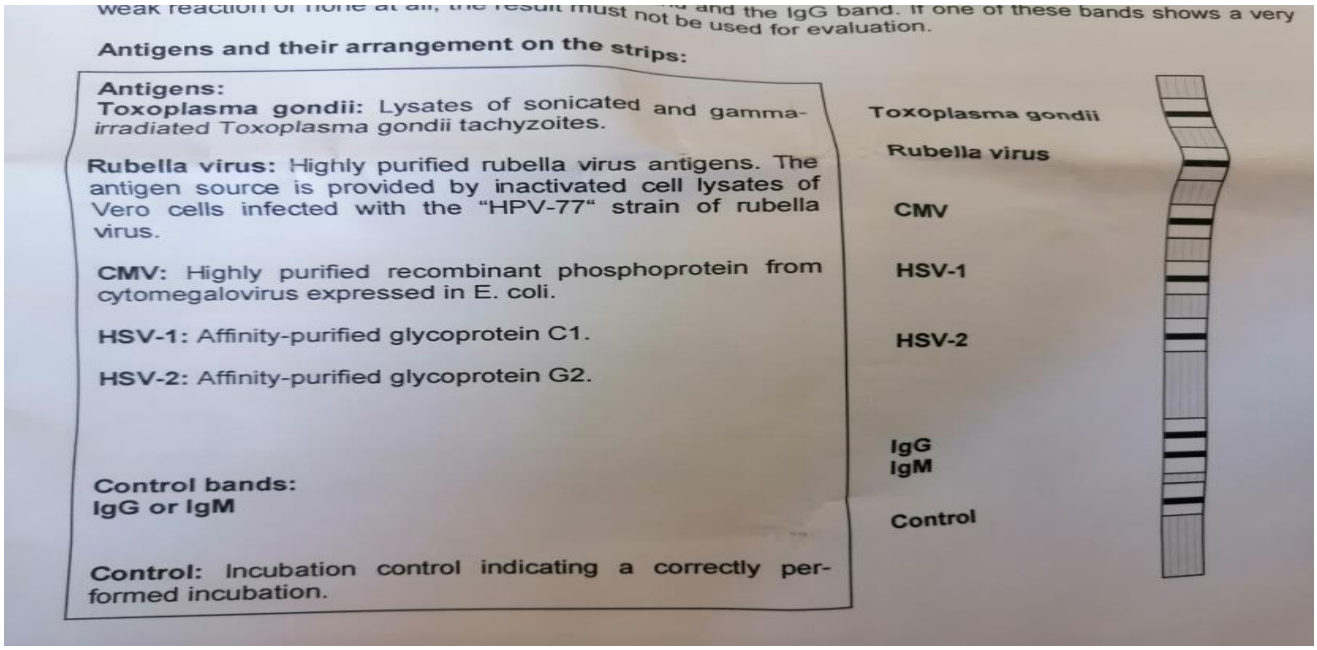

Figure 3.1 Antigen and their arrangement on the strips.

3.11.2.3. Evaluation of results

By using the EUROLineScan software. After stopping the reaction using deionised or distilled water, place incubated test trips onto the adhesive foiled of the green work protocol using a pair of tweezers. All test trips have been place onto the protocol, the should be pressed hard using filter paper and the left to air-

dry. After that, adhesive foil using. The dry test trips are scanned using a flatbed scanner and evaluated with EUROLineScan.

3.11.2.4. Interpretation of results

Signal	**Result**	
No signal or weak band	O	Negative
Weak band	(+)	Borderline
Medium to strong band	+	Positive

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of ninety six(n= 96) blood samples were obtained from pregnant women in two hospitals, which were Ombada Hospital (80 (83.3%)) and Fedail Hospital (16 (17%)) (Table 4.1). Out of 96 blood specimens; there were 65(67.7%) positive for anti-HSV-1 IgG antibodies and 31(32.3%) were negative. Of these positive, there were 29(44.6%) had history of miscarriage and 35(53.8%) hadn't previous miscarriage and according to P value, there no significant statistical difference between history of abortion in pregnant women and HSV-1 infection as show in (Table 4.3). concerning gestational age; there were 17(%) in first trimester, 33(%) in second trimester and 15(%) in third trimester were positive for anti-HSV-1 IgG antibodies and no significant association between HSV-1 infection and Gestational stage as show in (Table 4.4). regarding age groups; the highest frequency of anti-HSV-1 IgG antibodies in age group (25-33 years), followed by age group between (34-42 years) and the lowest frequency in age group (16-24years) as show in (Table 4.5).

