بسم الله الرحمن الرحيم

Sudan University of Science and Technology
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Seroprevalence of Cytomegalovirus among Pregnant Women in Khartoum State

الإنتشار المصلي لفيروس مضخم الخلايا بين النساء الحوامل في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of M.Sc in Medical Laboratory Science (Microbiology)

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الآيـــــــــة

قال تعالى :

(اللَّهُ الَّذِي جَعَلَ لَكُمُ الْأَرْضَ قَرَارًا وَالسَّمَاءَ بِنَاءً وَصَوَّرَكُمْ فَأَحْسَنَ صُوَرَكُمْ وَرَزَقَكُم مِّنَ الطَّيِّبَاتِ ۚ ذَٰلِكُمُ اللَّهُ رَبُّكُمْ ۖ فَتَبَارَكَ اللَّهُ رَبُّ الْعَالَمِينَ (٦٤) هُوَ الْحَيُّ لَا إِلَٰهَ إِلَّا هُوَ فَادْعُوهُ مُخْلِصِينَ لَهُ الدِّينَ ۗ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

سورة غافر الآية : ٦٣ــــ٦٥

DEDICATION

To my mother, father,

brother, sister

and

my friends

ACKNOWLEDGEMENT

My gratitude and prayers to ALMIGHTY Allah for the mercy which followed me during the long path of this research. I am owe so much to my supervisor **Prof. Humodi Ahmed Saeed** for his close supervision, valuable advices and stimulating suggestions. Pleasant personality made it easy for me to do this work together and immense efforts not only to accomplish this work inculcate the research's soul on me.

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ABSTRACT

Cytomegalovirus is the most important cause of congenital infection. Exposure to this virus for the first time during pregnancy may have a higher risk of miscarriage.

The aim of this study was to determine the prevalence of Cytomegalovirus among pregnant women in Khartoum State.

A total of 91 participated women with and without history of miscarriage were enrolled in this study. Out of the enrolled women 39(42.8%) were pregnant women with history of abortion, while 52(57.1%) were pregnant women without history of abortion. Five ml of blood specimens were collected from each participated women and dispensed into sterile EDTA blood container to obtain the plasma. The later was obtained by centrifugation at 3000 g for 5 minutes. The sera were examined for the presence of CMV IgG antibodies using enzyme linked immunosorbent assay (ELISA).

The results showed that out of 91 blood specimens investigated, 67(73.6%) were positive for CMV. The Pregnant women with history of miscarriage were highly infected with CMV than those without history of miscarriage (82.1% vs 67.3%) respectively. The seropositivity of CMV was more detected in those pregnant women within the second trimester (76.2%) of pregnancy. There was high prevalence rate (82.4%) of CMV infection among pregnant women within the age group (25–34).

The study concluded that the seroprevalence of CMV infections among pregnant women in Khartoum State was high. Further studies with large numbers of participants and more advanced techniques are required to validate the results of this study.

المستخلص

الفيروس المضخم للخلايا هو أهم أسباب العدوى الخلقية وأنه يؤدي إلى التخلف العقلي والإعاقة التنموية كما ان تعرض النساء للفيروس المضخم للخلايا لأول مرة خلال فترة الحمل قد يجعلهم أكثر عرضة للإجهاض. الغرض من هذه الدراسة هو الكشف عن انتشار الفيروس المضخم للخلايا من بين النساء الحوامل في ولاية الخرطوم.

شملت الدراسة 91 امرأة حاملا كان من ضمنهم 39 (42.8٪) نساء حوامل تعرضن لإجهاض، و52 (57.1٪) نساء حوامل لم يحدث لهن اجهاض. تم جمع خمسة مل من عينة دم من كل النساء الحوامل وتم الحصول على البلازما بواسطة جهاز الطرد المركزي عند 3000 دورة لمدة 5 دقائق. تم فحص بلازما الدم لوجود الأجسام المضادة باستخدام تقنية الانزيم المناعي المرتبط (الاليزا).

أظهرت النتائج أن من أصل 91 عينة دم، 67 (73.6٪) كانت ايجابية للاجسام المضادة لفيروس المضخم للخلايا. نسبة الاصابة بالفيروس المضخم للخلايا كانت اكثر لدى النساء الحوامل اللاتي سبق لهن اجهاض مقارنة بالنساء اللاتي لم يحدث لهن اجهاض من قبل (82.1٪ مقابل 67.3٪) على التوالي.

أكثر العينات ايجابية كانت في النساء الحوامل في الثلث الثاني من الحمل (76.2٪)، كما ان هنالك ارتفاع في معدل انتشار الفيروس المضخم للخلايا (82.4٪) لدى النساء الحوامل في الفئة العمرية (34-25).

خلصت الدراسة إلى أن الانتشار المصلي من عدوى الفيروس المضخم للخلايا لدى النساء الحوامل في ولاية الخرطوم كان مرتفعا ويلزم إجراء مزيد من الدراسات مع عدد كبير من العينات والمزيد من التقنيات المتقدمة للتحقق من صحة نتائج هذه الدراسة.

