Seroprevalence of Cytomegalovirus among Pregnant Women in Kassala State

الانتشار المصلي للفيروس المضخم للخلايا بين النساء الحوامل في ولاية كسلا

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

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قال تعالى:

(إن في خلق السماوات والأرض واختلاف الليل والنهار لآيات لأولى الألباب * الذين يذكرون الله قيامًا ونهوضًا وعلى جنوبهم ويتفكرون في خلق السماوات والأرض ربنا ما خلقته هذا باطلا سبحانك فقاء عذاب النار)

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DEDICATION

To my dear mother, dear father, brother, sister

and

my friends

To all those who helped and encouraged me
ACKNOWLEDGEMENT

All thank to ALMIGHTY ALLAH for giving me strength and courage to complete this work and made all the things possible.

I wish to express my deepest gratitude to my supervisor Dr. Ehssan Hssan Osman Moglad for her guidance.

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Last but not least, I thank everyone who contributed by any means to this research from the commence, during the processing of specimens or the final touches.
ABSTRACT

Cytomegalovirus (CMV) is the most important cause of congenital abnormalities. It is highly prevalent in human population in many parts of the world. Exposure to this virus for the first time during pregnancy may have a higher risk of miscarriage.

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

A total of 91 pregnant women with or without a history of abortion were included in this study. Of them, 29 (31.9%) were pregnant women with history of abortion, while 62 (68.1%) were pregnant women without history of abortion. Five ml of blood specimens were collected from each participated women dispensed into sterile EDTA blood container to obtain the plasma by centrifugation at 3000 rpm for 5 minutes. The sera examined for the presence of CMV IgG antibodies using an enzyme-linked immunosorbent assay (ELISA).

The result showed that out of 91 blood specimens investigated, 89 (97.8%) were positive for CMV. In this study, there was insignificant relationship between CMV IgG and age, history of abortion and insignificant association between gestation stage and IgG result (P.value >0.05), this is mainly due to sample size.

The study concluded that the seroprevalence of CMV infections among pregnant women in Kassala 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 من أهم الأسباب المسببة للتشوهات الخلقية. وهو منتشر بشكل كبير بين البشر في عدة أجزاء من العالم. تعرض النساء لهذا الفيروس لأول مرة خلال فترة الحمل قد يجعلهم أكثر عرضة للإجهاض.

الهدف من هذه الدراسة هو تحديد مدى انتشار الفيروس المضخم للخلايا بين النساء الحوامل في ولاية كسلا. شملت الدراسة 191 امرأة حاملًا كان من ضمنهم 29 (31.9%) نساء حوامل تعرضن للإجهاض، و 62 (68.1%) نساء حوامل لم يحدث لهن إجهاض. تم جمع خمسة مل عينة دم من كل النساء الحوامل.

تم 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 (الإليزا).

أظهرت النتائج أن من أصل 91 عينة دم، 89 (89.7%) كانت إيجابية للاجسام المضادة لفيروس المضخم للخلايا في هذه الدراسة كانت هناك علاقة ضئيلة بين الجسم المضاد للفيروس المضخم للخلايا والعمر، تاريخ الإجهاض والرابط غير الهام بين مرحلة الحمل ونتائج الال، ويرجع ذلك أساسا إلى حجم العينة.

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

AIDS: Aquired Immunodefiency Syndrome
CMV:Cytomegalovirus
CF assay: Complement Fixation assay
DNA:Deoxyribonucleic acid
dsDNA: double strand Deoxyribonucleic acid
EDTA: Ethylene diamine tetra acetic acid
ELISA: Enzyme-Linked ImmunoSorbent Assay
ERGIC: Endoplasmic reticulum- golgi Intermediate compartment
HCMV: Human Cytomegalovirus
HIG: Hyper immunoglobulin
HIV: Human immunodeficiency virus
IgG: Immunoglobulin gamma
IgM: Immunoglobulin mu
MT: Microtubule
OPF: Open reading frame
OR: Odds ratio
PCR: Polymerase Chain Reaction
QNAT: Quantitative Nucleic Acid Testing
RNA: Ribonucleic acid
TORCH: Toxoplasma, Rubella, Cytomegalovirus and Herpes Simplex virus
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1.1. Introduction

The human cytomegalovirus (CMV) or human herpes virus 5 is one of the major causes of congenital infections (De Paschale et al., 2009).

