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Sero-Frequancy of Cytomegalovirus Infections among Pregnant Women in El Dammer City at River Nile State, Sudan

التردد المصلي لفيروس مضخم الخلايا بين النساء الحوامل بمدينة الدامر، ولاية نهر النيل، السودان

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By:

Ahmed Osman Ahmed Hassan

B.Sc., Medical Laboratory Science (Microbiology-Clinical Chemistry)
Shendi University (2016)

Supervisor:

Dr. Hind Haidar Ahmed

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الآيـة

قال تعالي:

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَى إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْماً)

صدق الله العظيم

سورة طه - الآية (114)

DEDICATION

To my parents

Brothers

Friends

Colleagues

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I am extremely grateful to **AllAh** who helped me to complete this work. I would like to express my sincere thanks and good respect to my supervisor **Dr. Hind Haidar Ahmed** for stimulation, suggestions, knowledge, experience encouragement and helped me in all the times of study.

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ABSTRACT

Cytomegalovirus (CMV) is a leading healthcare problem associated with stillbirth and congenital abnormalities and determining the Sero-frequancy along with the possible risk factors associated with cytomegalovirus infections may play a cornerstone role in preventing its complications.

This study was conducted during the period from May 2019 to January 2020 to determine the Sero-frequancy of CMV among pregnant women at El Damer Teaching Hospital, River Nile State, Sudan.

A total of 93 pregnant women were included in this study, serum sample were collected from them, and then tested for CMV IgM and CMV IgG antibodies by Enzyme Linked Immuno Sorbent Assay (ELISA) technique.

Out of 93 pregnant women tested, 29(31.2%) and 81(87%) were CMV IgM and CMV IgG positive, respectively.The ages of pregnant women ranged from 21 to 38 years old, with mean of (28.5 ± 3.5 SD). This study showed that the pregnant women within the age group (20– 30) years were highly infected with CMV compared with the other age groups ,there were 23.6% and 66.6% positive for CMV IgM and IgG respectively and there was no association between CMV IgM and IgG and age (*p* value=0.8 and 0.18) respectively. In this study the level of CMV IgM and IgG antibodies was higher in those pregnant women with history of miscarriage than those without history of miscarriage. (30.1% vs 1.0%) for CMV IgM, and (60.2% vs 26.8%) for CMV IgG, and there was strong statistically difference between CMV IgM and IgG and past history of miscarriage (*P* value =0.00 and 0.00).

Moreover, there was high frequency rate of CMV IgM among pregnant women within the second trimester of pregnancy than other pregnancy stages which was 16(17.2%) and there was association between CMV IgM and gestational stage (*p* value=0.028).

This study concluded that there was highly frequency rate of CMV IgM among pregnant women in El Damer city. There was a strong association between CMV IgM, IgG and past history of miscarriage, and there was no association between CMV IgM, IgG and age and educational level.

ملخص الاطروحة

يعتبر فيروس مضخم الخلايا من المشاكل الصحيه التي تؤدي الي تشوهات خلقيه وولادة اطفال ناقصين.
تحديد الانتشار المصلي الوبائي مع تحديد العوامل التي تؤدي اليه وعلاقتها بالاصابه بالفيروس والتي تساعد علي الوقايه من المرض.

االجريت هذه الدراسة لتحديد الانتشارالمصلي الوبائي لفيروس مضخم الخلايا وسط النساء الحوامل بمستشفى الدامر التعليمي،ولاية نهر النيل، السودان. شملت الدراسه ثلاث وتسعون امراه حامل بغض النظر عن سوابق الاجهاض ام لا خلال الفتره من مايو 2019 الي يناير 2020 ، أعمارهن تتراوح بين 21 و38 سنه .

اخذت العينات وفحصت لمعرفة احتوائها على اجسام مضادة من النمط (IgM) والنمط (IgG) باستخدام اختبار الامتزاز المناعي المرتبط بالانزيم .