ABBREVIATIONS

AIDS: Acquired Immunodefiency Syndrome

CMV: Cytomegalovirus

CDC: Centre for disease control

CNS: Central Nervous System

DNA: Deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

ELISA: Enzyme linked immunosorbent assay

EC: Endothelial cell

Gp: Glycoprotein

HSV: Herpes simplex virus

HCMV:Human Cytomegalovirus

HHV-5: Human Herpes virus-5

HDACs: Histone Deacetylases

IgG: Immunoglobulin gamma

IgM: Immunoglobulin Mu

NK: Natural killer cell

ORFs: Open Reading Frames

PCR:Polymerase Chain Reaction

RNA: Ribonucleic acid

TORCH: Toxoplasma, Rubella, Cytomegalovirus and Herpes Simplex virus

TMB: Tetramethyl benzidine

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INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Human Cytomegalovirus (CMV) is a ubiquitous virus which is contracted either vertically and/or horizontally. Also, it can be transmitted through primary infection; re infection or reactivation (Mocarski *et al.*, 2007).

Human cytomegalovirus (HCMV), HHV-5, belongs to the beta herpes family and is one of the most common causes of congenital viral infections. Congenital HCMV infection is associated with permanent hearing loss, vision loss and neurological impairment (Cannon *et al*., 2010).

Human Cytomegalovirus (CMV) infection is one of the Congenital infections (toxoplasmosis, other Infections including syphilis, rubella, CMV, and HSV), which carry a risk of significant symptomatic disease and developmental defects in newborns The clinical syndrome of congenital cytomegalic inclusion disease includes jaundice, splenomegaly, thrombocytopenia, intrauterine growth retardation, microcephaly, and retinitis (Ljungman *et al.,* 2002).

Cytomegalovirus infection during pregnancy is a major cause of congenital infection worldwide with an incidence of 0.2 – 2.2% of live births . Up to 15% of such children have newborns following intrauterine CMV infection (Adler, 2011). Infection in the newborn can be acquired through close contact (via contaminated blood, urine, and secretions), vertically through transplacental transmission and postnatally through breast milk (Bhide, 2008).

Most symptomatic neonatal CMV infections occur when a woman is newly infected justprior to or during pregnancy (Adler, 2011).

Women who develop primary CMV infection in the first trimester are morelikely to deliver fetuses with sensorineural hearing loss (24% vs 2.5%) or other CNS sequelae (Pass *et al*., 2006).

Cytomegalovirus infections tend to be less severe and are usually asymptomatic for both mother and newborn. Infants born to such mothers can also have sequelae of congenital CMV (Boppana *et al*., 2001).

The seroprevalence of HCMV is generally high in developing countries and among those of lower socioeconomic status in developed countries (Mocarski *et al.,* 2007*).*

A relatively low seroprevalence, 40%-60%, is reported from Australia, Belgium, France, Germany and USA (Colugnati *et al*., 2007).

A high HCMV seroprevalence (>90%) is reported from Brazil, Taiwan (Spano *et al.*, 2004) and in regional countries including Turkey, Qatar and Saudi Arabia (Uyar *et al.*, 2008). Reports from USA and Israel indicate that the HCMV seroprevalence among women varies based on ethnical and/or racial groups (Colugnati *et al.,* 2007).

The drug of choice for treatment of CMV disease is intravenous ganciclovir, although valganciclovir may be used for nonsevere CMV treatment in selected cases ,also as drug of choice for prevention of solid-organ transplant patients (Hodson *et al*., 2005).

1.2. Rationale

Cytomegalovirus is a major cause of morbidity and occasional mortality in newborn infants. In recent years, it has become evident that CMV is the most important cause of congenital infection in the developed world, and that it frequently leads to mental retardation and developmental disability (Soderberg, 2006).

Some research shows that women who are exposed to cytomegalovirus for the first time during pregnancy may have a higher risk of miscarriage (Tanaka *et al.,* 2006).

1.3. Objectives

1.3.1. General objective

To determine the frequency of Cytomegalovirus (CMV) infection among pregnant women in Khartoum State.

1.3.2. Specific objectives

A. To detect CMV IgG antibodies among pregnant women.

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

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Background

Cytomegalovirus (CMV) is a genus of viruses in the order Herpesvirales, in the family Herpesviridae, in subfamily betaherpesvirinae. Human and monkeys serve as natural hosts . There are currently eight species in this family including the type species human herpesvirus 5 (Mattes *et al*., 2000).

Human cytomegalovirus (CMV) is 1 of 8 human herpesviruses ,which also includes human herpesvirus type 6 and human herpesvirus type 7 (Schleiss, 2009).