HCMV can be transmitted via saliva, sexual contact, placental transfer, breastfeeding, blood transfusion, solid-organ transplantation, or hematopoietic stem cell transplantation (Crough and Khanna, 2009).

Infection with CMV can occur in pregnant women by primary infection, reactivation, or reinfection with a different CMV strain during pregnancy (Alvarado-Esquivel et al., 2018).

CMV among congenitally-infected infants can cause permanent disabilities such as hearing loss, vision loss, and mental retardation (Kenneson and Cannon, 2007).

Congenital CMV infection is the most common congenital infection worldwide, with an estimated incidence in developed countries that ranges from 0.6 to 0.7% of all live births. Since the incidence of congenital CMV infection in developing countries is even higher, between 1 and 5% of all live births (Marsico and Kimberlin, 2017).

Vertical infection can occur antenatal through the placenta, during delivery through cervical secretions and blood and postnatal through breast milk (Bhide and Papageorghiou, 2008).

The majority of children with congenital CMV infection (approximately 85%–90%) do not have clinical findings at birth (asymptomatic infection). The remaining 10%–15% of infected fetuses are symptomatic with characteristic
clinical manifestations and complications, manifestations can range from mild
nonspecific findings to multiple organ system involvements, with the particular
predilection for the reticuloendothelial and central nervous system. The most
common symptoms are petechial rash, jaundice, and hepatosplenomegaly with
neurologic abnormalities such as microcephaly and lethargy. Also chorioretinitis
and/or optic atrophy in approximately 10% of symptomatic infants (Boppana et al.,
2013).

primary CMV infections are associated with the greatest risk of in-utero
transmission at 30–35%, while for non-primary infections the transmission rate is
significantly lower at 1.1–1.7% (Marsico and Kimberlin, 2017).

It has been reported that the risk of fetal damage is greater if the primary infection
occurs during the first trimester of pregnancy (De Paschale et al., 2009).

CMV seroprevalence varies between countries and tends to be higher in
developing countries ( >90% in Brazil, India and Turkey, 70–80% in Ghana, , 80–
90% in South Africa) and lower in developed countries (40–70% in Western
Europe, 60–70% in Australia and in Canada and 50–60% in the United States).
Even within countries, the rates of CMV seropositivity in women vary by
socioeconomic status and ethnicity (van Zuylen et al., 2014).

1.2. Rationale:

Cytomegalovirus (CMV) is responsible for substantial morbidity and mortality
among human immunodeficiency virus (HIV)-infected persons, transplant
recipients and congenitally infected children (Schoenfisch et al., 2011).

Human cytomegalovirus (HCMV) is a leading cause of congenital infections in
developed countries, where it is the most common non-genetic cause of childhood
hearing loss and an important cause of neurodevelopmental delay (Simonazzi et al., 2017).

Infections of human cytomegalovirus in pregnant women are the main causes of recurrent spontaneous abortion (Pandey et al., 2005).

There is a Lack in the documentation system for seroprevalence of HCMV in Sudan’s different states.

1.3. Objectives:

1.3.1. General Objective:

To determine the seroprevalence of Cytomegalovirus (CMV) infection among pregnant women in Kassala state.

1.3.2. Specific Objectives:

1- To detect CMV IgG antibodies among pregnant women.

2- To determine the association between the CMV infection and frequency of abortion.
CHAPTER TWO
LITERATURE REVIEW

2.1. Background:

Human cytomegalovirus (HCMV) is a herpesvirus (Human herpesvirus 5, genus Cytomegalovirus, subfamily Betaherpesvirinae, family Herpesviridae) that causes widespread, persistent human infection (Sinclair and Sissons, 2006). It is a member of the Herpesviridae family of viruses, which includes herpes simplex virus type 1 and type 2, Varicella Zoster Virus, Epstein–Barr virus, Roseolovirus (HHV-6 and HHV-7), and Kaposi’s sarcoma-associated herpesvirus or HHV-8. (van Zuylen et al., 2014).

HCMV was isolated in 1956 for the first time. The name is derived from the fact that it causes enlargement of the infected cell (cytomegaly) and induces characteristic inclusion bodies (Schottstedt et al., 2010).

Although HCMV infects only humans, CMV-like viruses have been isolated from a variety of mammalian hosts, including other primates and rodent (Louten, 2016).

The virus generally does not cause disease in healthy individuals, but it can cause life-threatening disease in immunologically immature or compromised individuals, including neonates, AIDS patients, allogeneic-transplant recipients, and cancer patients undergoing chemotherapy (Feng et al., 2006).