Table 4.1. Distribution of Specimens according to the hospital

Hospital	No.	Percentage
Ombada Hospital	80	83.3
Fedail Hospital	16	16.6
Total	96	100

Table 4.2. Frequency of anti-HSV-1 IgG antibodies among pregnant women

Results	No.	Percentage
Positive	65	67.7
Negative	31	32.2
Total	96	100

Table 4.3. Frequency of anti-HSV-1 IgG according to history of miscarriage

Miscarriage	Result	No.	Percentage	P. value
Yes (n: 38)	Positive	29	44.6	0.2
	Negative	9	29.0	
No (n: 57)	Positive	35	53.8	
	Negative	22	70.9	
Total		96	100	

Table 4.4. Frequency of HSV-1 according to gestational stages

Gestational stage	No.	Result of anti-HSV-1 IgG antibodies		P. value
		Positive	Negative	
First trimester	23	17 (26.1%)	6 (19.3%)	0.08
Second trimester	44	33 (50.7%)	11 (35.4%)	
Third trimester	29	15 (23.0%)	14 (45.1%)	
Total	96	65 (67.7%)	31 (32.2%)	

Table 4.5. Frequency of anti-HSV-1 IgG antibodies according to age group

Age groups/ years	No.	Results	
		Positive	negative
16 – 24 years	22	13 (20%)	9 (29%)
25 – 33 years	41	31 (47.6%)	10 (32.2%)
34 – 42 years	33	21 (32.3%)	12 (38.7%)

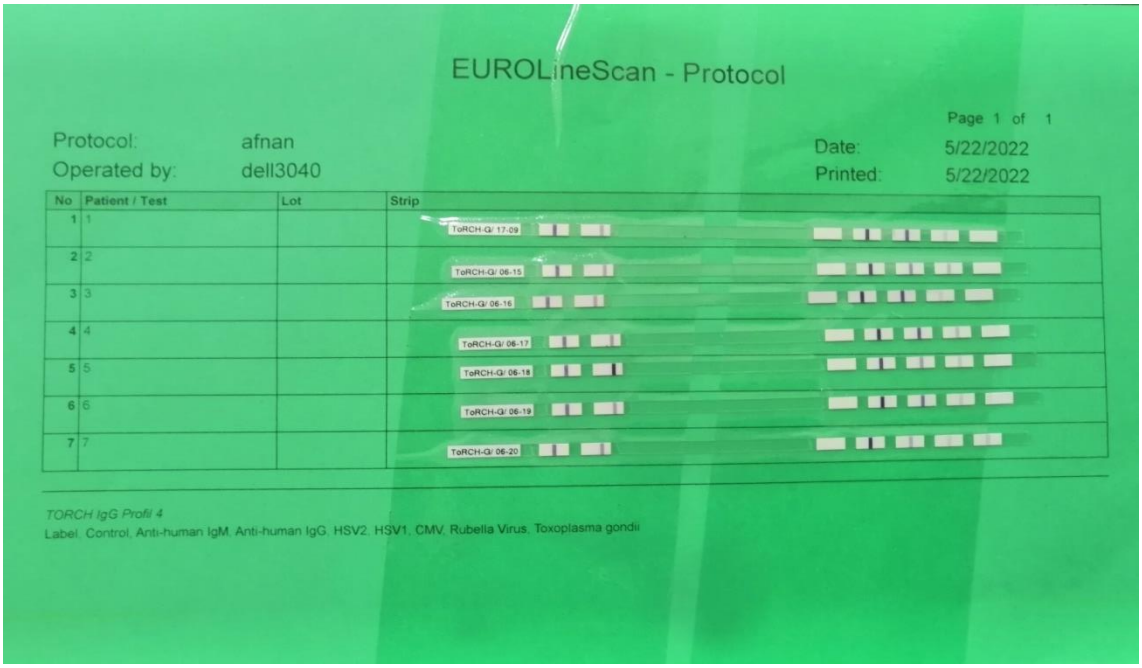

Figure 4.2 Results of immunoblot TORCH IgG screening. From 1 to 7 samples the lines fourth from left show bands that mean positive to HSV-1 IgG antibodies. The first and second lines for control to ensure validity of strips.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