In 1920 ,Goodpasture used the term cytomegalia to refer to the enlarged, swollen nature of the infected cells .Human CMV was first isolated in tissue culture in 1956 and the propensity of this organism to infect the salivary gland led to its initial designation as a salivary gland virus (Weller and Hanshaw, 1962).

Infection with CMV is ubiquitous and generally asymptomatic in healthy children and adults. However, several high–risk groups, including: immunocompromised organ transplant recipient and individual infected with human immunodeficiency virus (HIV) ,are at risk of developing life-threatening and slight–threatening CMV disease (Soderberg, 2006).

Cytomegalovirus (CMV) is transmitted person to person via close non–sexual contact ,sexual activity ,breastfeeding , blood transfusions and organ transplantation (Stango, 2001).

Cytomegalovirus infection during pregnancy is a major cause of congenital infection worldwide with an incidence of 0.2 – 2.2% of live births . Up to 15% of such children have newborns following intrauterine CMV infection (Adler, 2011).

2.2. Structure and composition

The human cytomegalovirus structure consists of an outer lipid bilayer envelope, composed of various viral glycoproteins, followed by the tegument, a proteinaceous matrix, which holds double stranded linear DNA core in an icosahedral nucleocapsid. The virion is usually spherical in composition. The average size can range from 200 to 300 nanometers. The glycoproteins, including glycoprotein B (gB), gH, gL, gM, gN, and gO, are involved in cell attachment and penetration. The tegument contains two major types of proteins, in addition to some cellular and viral RNA. One class of proteins serves a structural role and is integral in the assembly and disassembly of the virion during entry (Crough and Khanna, 2009).

The human cytomegalovirus genome consists of approximately 230kb of linear double stranded DNA, making it the largest out of all the human herpesviruses (Dunn *et al.*, 2003).

It contains approximately 150 open reading frames (ORFs) that encode proteins. Out of these, it has been found that 41 are essential and 109 are nonessential for HCMV replication. Whether an ORF is essential or nonessential depends on the type of cell the virus is grown in, which suggests that ORFs may play a role in tissue tropism (Shenk *et al.*, 2003).

2.3. Replication

Cytomegalovirus replicates within infected endothelial cells at a slow rate, taking about 5 days in cell culture. Like other herpes viruses, HCMV expresses genes in a temporally controlled manner. Immediate early genes (0–4 hours after infection) are involved in the regulation of transcription, followed by early genes (4–48 hours after infection) which are involved in viral DNA replication and further transcriptional regulation. Late genes are expressed during the remainder of infection up to viral egress and typically code for structural proteins. While HCMV encodes for its own functional DNA polymerase, the

virus makes use of the host RNA polymerase for the transcription of all of its genes **(**Stern *et al.,* 2012).

Following its entry into cells, the viral genome is delivered to the nucleus, where it associates with cellular histones (Nitzsche *et al*., 2008).

These histones are heavily posttranslationally modified with modifications regulated by various chromatin remodeling enzymes. Among these enzymes are histone deacetylases (HDACs), which have recently been shown to modulate the viral gene expression for several herpesviruses. Assembly of the viral core and capsid takes place within the nucleus. This is followed by envelopment at the nuclear membrane and transport out of the nucleus through the endoplasmic reticulum and the Golgi apparatus. Glycosylation of the viral membrane occurs in the Golgi apparatus. Mature virions are transported to the outer membrane of the host cell inside vesicles. Release of progeny virus is accompanied by cell death (Nitzsche *et al.,* 2008*).*

2.4. Transmission

The mode of human CMV transmission from person to person is entirely unknown but is presumed to occur through bodily fluids (Koichi *et al.,* 2007) .

Infection requires close intimate contact with a person secreting the virus in their saliva, urine, or other bodily fluids. CMV can be transmitted sexually and via breast milk, and also occurs through receiving transplanted organs or blood transfusions (Taylor, 2003).

2.5. Epidemiology

Cytomegalovirus (CMV) is a universally distributed pathogen with approximately 40-100% of the world's population having CMV antibody present in blood as evidence of infection (Mocarski *et al.,* 2007).

In the United States, >90% of healthy adults have become infected with CMV by the age of 80 years. Immunocompromised patients (AIDS patients or organ transplant recipients),

premature infants, and newborns with congenital CMV are at a high risk of developing serious, life threatening illness with CMV infection (Ross *et al.,* 2006).

In the developed world, CMV is the most common congenital viral infection. An overall rate of congenital CMV transmission of approximately 1% (ranging from 0.25–2%, depending on the population studied) has been estimated in newborn infants in the developed world in most reviews. This translates to about 80,000 congenital CMV infections per year in the United States and Europe. A recent meta-analysis of published studies concluded that the overall birth prevalence of congenital CMV infection was 0.64%, but noted that rates varied considerably among different study populations (Kenneson and Cannon, 2007).

In particular, a study reported 5 cases of severe morbidity and mortality in very low birth weight infants with CMV infection acquired postnatally through breast milk (Hamele *et al.,* 2010).