CMV is transmitted from person to person via close nonsexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation. For pregnant women, the important source of infection include sexual activity and contact with the urine or saliva of young children, especially their own children (Colugnati et al., 2007).
Human cytomegalovirus (CMV) is an endemic and ubiquitous beta-herpesvirus that leads to congenital infection in 0.3–2.3% of all live births (Lazzarotto et al., 2011).

2.2. Structure and composition:

HCM structure is consist of a core capsid containing dsDNA. The capsid is covered with a tegument layer containing structural proteins and is further surrounded by a lipid bilayer containing several viral glycoproteins. Virions size can range from 200–300 nm in diameter (Mohammad et al., 2017).

The glycoproteins include glycoprotein B (gB), gH, gL, gM, gN, and gO that are involved in cell attachment and penetration. The tegument contains the majority of the virion proteins, and also contains additional proteins that are present in small amounts and some cellular and viral RNA. The function of the tegument proteins: (i) proteins that play a structural role and are important for the assembly of virions and the disassembly of the particle during entry and (ii) proteins which modulate the host cell response to infection (Crough and Khanna, 2009).

The HCMV genome is consist of 235,645-base pair (bp) linear double-stranded DNA molecule with a coding capacity of 165 genes. It is the largest genome among the human herpesviruses (Douglas. Richman, 2017).

2.3. Transmission:

Cytomegalovirus (CMV) transmissions occur among people of all ages, races, and socioeconomic classes, throughout both the modernized and developing parts of the world (IFeanyi and Ogbonnaya, 2017).
This virus can be transferred between individuals via all bodily fluids (e.g. saliva, breast milk, semen, blood products), as well as by bone marrow grafts and solid organ transplants (Soderberg-Naucler, 2006).

Transmission of CMV can occur through sexual contact is an important means of spread. High rates of incident CMV infection have been reported in clinics for sexually transmitted diseases and among sexually active adolescents. Also, vertical transmission occurs in that transmission from mother to fetus or newborn not only occurs but is common and plays an important role in maintaining CMV infection in the population. Human CMV is spread from mother to baby by three routes: transplacental, intrapartum, and human milk (Knipe et al., 2001)

2.4. Replication:

The virion attaches to the cellular surface via glycoproteins in the envelope and then penetrates the cell. It is hypothesized that the capsid travels to the nucleus via the microtubule (MT) network, where it docks and releases the viral DNA at a nuclear pore complex. Upon release of DNA into the nucleus, products responsible for various functions including but not limited to, viral replication, immunomodulation, and inhibition of apoptosis are then expressed. The linear viral DNA circularizes and replicates via a rolling circle mechanism. The new DNA strands are then packaged into capsids formed within the nucleus. Nuclear egress of the nucleocapsid occurs by a primary envelopment and deenvelopment, which releases the nucleocapsid into the cytoplasm. In the cytoplasm, the nucleocapsid obtains its tegument proteins and buds into the Endoplasmic Reticulum-Golgi Intermediate Compartment (ERGIC) to obtain its envelope. After secondary envelopment, the mature virion-containing vesicles then fuse with the plasma membrane, resulting in the release of the progeny into the periphery (Yu et al., 2005).
The ORFs responsible for viral DNA replication is critical for the production of viral progeny. These ORFs include UL84 and UL122 (IE2), which express transcriptional transactivators that form protein complexes across the oriLyt to start viral replication. Following the complex formed by UL84 and IE2, six core replication proteins are then recruited to the site of DNA synthesis. These proteins include DNA polymerase (UL54); C-clamp DNA processivity factor (UL44); helicase (UL105); single-stranded DNA-binding protein (UL57), which is believed to aid in strand separation during DNA replication; primase (UL70); and primase-associated factor (UL102) (Pari, 2008).

2.5. Epidemiology:

Cytomegalovirus (CMV) is common in all human populations, with seroprevalence rates ranging from 40% to 100%. Thus, at-risk populations include transplanted patients, HIV-positive patients and, more largely, patients receiving immunosuppressive therapy (Delvincourt et al., 2014).

Seroprevalence rates vary by socioeconomic class and geographic location, but the overall seroprevalence in developed countries is estimated to be in the range of 30-70% (Biron, 2006). CMV is now the most common viral etiology of mental retardation and auditory disorder of children in developed countries (Abdullahi Nasir et al., 2016). Congenital CMV infection, occurring in approximately 1% of all live births (Zhang et al., 2014).