29(31.2%) من 93 امراه حامل كانت لديها اجسسام مضاده من النمط (IgM) ،وبينما 81(87%) من اصل 93امراه حامل كانت لديها اجسام مضاده من النمط (اي جي جي).كاانت اعمارهن تتراوح ببين 21و38 سنه(المتوسط=28.5±3.5 الانحراف المعياري)،اظهرت الدراسه ان الحوامل التيي تتراوح اعمارهن بين 20و30 سنه اكثر عرضه للاصابه بالفيروس،ححيث وجد انا 23.6% لديهن اجسام مضاده من النوع (اي جي ام)بينما 66.6% تحتوي علي النوع (IgG) من الاجسام المضاده،كما وجد لاتوجد علاقة ذات دلاله احصائيه بين الاصابه بالفيروس والعمر (القيمه الاحتماليه=0.80و0.18) للاجسام المضاده (IgM) و(IgG) علي التوالي.

كما وجد ان النساء الاتي اجهضن سابقا اكثر عرضه للاصابه بالفيرروس من غيرهن حيث كانت النسبه(1% ضد 30.1%)بالنسبه للاجسام المضاده (IgM) بينما (26.8% ضد 60.2%) بالنسبه للاجسام المضاده من النوع (اي جي جي)، ووجد ان هنالك علاقة قويه ذات دلاله احصائيه بين الاصابه بالفيروس والاجهاض السابق(القيمه الاحتماليه=0 و0)، كما وجد ان الاجسام المضاده من النوع (IgM) في السيرم كان كثيرا في الثلاثة اشهر الثانيه من الحمل ووجد ان هنالك علاقه ذات دلاله احصائيه بين الاجسام المضاده من النوع (IgM) واشهر الحمل (القيمه الاحتماليه=0.028).

كما وجد انه لاتوجد علاقة ذات دلاله احصائيه بين الاجسام المضاده (IgM) و(IgG) والمستوى التعليمي (القيمه الاحتماليه= 0.14 و0.38) علي التوالي.

توجد علاقة بين الاصابه بالاجهاض سابقا ووجود الاجسام المضاده (IgG) و(IgG) ، كما توجد علاقه بين الاجسام المضاده (IgG) وعمر الحمل.

خلصت هذه الدراسة الي ان هنالك تردد عالي لفيروس مضخم الخلايا من خلال وجود الاجسام المضاده من النمط IgM بين الحوامل بمدينه الدامر.

أيضا وجد ان هنالك علاقه قويه بين وجود الاجسام المضاده (IgM و IgG) والاصابه السابقه بالاجهاض. كما لاتوجد علاقه ذات دلاله احصائيه بين وجود الاجسام المضاده (IgM و IgG) والعمر والمستوى التعليمي.

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LIST OF ABBREVIATIONS

CDC	Centres for Disease Control and Prevention
CMIs	Cell Mediated Immunities
CMV	Cytomegalovirus
C CMV	Congenital cytomegalovirus
CNS	Central Nervous System
CPE	Cytopathic effect
DC	Seropositive Donor
DNA	Deoxyribonucleotide
EBV	Epstein-Barr virus
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immunosorbent Spot
EM	Electron Microscopy
GCSF	Granulocyte Colony Stimulating Factor
GvHD	Graft –versus Host Disease
HAART	Highly active antiretroviral therapy
HCMV	Human cytomegalovirus
HHV-5	Human herpesvirus 5
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HSCT	Haematopoietic Stem Cell Transplant
HSV	Herpes simplex virus
ICU	Intensive Care Unite
IFN-g	Interferon-Gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IGRA	Interferon Gamma Release Assays
IL6	Interleukin-6
IUGR	Intrauterine Growth Restriction
MCMV	Murine Cytomegalovirus
mDC	Monocyte and Myeloid Dendritic Cells
NASBA	Nucleic Acid sequence-based amplification
PCEHL	Permeant congenital oready-onset
PCR	Polymerase chain reaction
pp65	Phosphoprotein 65
QFT	Quani FERON