Seroprevalence status among pregnant and childbearing age women is the main focus of various worldwide studies due to the severe consequences to offspring. A relatively low seroprevalence, 40%-60%, is reported from Australia, Belgium, France, Germany and USA (Seale *et al.,* 2006).

A high HCMV seroprevalence (>90%) is reported from Brazil, Taiwan (Spano *et al.,* 2004*)* and in regional countries including Turkey, Qatar and Saudi Arabia (Uyar *et al.,* 2008) Reports from USA and Israel indicate that the HCMV seroprevalence among women varies based on ethnical and/or racial groups (Colugnati *et al.,* 2007).

2.6. Pathogenesis

Most healthy people who are infected by HCMV after birth have no symptoms. Some develop a syndrome similar to infectious mononucleosis or glandular fever, with prolonged fever, and a mild hepatitis. A sore throat is common. After infection, the virus remains latent in lymphocytes in the body for the rest of the person's life. Overt disease

rarely occurs unless immunity is suppressed either by drugs, infection or old age. Initial HCMV infection, which often is asymptomatic, is followed by a prolonged, inapparent infection during which the virus resides in mononuclear cells without causing detectable damage or clinical illness (Elizabeth and Caitlin, 2006).

Infectious CMV may be shed in the bodily fluids of any infected person, and can be found in urine, saliva, blood, tears, semen, and breast milk. The shedding of virus can occur intermittently, without any detectable signs or symptoms.The infected cell with CMV becomes enlarged and has characteristics of intranuclear inclusion bodies (owl's eye), which detected microscopically using H&E stain (Mattes *et al.,* 2000*).*

Lytically replicating viruses disrupt the cytoskeleton, causing massive cell enlargement, which is the source of the virus' name (Bennekov *et al.,* 2004).

A study published in 2009 links infection with CMV to high blood pressure in mice, and suggests that the result of CMV infection of blood vessel endothelial cells (EC) in humans is a major cause of atherosclerosis. Researchers also found that when the cells were infected with CMV, they created renin, a protein known to contribute to high blood pressure (Cheng *et al.,* 2009).

CMV encodes a protein, UL16, which is involved in the immune evasion of NK cell responses. It binds to ligands ULBP1, ULBP2 and MICB of NK cell activating receptor NKG2D, which prevents their surface expression. These ligands are normally upregulated in times of cellular stress, such as in viral infection, and by preventing their upregulation, CMV can prevent its host cell from dying due to NK cells (Welte *et al.,* 2003).

2.7. Clinical significance

2.7.1. Pregnancy and congenital infection

Human Cytomegalovirus is one of the vertically transmitted infections that lead to congenital abnormalities. (Others are:Toxoplasmosis, Rubella and Herpes simplex). Congenital HCMV infection occurs when the mother suffers a primary infection or reactivation during pregnancy. Up to 5/1000 live births are infected. 5% develop multiple handicaps, and develop cytomegalic inclusion disease with nonspecific signs that resemble rubella. Another 5% later develop cerebral calcification (decreasing IQ levels dramatically and causing sensorineural deafness and psychomotor retardation) (Caruso *et al.*, 2009).

Most (90 of every 100) infants who are infected with cytomegalovirus (CMV) at birth (Congenital CMV infection) appear healthy at birth. Health problems or disabilities due to congenital CMV infection may appear 2 or more years after birth, or they may never appear—80 of every 100 infants with congenital CMV infection never develop symptoms or disabilities (Pediatr, 2003).

2.7.2. Immunocompromised adults

Primary CMV infection in patients with weakened immune systems can lead to serious disease. However, a more common problem is reactivation of the latent virus.Infection with CMV is a major cause of disease and death in immunocompromised patients, including organ transplant recipients, patients undergoing hemodialysis, patients with cancer, patients receiving immunosuppressive drugs, and HIV-infected patients. Infections include hepatitis, cytomegalovirus retinitis, cytomegalovirus colitis, pneumonitis and esophagitis (Guido *et al.*, 2010*).*

2.7.3. Immunocompetent adults

CMV infections can still be of clinical significance in adult immunocompetent populations. However infection is typically asymptomatic or persists as a mononucleosis syndrome. The mononucleosis syndrome associated with CMV typically lacks signs of enlarged cervical lymph nodes and splenomegaly. CMV has been also been associated with Guillain–Barré syndrome, type 1 diabetes, and type 2 diabetes ,also infection may be linked to the development of arterial hypertension (Cheng *et al.*, 2009).