Each year in the USA, an estimated 40,000 pregnant women acquire a primary CMV infection (seroconvert) during pregnancy. Of the 40,000 women who seroconvert approximately 6,000 to 8,000 of their infants will develop severe and permanent neurologic damage from this infection. Another less frequent effect is
fetal death or neonatal death which occurs in about 10% of fetuses or newborns following an intrauterine CMV infection (Adler, 2011).

The rate of seropositivity of anti-CMV immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) antibodies of pregnant women in Turkey was reported to be 98.5% and 84% in Spain. These rates are much higher than the typical European rate but similar to the rate obtained amongst Black pregnant women. An overall rate of 87% of anti-CMV IgG ELISA antibodies in pregnant women was reported in Singapore, 100% in Thailand, and 93% in Iranian women of childbearing age (Akinbami et al., 2011).

2.6. Pathogenesis:

Cytomegalovirus (CMV) can cause severe disease in immunocompromised patients, either via reactivation of latent CMV infection or via the acquisition of primary CMV infection. Furthermore, CMV infection affecting the human embryo, a host with the immature immunological response, is often associated with serious complication (Rafailidis et al., 2008). To contrary in immunocompetent patients, primary CMV infection typically runs an undifferentiated viral syndrome or is manifested by a mononucleosis-like syndrome. Symptomatic CMV infection in immunocompetent hosts has traditionally been considered to have a benign, self limited course (Rafailidis et al., 2008).

CMV is a major cause of morbidity in immunosuppressed patients, causing significant disease in transplant patients and, prior to the introduction of highly active antiretroviral therapy (HAART), in HIV. In such patients, end-organ involvement following the viraemic spread of CMV may lead to damage to a single organ, as seen in, for example, colitis, retinitis or severe pneumonitis (Goodman et al., 2015).
Primary infection with CMV may be acquired at any time, possibly from conception onwards and, similar to other herpesviruses, CMV persists in the host for life. Reactivation is common, and virus shed in body secretions such as urine, saliva, semen, breast milk, and cervical fluid. Mononuclear cells carry the latent virus genome and viral RNA transcripts of early genes have been detected in such cells. Bone marrow progenitor cells of the myeloid line may be the prime site of latency. Once their descendants have been activated to differentiate into tissue macrophages, the virus can enter the replication cycle. Recurrent infections may follow the reactivation of latent (endogenous) virus, or re-infection with another (exogenous) strain (Greenwood, 2012)

**2.7. Clinical significance:**

**2.7.1. Pregnancy and congenital infection:**

Some of the most common infections associated with congenital anomalies are summarized in TORCH testing. TORCH includes Toxoplasmosis, Rubella, Cytomegalovirus (CMV), and Herpesvirus infections. Most of the TORCH infections cause mild maternal morbidity (Neirukh et al., 2013).

Congenital infections are the result of transplacental transmission of CMV. Transmission to the fetus may occur because of primary or secondary maternal infection. Ten to fifteen percent of congenitally infected infants will have symptoms a birth including intrauterine growth restriction, microcephaly, hepatosplenomegaly, petechiae, jaundice, chorioretinitis, thrombocytopenia, and anemia, and 20% to 30% of them will die, mostly of disseminated intravascular coagulation, hepatic dysfunction, or bacterial superinfection. Most of the congenitally infected infants (85–90%) have no signs or symptoms at birth, but 5%
to 15% of them will develop sequelae such as sensorineural hearing loss, delay of psychomotor development, and visual impairment (Yinon et al., 2010).

2.7.2. Immunocompromised adults:

Immunocompromised patients may develop symptoms as a result of primary, or recurrent CMV infection. Dissemination of the virus in the blood, as indicated by a hectic fever, is a bad prognostic sign. The complications of CMV infection in cellular immunodeficiency include: pneumonitis: a high mortality rate in recipients of bone marrow allografts, encephalitis, retinitis: may occur on its own (10–40% of patients with AIDS) oesophagitis/colitis: 5–10% of patients with AIDS, hepatitis, pancreatitis and/or adrenalitis. CMV infection in transplant recipients is a significant cause of direct (caused by the virus) and indirect (caused by virus interactions with the immune system) morbidity that may culminate in loss of the grafted organ and even death (Greenwood, 2012).