Herpes simplex virus types 1 is one of the vertically transmitted infections that lead to congenital abnormalities. Studies showed that women who are exposed to HSV-1 for the first time during pregnancy may have a higher risk of miscarriage (Avgil and Ornoy, 2006).

Out of 96 investigated blood specimens, 65 (67.7 %) were positive for anti-HSV-1 IgG antibodies. This result was lower than obtained in Iran, (42%) by (Mina *et al*., 2016). But higher than those obtained in Venezuela, (97.2%) by (Monsalve-castillo *et al*., 2012), in China, (92%) by (Gao *et al*., 2018) and in Saudi Arabia, (80.8%) by (Althaqafi *et al*., 2020). These differences may be due to endimicity variations of these countries with HSV-1 infections.

In the present study pregnant women without history of miscarriage were highly infected with HSV-1 than those with history of miscarriage and anti-HSV-1 IgG positive (70.9% vs 44.6%). In this study seropositivity of HSV-1 was more detected in those pregnant women within the second trimester (34.4%) of gestation. In the present study there was high prevalence rate (32.2%) of HSV-1 infection among pregnant women within the age group (25–33). In study carried out by El-Amin *et al*., (2011) in which the result was low prevalence rate (19%) among pregnant women between the ages of (16 – 25) years and this may agree with the result obtained by this study.

5.2. Conclusion

There is high prevalence rate of HSV-1 infections among pregnant women in Khartoum State.

The level of infection is higher in those pregnant women with history of abortion than those no aborted women.

Statistical analysis shows that there is no significant association between abortion and HSV-1 infection.

High prevalence rate was found in those women within the second trimester of gestation.

Pregnant women within the age group (25–33) were highly infected with HSV-1.

5.3. Recommendations

1. Routine HSV-1 screening for each pregnant women must be done with high sensitive and specific approach.
2. Antiviral prophylaxis must be run to reduce the risk for HSV-1 infection.
3. Health educational programs must be improved to facilitate in prevention and control of HSV-1 infections.
4. Detection of HSV-1 IgM antibodies is needed to detect the recent infection.
5. Further studies in different geographical locations with large sample size and more advanced techniques such as RT-PCR are required to validate the results of the present study.

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Appendix

Questionner

Sudan university of Science and technology
College of Graduates Studies

Questionner No.( )

Detection of HSV-1 IgG among pregnant women in Khartoum state.

No.( )

Age ……..

Date …….