2.8. Laboratory diagnosis

The most frequently used tests for the diagnosis of CMV infection are detection of antigen (the pp65 antigenemia assay), 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 methods to document CMV disease include rapid cultures, direct fluorescent antibody tests, DNA hybridization, and cytology (Ljungman *et al.*, 2002*).*

2.8.1. Specimens

Cytomegalovirus detection may be done on a variety of samples including urine, blood or sputum. Some samples may require a special procedure to collect, such as amniotic fluid, duodenal fluid, cerebrospinal fluid, or body tissue (biopsy) (Cheng *et al.*, 2009*).*

2.8.2. Antigen testing

Detection of the CMV pp65 antigen in leukocytes with either immunofluorescence assay or messenger RNA amplification. These proteins are typically expressed only during viral replication.Antigen tests are often the basis for institution of antiviral therapy in transplant recipients and may allow for the detection of subclinical disease in high-risk patients. The assay is sensitive and specific yields results quickly. Antigen assays cannot

be used in patients with leukopenia, as these tests detect antigen within neutrophils. In immunocompromised patients, low or moderate CMV antigenemia may indicate reactivation or infection (Cunha, 2010).

2.8.3. Shell vial assay

The shell vial assay is performed by adding the clinical specimen to a vial that contains a permissive cell line for CMV. The shell vials are centrifuged at a low speed and placed in an incubator. After 24 and 48 hours, the tissue culture medium is removed and the cells are stained using a fluorescein-labeled anti-CMV antibody. The cells are read using a fluorescent microscope. Alternatively, the cells are stained with an antibody against CMV, followed by a fluorescein-labeled anti–immune globulin. This test has been found to be as sensitive as traditional tissue culture (Smith *et al.*, 2007).

2.8.4. Nucleic acid detection

Qualitative PCR is used to detect CMV in blood and tissue samples. PCR depends on the multiplication of primers specific for a portion of a CMV gene. The primers usually bind to the area of virus that codes for early antigen. Qualitative PCR is extremely sensitive, but, because CMV DNA can be detected in patients with or without active disease, the clinical utility of qualitative PCR is limited (Martín *et al.*, 2005).

Quantitative PCR has been used to detect plasma CMV. Ideally, quantitative PCR is as sensitive as qualitative PCR and provides an estimate of the number of CMV genomes present in plasma. Quantitative real-time PCR for CMV DNA could be used to detect antigenemia for monitoring CMV infection (viral load) and determining when to initiate preemptive treatment (Sanghavi *et al.*, 2008).

2.8.5. Serological tests

The enzyme-linked immunosorbent assay (ELISA) is the most commonly available serologic test for measuring antibody to CMV. Various fluorescence assays and indirect hemagglutination and latex agglutination tests are also available. The presence of CMV

IgM is not solely indicative of primary infection. CMV IgM is detectable when a person is newly infected or has been infected in the past but recently re-exposed to CMV or is undergoing reactivation of CMV infection that was acquired in the past, or has a false positive test result. Thus, the presence of CMV IgM should not be used by itself to diagnose primary CMV infection (CDC, 2010).

A positive test for CMV IgG indicates that a person was infected with CMV at some time during their life , but the IgG test cannot determine when a person was infected. Recently, IgG avidity assays, which measure antibody maturity, have been shown to reliably detect recent primary CMV infection. When a person is infected with CMV for the first time, the body produces low-avidity IgG. After 2-4 months, the body begins to produce high avidity CMV IgG. Low CMV IgG avidity suggests a primary CMV infection occurred within the past 2-4 months. High CMV IgG avidity suggests that CMV infection occurred at some point in the past (Zhang *et al.,* 2009).

2.9. Prevention of CMV infection

The practice of using "CMV-safe" blood products for recipients that are CMV seronegative is widely accepted. Two options exist for reducing the risk of CMV transmission via blood products: blood products from CMV-seronegative donors and leukocyte-reduced, filtered blood products. Both strategies are widely used. Leukocyte filtration should be performed at the blood bank and the established quality standards should be followed (Ratko *et al.,* 2001*).*

CMV-seronegative donors are generally selected for CMV-negative recipients in an HLA-identical sibling situation if multiple donors are available (Boeckh and Ljungman, 2009). Treatment with hyperimmune globulin in mothers with primary CMV infection has been shown to be effective in preventing congenital disease in several studies (Nigro *et al.,* 2012*).*

High-dose acyclovir and (500 mg/m2 3 times daily) valacyclovir prophylaxis (2 g: 3-4 times daily) have been shown in earlier studies to reduce the risk for CMV infection,

CMV disease, and survival. However, the most recent trial in the preemptive therapy era did not demonstrate an effect on CMV disease and survival (Griffiths *et al.,* 2008*).*

2.10. Treatment

CMV disease should be treated with antiviral agents such as ganciclovir or foscarnet. Induction doses for at least 2 weeks (preferably 3 weeks, if tolerated) are generally recommended, followed by maintenance dosing for another 3-4 weeks.13 Treatment should be continued until resolution of symptoms and negativation of the viral load. In patients with continued immunosuppression, continued maintenance or close virologic monitoring is recommended and additional treatment courses may be necessary.Gastrointestinal disease is generally treated with antiviral agents alone. CMV pneumonia treatment includes the use of intravenous immunoglobulin (Boeckh and Ljungman, 2009).