RC	Seropositive Recipient
RNA	Ribonucleotide
SCT	Stem cell transplantation
SNHL	Sensorineural Hearing Loss
SOT	Solid-organ transplantation
SPSS	Statistical Package of Social Sciences
TH1	T helper Lymphocyte -1
TH2	T helper Lymphocyte-2
TLRs	Toll-like receptors
TMB	Tetramethylbenzidine
TNF	Tumor Necrosis Factor

CHAPTER ONE
INTRODUCTION

1.1. Introduction

Cytomegalovirus belongs to the family herpesviridae of betaherpesvirnae subfamily. It is also known as HHV-5 (Abdul Wahab, 2012). Compared to other human herpes viruses, it is the largest, with a genome of 235 kb encoding 165 genes. The virion consists of a double-stranded linear DNA core in an icosahedral nucleocapsid enveloped by a proteinaceous matrix, which are enclosed in a lipid bilayer envelope that is derived from the nuclear membrane (Hama and Abdurahman,2013).

The virus is highly species-specific and only human strains are known to produce human disease. Initial infection with HCMV commonly occurs during childhood and depending on geographic location and socioeconomic group (35-90%) of the population have antibody against the virus by adult hood (Hama and Abdurahman,2013 .

The virus is ubiquitous virus infection with worldwide distribution and association with opportunistic disease that has been recognized in more highly developed areas of the world and it covers larger areas in developing countries and in communities with lower socioeconomic status (Elamin and Omer., 2015). Worldwide distribution, infects humans of all ages and all socioeconomic groups, and with no seasonal or epidemic patterns of transmission. It is the most common cause of congenital infection with birth prevalence of about 0.5% (range 0.2-2.5 percent), and a common cause of deafness and intellectual impairment worldwide In utero transmission of CMV can occur following primary maternal infection during pregnancy but can also occur in women with natural immunity, either because of the reactivation of latent virus or by re-infected with a different strain postnatally. CMV is also transmitted from mother to child through breastfeeding and close contact. The transmission risk is the proportion of mothers undergoing a primary infection in a given trimester and/or the preconception period who transmitted CMV to the fetus (Mamuye *et al*., 2016). 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 new born. Congenital HCMV infection is associated with permanent hearing loss, vision loss and neurological impairment (Cannon *et al*., 2010). CMV is an opportunistic infection with global prevalence that can act as a dangerous pathogen in immunocompromised persons. Especially, transplant recipients, patients with

acquired immune deficiency syndrome and congenital infected new-borns are at risk of developing CMV disease (Talkhapifard e*t al*., 2017).

1.2. Rationale

CMV is considered as one of the serious infections with a high impact on the fetus causing different types of neonatal abnormalities, actually it's the most common and serious congenital infection because it occurs after both primary or recurrent infection in pregnancy and it is the major cause of childhood deafness, and neurologic handicap (Babiker, 2018).

The aim of this study to investigate the serofrequancy of CMV infection among pregnant women and its association with possible risk factors in El Damer Teaching Hospital as there was lacking of previous data and there are clinical observations that reflect the importance of this issue and the emerging of congenital CMV among new-borns. Furthermore the epidemiological data will give a valuable data on either the possible risk factors for transmission or the preventive measures. All of this compared to the wide distributed of CMV as the previous reported researches in other cities in Sudan that show seroprevalence of CMV IgG antibodies among pregnant women up to 95% (Kafi *et al.*, 2013), with a huge variety between recent and chronic infections by determining IgG and IgM which show 72.2% and 2.5% respectively (Hamdan *et al.*, 2011).

1.3. Objectives

1.3.1. General objective

To detect the Sero-frequancy of CMV among pregnant women in El Damer city, River Nile State- Sudan.

1.3.2. Specific objectives

1. To detect CMV IgM and IgG antibodies among pregnant women by ELISA technique.
2. To determine the frequency of CMV IgM and IgG antibodies among pregnant women.
3. To associate between possible risk factors including; age, educational level, history of miscarriage and gestational stage of pregnancy and CMV infection.