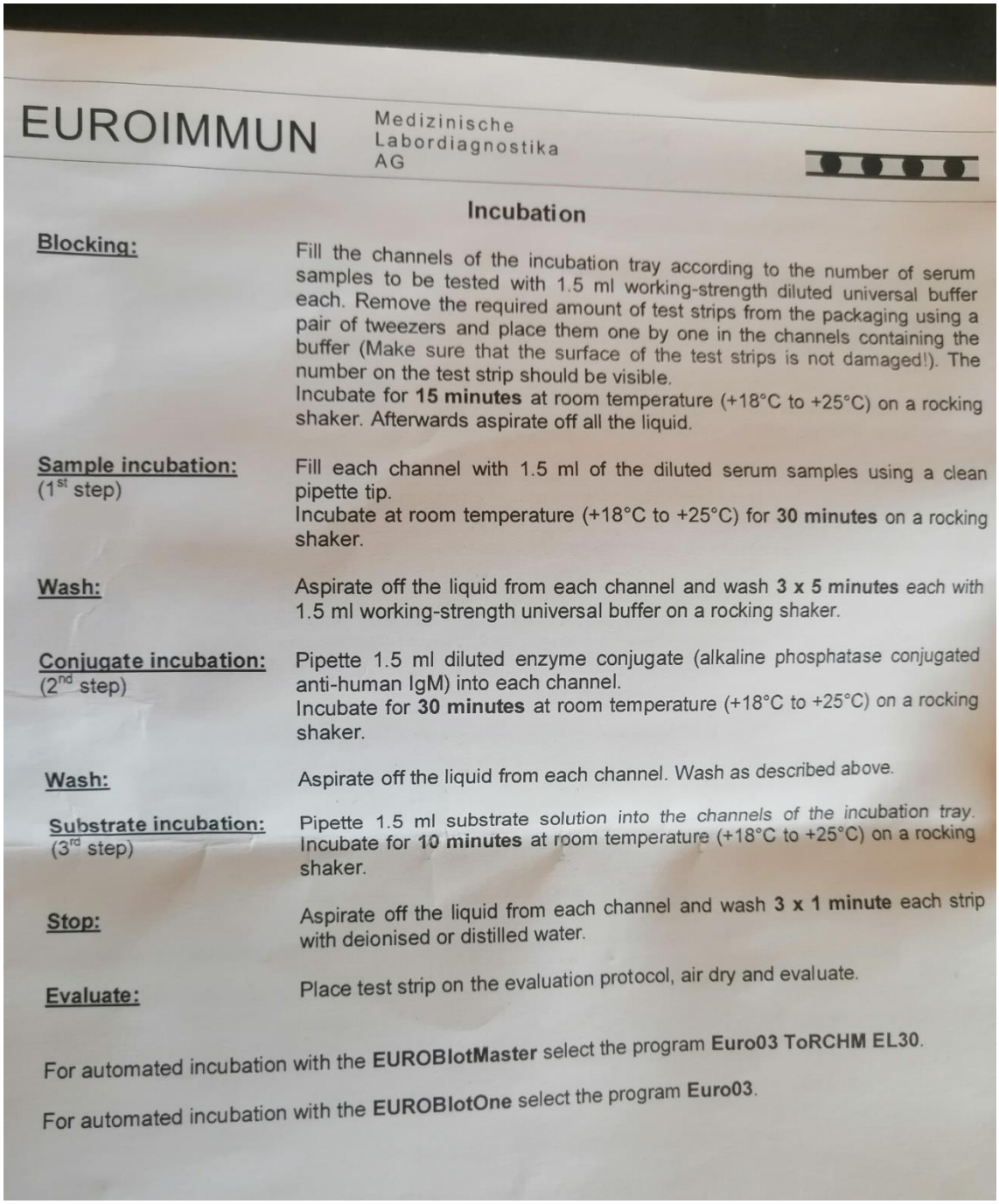
History of abortion :

- Yes ☐
- No ☐

Time of gestational stage:

- First trimester ☐
- Second trimester ☐
- Third trimester ☐

Box of EUROIMMUN Anti-TO.R.C.H profile (IgG) test instruction



EUROIMMUN Medizinische Labordiagnostika AG

Incubation

Blocking: Fill the channels of the incubation tray according to the number of serum samples to be tested with 1.5 ml working-strength diluted universal buffer each. Remove the required amount of test strips from the packaging using a pair of tweezers and place them one by one in the channels containing the buffer (Make sure that the surface of the test strips is not damaged!). The number on the test strip should be visible.
Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.

Sample incubation: (1st step) Fill each channel with 1.5 ml of the diluted serum samples using a clean pipette tip.
Incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.

Wash: Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working-strength universal buffer on a rocking shaker.

Conjugate incubation: (2nd step) Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase conjugated anti-human IgM) into each channel.
Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Wash: Aspirate off the liquid from each channel. Wash as described above.

Substrate incubation: (3rd step) Pipette 1.5 ml substrate solution into the channels of the incubation tray.
Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.

Stop: Aspirate off the liquid from each channel and wash **3 x 1 minute** each strip with deionised or distilled water.

Evaluate: Place test strip on the evaluation protocol, air dry and evaluate.

For automated incubation with the **EUROBlotMaster** select the program **Euro03 ToRCHM EL30**.

For automated incubation with the **EUROBlotOne** select the program **Euro03**.

Procedures of EUROIMMUN Anti-TO.R.C.H profile (IgG) test instruction.