Recent studies suggest that CMV hyperimmune globulin may reduce the risk of congenital infection and disease when given to pregnant women experiencing a primary CMV infection.There is limited data on using antiviral medications such as ganciclovir to treat congenital CMV infection with central nervous system (CNS) involvement. There is some evidence that ganciclovir may prevent hearing loss and developmental outcomes in infants with symptomatic congenital CMV infection with CNS involvement (CDC, 2010).

2.11. Previous studies

A study conducted to detect the prevalence of CMV among pregnant women attending Murtala Mohammed Specialist Hospital Kano, Nigeria ,shows that out of 180 pregnant women, 164 (91.1%) were (IgG) seropositive (Hamid *et al.,* 2014).

Another study conducted to estimate the Seroprevalence of cytomegalovirus among pregnant women in western Sudan, shows that out of 231 pregnant women, 167 (72.2%) were CMV-IgG positive (Hamdan *et al.,* 2011).

Comprehensive study achieved to detect Seroprevalence of Cytomegalovirus among pregnant women and hospitalized children in Palestine . HCMV IgG was positive in 96.6% of pregnant women, in 88% of hospitalized children and in 98.4% of hospitalized newborns (Tahani *et al.,* 2013).

The estimation of Seroprevalence of cytomegalovirus (IgG) antibodies among normal pregnant women in Nigeria ,shows that there are high prevalence rate (50.8%) among pregnant women between the ages of 25–30 years (Akinsegun *et al.,* 2011).

Study aimed to determine the provincial population-based seroprevalence in pregnant women and to further explore the association of maternal CMV infection status and adverse pregnancy/neonatal/growth outcomes in Jiangsu, China , 98.7% was positive to CMV (IgG) antibodies (Lingqing *et al.,* 2014).

The first seroepidemiology study of CMV infection in pregnant women in Mexico, to determine seroprevalence association with socio-demographic, clinical and behavioral characteristics of pregnant women . Out of 343 pregnant women , 225 (65.6%) were CMV-IgG positive (Luis *et al.,* 2014).

Study conducted in Thika, Kenya showed high prevalence rate of cytomegalovirus (CMV) among pregnant those on the age group between 31–35 year old. More than third (39.4%) of these women were literate and either in business 68(26.2%) or employed 70(26.9%) (Zakayo and Anthony, 2014).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This was 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 Specialized Hospital and Ultra lab Diagnostic Centre. The practical part of this study was done in the 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 and without history of miscarriage were included.

3.2. Sample size

A total of ninety one (n=91) pregnant women were participated in this study.

3.3. Data collection

Informations such as (age, gestational stage and history of miscarriage) were obtained from patients by questionnaire (Appendix 1).

3.3.1. Data analysis

The data that collected from questionnaire and laboratory results were analyzed by Statistical Package for Social Sciences (SPSS) version 11.5 computerized program.

3.4. Ethical consideration

This study was approved by college of Medical Laboratory Science ethical committee, SUST. Permission from hospital was applied and verbal conset was taken from participants involved in the study.

3.5. Laboratory methods

3.5.1. Collection of blood specimens

A volume of 5 ml blood were collected from each patient through venipunctures technique then displaced into Ethylenediaminetetraacetic acid (EDTA) container.

3.5.2. Sample processing

Each blood specimen was centrifuged at 3000 g for 5 minutes to obtain the plasma. The later was gently collected into plain container and stored at -20 °C until the serological analysis.

3.5.3. Analysis of specimens

The specimens were analyzed for qualitative detection of CMV IgG antibodies by commercially available enzyme–linked immunosorbent assay ‘CMV IgG ELISA’ kit (Foresight, Acon laboratories, Inc., 10125 Mesa Rim Road, san Diego, CA 92121, USA). The assays were performed following the instructions of the manufacturer. According to the information included in the kit’s insert, the immunoassay used has 98.0% sensitivity and 98.3% specificity.

3.5.4. Principle of CMV ELISA

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

After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A (hydrogen peroxide) and substrate B (Tetramethyl benzidine) are added and then incubated to produce blue colour indicating the amount of CMV IgG antibodies present in the specimens. Sulfuric acid solution is added to microwell plate to stop the reaction producing a color change from blue to yellow. The colour intensity is measured using microwell plate reader (Appendix 3).

3.5.5. Procedure

1. All reagents and specimens were settled to reach the room temperature.

2. 100 µl of calibrator 1, 2, 3 and 4 were added to their respective wells.

3. 100 µl of sample diluents was added to each well except the blank.

4. 5 µl of sample was added to each well.

5. The microwell plate was mixed gently and covered by plate sealer and incubated for 30 minutes at 37 °C.

6. The microwell plate was washed 5 times using diluted wash buffer.

7. 100 µl of conjugate was added to each well except the blank.