2.7.3. Immunocompetent adults:

Although primary infection in immunocompetent persons is normally subclinical, a mononucleosis-like syndrome that is characterized by malaise, protracted fever, mild liver-function abnormalities, and lymphocytosis with atypical lymphocytes occurs in 10% of immunocompetent adults. This syndrome is generally mild and self-limiting, but rarely patients may develop a fulminant infection that manifests with multiple organ involvement and marked constitutional symptoms (Eddleston, 1997).

2.8. Laboratory diagnosis:

Diagnostic tests for CMV include serology, tests for the active disease, including quantitative nucleic acid testing (QNAT), antigenemia, culture, and histopathology, as well as newer immunology assays reflecting the cellular immune response to
CMV (Michael J. Loeffelholz 2016). Samples should include urine, saliva, broncho-alveolar lavage fluid, and/or biopsy tissue (if available), and peripheral blood collected in suitable (usually EDTA) anticoagulant (Greenwood et al., 2012)

2.8.1. **Nucleic acid detection:**

PCR Due to its high sensitivity and rapid turn around time, PCR is considered the gold standard method of diagnosing CMV infection. Both qualitative and quantitative (viral load) PCR assays are available. However, quantitative tests are preferred as it quantifies viral load, which has prognostic importance. Whole blood testing is more sensitive than plasma testing because it enables the detection of cell-free and intracellular viruses (Al-Omari et al., 2016).

2.8.2. **Antigen detection:**

PP-65 antigenemia test in which specific monoclonal antibodies are used to detect, a CMV matrix phosphoprotein known as pp-65 in leukocytes. Since its initial description, the pp65 antigenemia assay has represented a major breakthrough for the diagnosis of CMV infections. The test has several advantages from the clinical perspective, and also in terms of laboratory practices. Method of detection of pp65 is very fast, allowing viral detection after 4-5 hour of blood sampling. CMV antigen positive blood cells appeared 1-3 weeks on average nine days before serologic signs of active infection. Thus detection of CMV pp65 antigens appeared to be an earlier indicator of active infection than CMV IgM antibody. This assay is sensitive and specific and yields result within 5 hours. In addition, the antigenemia assay is quantitative and has been useful for estimating the likelihood of disease progression, as well as for monitoring the response to therapy (Jahan, 2012).

2.8.3. **Serology:**
Serologic tests that detect CMV antibodies (IgM and IgG) are widely available. Enzyme immunoassays are usually employed to detect anti-CMV antibodies. Various fluorescence assays and indirect hemagglutination and latex agglutination tests are also available for measuring antibody to CMV. Detection of CMV IgG antibody is used for the diagnosis of CMV infection and not a disease since a majority of individuals are seropositive for CMV. Antibody tests of paired acute- and convalescent-phase serum samples showing a fourfold rise in IgG antibody and CMV IgM antibody can indicate active CMV disease. The presence of CMV IgM is not solely indicative of primary infection. CMV IgM is detectable when a person is newly infected; has been infected in the past but has been recently re-exposed to CMV; 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 (Michael. Loffleholz 2016).

ELISA for detection of CMV antibody is available from several manufacturers. The ELISAs give higher antibody titers and is as accurate as CF assays in determining serologic status, are much easier to perform and eliminate the problem of anticomplementary sera. Results are typically available in a few hours (Jahan, 2012).

-2.8.4. The IgG Avidity Test:

The functional binding affinity of anti-CMV IgG antibodies increases progressively over time after immunity by infection; it is otherwise referred to as maturation of the humoral immune response. Low IgG antibodies avidity indices may indicate primary infection whereas high avidity indices indicate non-primary infection (Abdullahi Nasir et al., 2016).
2.8.5. Culture test:

CMV culture can be slow, expensive and less sensitive. Seropositive humans may shed CMV in their secretions, especially during times of stress, rendering positive cultures that do not necessarily reflect active disease. Viral culture of blood for CMV has poor sensitivity, while CMV urine, stool, and sputum cultures have poor specificity. The culture of tissue specimens remains an important option for diagnosis of tissue-invasive disease, particularly for gastrointestinal samples (i.e. colonic biopsies) (Kotton, 2013).

2.8.6. Shell vial assay:

Shell vial culture is a modification of conventional cell culture technique for rapid detection of viruses in vitro. The technique involved inoculation of the clinical specimen onto cell monolayer grown on a coverslip in a shell vial culture tube, followed by low-speed centrifugation and incubation. This system works on the principle that the low-speed centrifugation enhances viral infectivity to the susceptible cells. It is thought that the minor trauma to cell surface produce as a result of low-speed centrifugation mechanical force enhance the viral entry into the cells, which in turn reduces the total time taken for the virus to produce infection of cells (Jayakeerthi et al., 2006).