8. The plate was covered and incubated for 30 minutes at 37 °C.

9. The microwell plate was washed 5 times with diluted wash buffer by automated ELISA washer (Appendix 4).

10. 50 µl of substrate A and substrate B were added to each well including the blank.

11. The plate was covered and incubated for 10 minutes at 37 °C .

12. 50 µl of stop solution was added to each well to stop the reaction.

13. The optical density was read at 450 nm within 30 minutes.

3.5.6. Quality control and calculation of results

3.5.6.1. Quality control

Reagents and calibrators were checked for storage, stability and preparation before starting work.

- Blank absorbance must be < 0.100 at 450 nm
- Calibrator 1 absorbance must be < 0.150 at 450 nm
- Calibrator 2 absorbance must be > 0.150 and < 0.400
- Calibrator 3 absorbance must be > 0.400 and < 1.200
- Calibrator 4 absorbance must be > 1.200

3.5.6.2. Calculation of results

The results were calculated by relating each specimen absorbance to index value.

Cut-off value = absorbance of calibrator 2 – Blank absorbance

Index value = Specimen absorbance / cut-off value

3.5.6.3. Interpretation of results

Index value > 1.1 : Positive

Index value < 0.9 : Negative

Index value $\geq 0.9 \leq 1.1$: Equivocal

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of ninety one blood specimens (n=91) were obtained from pregnant women in two hospitals and one diagnostic centre in Khartoum State. These were Omdurman Friendship Hospital 55(60.4%), AL-Saudi Specialized Hospital 23(25.2%) and Ultra lab Diagnostic Centre 13(14.2%) (Table 1). All specimens were examined for the presence of CMV IgG antibodies using ELISA kit. The results showed that out of 91 blood specimens investigated, 67(73.6%) were positive for CMV, while the rest 24(26.4%) were negative (Table 2). Out of 39 of the pregnant women with history of abortion 32(82.1%) were positive for CMV, while the rest 7(17.9%) were negative. Moreover, out of 52 of the pregnant women without history of abortion 35(67.3%) were positive for CMV, while the rest 17(32.7%) were negative (Table 3). According to gestational stages of pregnancy positive specimens were 12(70.5%) in the first trimester, 16(76.2%) in the second trimester and 39(73.6%) in third trimester (Table 4). Moreover, according to age group of each participated women, the positive specimens were 16(66.7%) within the age group (15 – 24), 42(82.4%) within the age group (25 – 34) and 9(56.3%) within age group (35–44) (Table 5).

Table 1. Distribution of Specimens according to the hospital

Hospital	Specimens	
	Number	%
Omdurman Friendship Hospital	55	60.4
Al-Saudi Specialized Hospital	23	25.2
Ultra lab. Diagnostic Centre	13	14.2
Total	91	100

Table 2. Serological results of CMV among participated women

Results	CMV	
	Number	%
Positive	67	73.6
Negative	24	26.4
Total	91	100

Table 3. Frequency of CMV among participated women according to the history of abortion

Abortion	Number	CMV	
		Positive	%
Yes	39	32	82.1
No	52	35	67.3
Total	91	67	73.6

Table 4. Frequency of CMV among participated women according to the gestational stages

Gestational stage	Number	CMV	
		Positive	%
First trimester	17	12	70.5
Second trimester	21	16	76.2
Third trimester	53	39	73.6
Total	91	67	73.6

Table 5. Frequency of CMV among participated women according to the age group

Age group	Number	CMV	
		Positive	%
15 ─ 24	24	16	66.7
25 ─ 34	51	42	82.4
35 ─ 44	16	9	56.3
Total	91	67	73.6

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Human cytomegalovirus is one of the vertically transmitted infections that lead to congenital abnormalities. Studies show that women who are exposed to cytomegalovirus for the first time during pregnancy may have a higher risk of miscarriage. The present study aimed to detect CMV among pregnant women in Khartoum State. Out of 91 blood specimens investigated, 67(73.6%) were positive. This result is similar to that obtained in western Sudan by (Hamdan *et al*., 2011), who reported that 72.2% of participated women were CMV-IgG positive. It is more than result that obtained in Mexico by (Luis *et al*., 2014) (65.6%), but less than those obtained in Nigeria by (Hamid *et al.*, 2014) (91.1%), in Palestine by (Tahani *et al.*, 2013) (96.6%) and in China by (Lingqing *et al.*, 2014) (98.7%). These differences may be due to endimicity variations of these countries with CMV infections. In this study the participated women with history of miscarriage were highly infected with CMV than those without history of miscarriage (82.1% vs 67.3%) and this may explain the association of CMV with miscarriage among pregnancy. The seropositivity of CMV was more detected in those pregnant women within the second trimester (76.2%) of gestation. Moreover, there was high prevalence rate (82.4%) of CMV infection among pregnant women within the age group (25–34). In study carried out by (Akinsegun *et al.*, 2011) showed that (50.8%) of CMV infected women were within the age group (25–30) and this may agree with the result obtained by this study.

5.2. Conclusion

This study concluded that there was high prevalence rate of Human cytomegalovirus infections among pregnant women in Khartoum State. In the present study the level of CMV infection is higher in those pregnant women with history of miscarriage than those women without history of miscarriage. There was high prevalence rate among

participated women within the second trimester of pregnancy than other pregnancy stages. Moreover, this study showed that the participated women within the age group (25– 34) were highly infected with CMV compared with the others age groups.

5.3. Recommendations

1. Pregnancy health care centers should be improved and routine CMV 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 CMV infection.
3. Health educational programs must be improved to facilitate in prevention and control of CMV infections.
4. Further studies in different geographical locations with large numbers of samples and more advanced techniques are required to validate the results of the present study.

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APPENDICES

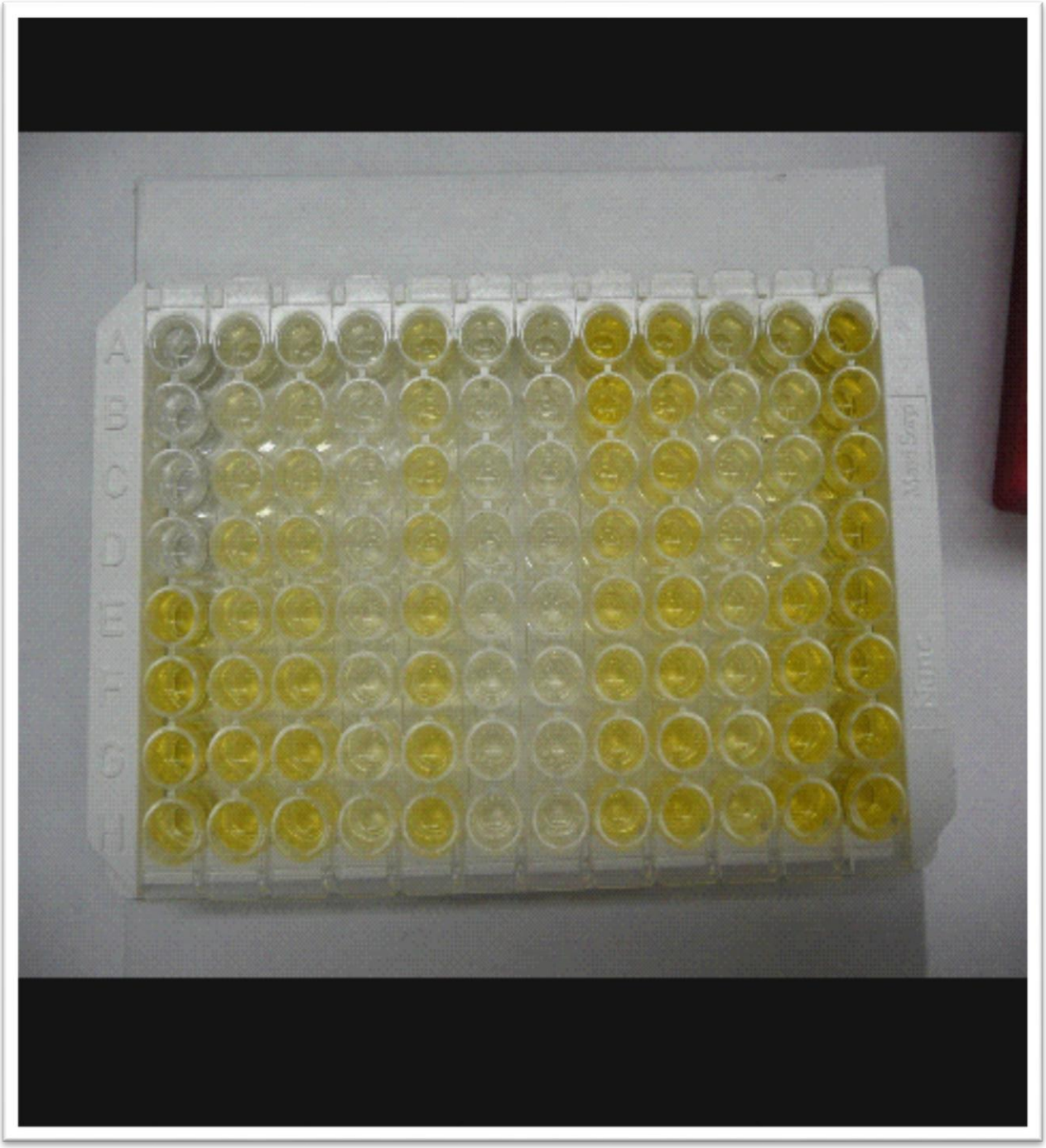
Appendix 1

Questionnaire

Number	Age	Gestational stage	History of abortion

Appendix 2

Microtiter plate



Appendix 3

Microwell plate reader

Appendix 4

Microwell plate washer