2.8.7. Histopathology test:

Immunohistochemistry for CMV should be routinely performed on all biopsy specimens where CMV is suspected, to maximize diagnostic sensitivity. Identification of inclusion bodies or viral antigens in biopsy material or in Broncho alveolar lavage specimens’ cells is very specific for CMV disease, especially with a positive culture (Kotton, 2013).
2.9. Prevention of CMV Infection

CMV infection has been associated with numerous neurologic debilitating effects, especially in infants and immunocompromised individuals. It is, therefore, necessary to prevent rather than to treat the disease. This could be achieved by avoiding transplantation of CMV seropositive blood, fluid or organ to seronegative patients (IFnanyi and Ogbonnaya, 2017).

The rationale for passive immunization of seronegative mothers comes from the observed lower risk of fetal infection in mothers with preexisting antibodies. This is further supported by evidence that CMV hyperimmune globulin (HIG) can inhibit viral spread in vitro, restore placental health in mothers with primary infection, and lead to regression of cerebral ultrasound abnormalities. A recent retrospective study has demonstrated that monthly intravenous infusions of CMV HIG to mothers with confirmed primary infection (including those with virological evidence of fetal infection) are safe and can both prevent (adjusted odds ratio [OR], 0.32) and treat (adjusted OR, 0.02) fetal infection (Manicklal et al., 2013).

Ganciclovir cannot be used for prenatal therapy due to its mutagenic potential in animals, but oral valaciclovir administered to mothers with evidence of fetal infection appears to be safe and decreases the circulating fetal viral load (Manicklal et al., 2013).

2.10. Treatment:

Although specific antiviral drugs, such as ganciclovir and foscarnet, have been available for several years for treatment of life-threatening or sight-threatening HCMV disease in immunocompromised patients, their use for treatment of congenital HCMV infection remains undefined due to a paucity of data. A few anecdotal reports on the use of ganciclovir in congenitally infected infants have
also been discouraging. In these reports, indications for treatment were acute symptoms of HCMV organ localization (pneumonia, hepatitis) or generalized congenital disease (Revello and Gerna, 2002).

Additionally, recent studies have focused on passive immunization of pregnant women with CMV infection with hyperimmune globulin (CMV HIG) to reduce the rate of vertical CMV transmission and improve the outcome of the newborn (van Zuylen et al., 2014).

2.11. Previous studies:

A study conducted to detect the 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 (Neirukh et al., 2013).

A 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).

A study conducted to detect seroprevalence of cytomegalovirus among normal pregnant women in Nigeria shows that 97.2% were CMV-IgG positive, and shows that there is high prevalence rate (50.8%) among pregnant women between the ages of 25-30 year (Akinbami et al., 2011).

The estimation of seroprevalence of Cytomegalovirus among pregnant women in the central Mexican city of Aguascalientes, shows that out of 289 pregnant women, 259 (89.6%) were (IgG) seropositive (Alvarado-Esquivel et al., 2018).
A study conducted to estimate the prevalence of cytomegalovirus antibodies among pregnant women in northern Turkey shows that out of 600 pregnant women, 566 (97.3%) were CMV IgG positive (Uyar et al., 2008).

A study aimed to determine the seroprevalence of Cytomegalovirus among pregnant women in Khartoum state shows that out of 91 pregnant women, 67 (73.6) were CMV IgG positive (Ali, 2016).
CHAPTER THREE
MATERIALS AND METHODS

3.1. Study design:

3.1.1. Type of study:

This is a cross-sectional study.

3.1.2. Study area:

This study was conducted in Al-Saudi Hospital, Kassala State. The practical part of this study was done in the Department of Immunology, Tropical Medicine Research Institute, National Central Laboratory.

3.1.3. Study duration:

This study was carried out during the period from February to May 2018.

3.1.4. Study population:

This study was done on pregnant women with or without a history of abortion in Kassala State.

3.2. Sample size:

A total of ninety-one pregnant women (n=91).

3.3. Data collection

The data collected from patients by the data collection form containing information like age, gestation stage, and history of abortion.
3.3.1. Data analysis

The data analyzed by a statistical package for social sciences (SPSS) software programme by used chi-square tests.

3.4. Ethical consideration:

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

3.5. Laboratory methods:

3.5.1. Sample collection:

5ml of whole blood samples collected by vein puncture from each patient into Ethylene diamine tetra acetic acid (EDTA) container.

3.5.2. Sample processing:

Each blood specimen centrifuged at 3000rpm for 5 minutes to obtain the plasma. Then collected into plain container and stored at -20 °C until tested.

3.5.3 Analysis of specimens:

The specimens analyzed by using commercially available Enzyme-linked Immunosorbent assay (CMV IgG ELIZA Kits) for qualitative detection the specific CMV IgG antibody.

3.5.4 Principle of CMV ELISA:

The CMV IgG ELISA Test Kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative detection of IgG antibodies to CMV in human serum or plasma. The micro well plate is coated with CMV antigens. During testing, the specimen diluent and the specimens are added to the antigen-coated
micro well plate and then incubated. If the specimens contain IgG antibodies to CMV, it will bind to the antigens coated on the micro well 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 micro well plate washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies added to micro well plate and then incubated. The enzyme-conjugated anti-human IgG antibodies bind to the immobilized antigen CMV IgG antibody complexes present. After the second of the incubation, the micro well plate washed to remove unbound materials. Substrate A (hydrogen peroxide) and substrate B (Tetramethylbenzidine) added and then incubated to produce a blue color indicating the amount of CMV IgG antibodies present in the specimens. Sulfuric acid solution added to the micro well plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of CMV IgG antibodies present in the specimens, measured with a micro plate reader at 450 nm.

3.5.5. Storage and stability:

Test Kits should be stored at 2-8°C. All reagents are stable through the expiration date on box if stored between 2-8°C.

3.5.6. Test procedure:

1. Reagents and specimens was allowed to reach room temperature prior to testing.

2. Working wash Buffer was prepared by diluting the concentrated wash Buffer 1:25 and leave A1 as Blank well.

3. 100µl of calibrator 1.2.3 and 4 were added to their particular well.

4. 100 µl of specimen diluents was added to each well except the blank.
5. 5 µl of sample was added to each well.

6. The micro well plate was mixed gently for and covered with plate sealer and incubate at 37°C for 30 minutes.

7. The plate sealer was removed and each well washed 5 times with working wash buffer.

8. 100 µl of conjugate added to each well except the blank well.

9. The micro well plate covered by plate sealer and incubated at 37°C for 30 minutes.

10. The micro well plate washed 5 times with working wash buffer.

11. 50 µl of substrate A and B were added to each well including the blank.

12. The micro well plate mixed gently, covered with plate sealer and incubated at 37°C or 10 minutes.

13. 50µl of stop solution added to each well to stop the reaction.

14. The color intensity was read at 450 nm within 30 minutes.

**3.5.7. Validation requirements and quality control:**

- Blank absorbance should be < 0.100 at 450nm

- Calibrator 1 absorbance should be < 0.150 at 450 nm

- Calibrator 2 absorbance should be > 0.150

- Calibrator 3 absorbance should be > calibrator 2

- Calibrator 4 absorbance should be >1.200
3.5.7.1. Calculation of results:

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

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

3.5.7.2. Interpretation of result:

Positive results: Index value > 1.1

Negative results: Index value < 0.9

Equivocal results: Index value ≥0.9 ≤ 1.1
CHAPTER FOUR

RESULTS

A total of ninety-one blood specimens (n=91) obtained from pregnant women in AL-Saudi Hospital in Kassala State. All specimens were examined for the presence of CMV IgG antibodies using ELISA Kits. The results showed that out of 91 blood specimens investigated, 89 (97.8%) were positive for CMV, while the rest 2 (2.2%) were negative(Table 2). According to the age group of each participated women, the positive specimens were 33 (36.3%) within the age group (15-24), 37 (40.7%) within the age group (25-34%) and 19 (20.9%) within the age group (35-44)(Table3) . All the 29 pregnant women with a history of abortion revealed positive results. Moreover, 60 out of 62 of the pregnant women without a history of abortion were positive for CMV, while the remained two were negative(Table 4) Furthermore, according to gestational stages of pregnancy positive specimens were 23(25.3% review it) in the first trimester, 25(27.5%) in the second trimester and 41(45.1%) in the third trimester(Table 5).
Table 1: Sociodemographic characteristic of pregnant women enrolled in the study

<table>
<thead>
<tr>
<th>Sociodemographic data</th>
<th>Count</th>
<th>Result</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Age group:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-24</td>
<td>35</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>25-34</td>
<td>37</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>35-44</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>History of abortion:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Gestational stage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First trimester</td>
<td>24</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Second trimester</td>
<td>25</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Third trimester</td>
<td>42</td>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Serological results of CMV among participated women

<table>
<thead>
<tr>
<th>Results</th>
<th>Participants</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>89</td>
<td>97.8</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 3: The frequency of CMV among participated women according to the age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>CMV IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>15-24</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>36.3%</td>
<td>2.2%</td>
</tr>
<tr>
<td>25-34</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40.6%</td>
<td>0.0%</td>
</tr>
<tr>
<td>35-44</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20.9%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>97.8%</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

*P.value: 0.195*
Table 4: Frequency of CMV among participated women according to the history of abortion

<table>
<thead>
<tr>
<th>Abortion</th>
<th>CMV IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Yes Count</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>% of Total</td>
<td>31.9%</td>
<td>0.0%</td>
</tr>
<tr>
<td>No Count</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>% of Total</td>
<td>65.9%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Total Count</td>
<td>89</td>
<td>2</td>
</tr>
<tr>
<td>% of Total</td>
<td>97.8%</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

*P.value: 0.328
Table 5: Frequency of CMV among participated women according to the gestation stage:

<table>
<thead>
<tr>
<th>Gestation stage</th>
<th>CMV IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>First trimester</strong></td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Count</td>
<td>25.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Second trimester</strong></td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Count</td>
<td>27.5%</td>
<td>0.0%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Third trimester</strong></td>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td>Count</td>
<td>45.0%</td>
<td>1.1%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>89</td>
<td>2</td>
</tr>
<tr>
<td>Count</td>
<td>97.8%</td>
<td>2.2%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P.value: 0.606
CHAPTER FIVE.

DISCUSSION

5.1. Discussion

Human Cytomegalovirus is a common cause of congenital infection. It is an important cause of intra-uterine fetal and neonatal infection when transmitted from mother to fetus. The present study aimed to detect CMV among pregnant women in Kassala state. Out of 91 blood specimens investigated, 89 (97.8%) were positive. This high percent of seroprevalence is completely similar to those obtained in Nigeria by (Akinbami et al., 2011) who reported that 97.2% of participated women were CMV-IgG positive, and in northern Turkey (97.3%) by (Uyar et al., 2008).

This seroperevelance results is more than that obtained in Western Sudan (Hamdan et al., 2011), in Palestine by (Neirukh et al., 2013) (96.6%), in Central Mexican City of Aguascalientes by (Alvarado-Esquivel et al., 2018) (89.6%) and in Khartoum State by (Ali, 2016) (73.6%) . These differences may be due to endemicity variations of these countries with CMV infections. In this study, although all women that have a history of abortion resulted positive CMV, there were an insignificant association between CMV infection and history of abortion (p. value 0.328). This is insignificant association may be due to sample size and is highly prevalence may be due to Socioeconomic status and hygienic practics.

5.2. Conclusion

This study concluded that, there was a high prevalence rate of human Cytomegalovirus infection among pregnant women in Kassala State. The present CMV IgG positive results in pregnant women indicate the exposure of those women to the virus and this high percentage confirms the importance of screening
pregnant women for CMV IgG antibodies. Prevention of CMV infection in pregnant women will prevent congenital infections in their infants.

5.3. Recommendations

1. Routine screening of CMV infection in pregnant women should be carried out in maternity health care.

2. Antiviral prophylaxis should be run to reduce the risk for CMV infection.

3. Pregnant women should be prevented from getting CMV, prevention should be based on correct hygienic and behavior, and education of pregnant women about the implication of acquiring CMV infection is vital.

4. Future research should involve a larger sample size and more advanced techniques are required to validate the results of the present study.
REFERENCES


Appendix 1

Data collection form

1- Date: ........................................

2- Name: ........................................................................................................ No. ........................................

3- Age: ........................................................................................................

4- History of abortion:
   a- Yes: □ b- No □

5- Gestation stage:
   a- 1st trimester: □ b- 2nd trimester □
   b- 3rd trimester □
Appendix 2

Figure 1: CMV ELISA Plate
Figure 2: Microwell plate reader (Thermo Scientific).
Figure 3: Microwell plate washer (Thermo Scientific).