CHAPTER TWO
LITERATURE REVIEW

2.1. Cytomegalovirus

2.1.1 Background

Cytomegalovirus (CMV) is an enveloped DNA virus and a member of the family Herpesviridae and belongs to the subfamily beta herpesviridae. CMV has worldwide distribution, infects humans of all ages and all socioeconomic groups, and with no seasonal or epidemic patterns of transmission (Alghalibi *et al.,* 2016). CMV infection during pregnancy is a major cause of congenital infection in developing countries with an incidence of 0.2-2.4% of live births (Alghalibi *et al.,* 2016).

Human cytomegalovirus (HCMV) is one of 8 human herpesviruses, which also includes human herpesvirus type 6 and human herpesvirus type7. In 1920, Good pasture 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). Cytomegalovirus (CMV) is the commonest among viral infections during perinatal period that cause congenital CMV infections. Its clinical manifestations range from asymptomatic forms (90% of cases) to severe fatal damage that may include permanent hearing, vision loss, neurological impairment and, in rare cases, death due to abortion The fatal consequences of CMV infection have made it one of the most serious infections contracted during pregnancy. Despite the posed teratogenic risk during pregnancy, there is no national screening test for CMV infection is available during pregnancy. Thus, little is known on its epidemiological data that is necessary for health planners and care providers (Maingi and Nyamache, 2014).

2.1.2. Properties of CMV

Cytomegalovirus has the largest genetic content of the human herpesviruses. Its DNA genome (240kbp) is significantly larger than that of HSV. Only a few of the many proteins encoded by the virus (over 200) have been characterized. One, a cell surface glycoprotein, acts as an Fc receptor that can non-specifically bind the Fc portion of immunoglobulins. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins (Azim *et al.,* 2018).

The major immediate early promoter-enhancer of cytomegalovirus is one of the strongest known enhancers, due to the concentration of binding sites for cellular transcription factors. It is used experimentally to support high-level expression of foreign genes (Azim *et al*., 2018).

Many genetically different strains of cytomegalovirus are circulating in the human population. The strains are sufficiently related antigenically, however, so that strain differences are probably not important determinants in human disease. Cytomegaloviruses are very species-specific and cell type-specific. All attempts to infect animals with human cytomegalovirus have failed. A number of animal cytomegaloviruses exist, all of them species-specific (Azim *et al*., 2018).

Human cytomegalovirus replicates in vitro only in human fibroblasts, although the virus is often isolated from epithelial cells of the host. CMV replicates very slowly in cultured cells, with growth proceeding more slowly than that of HSV or varicella-zoster virus. Very little virus becomes cell-free; infection is spread primarily cell-to-cell. It may take several weeks for an entire monolayer of cultured cells to become involved (Azim *et al*., 2018).

2.1.3. Structure

CMV is a member of the subfamily Beta herpesvirinae. It has the largest genome of the human herpesviruses. In contrast to the traditional definition of a virus, which states that a virion particle contains DNA or RNA, CMV carries specific mRNAs into the cell in the virion particle to facilitate infection (Murray *et al.*, 2013).

2.1.4. Replication

Human CMV replicates only in human cells. Fibroblasts, epithelial cells, granulocytes, macrophages, and other cells are permissive for CMV replication. CMV has been generally regarded as a slowly replicating virus on the basis of time to appearance of cytopathic effect in cell culture, especially when compared with other members of the Herpesviridae such as herpes simplex virus type 1 (Emery *et al.*, 1999). This may facilitate the establishment of latent infection in myeloid stem cells, monocytes, lymphocytes, the stromal cells of the bone marrow, or other cells (Murray *et al*., 2013).

Herpesvirus replication is initiated by the interaction of viral glycoproteins with cell surface receptors with relatively variable tissue tropism. The deletion of glycoprotein B (g B) renders HCMV incapable of entering cells unless a chemical fusogen (ie polyethylene glycol) is added These data, along with analogous studies of gB in other herpesviruses, show that gB is important for virus fusion (Vanarsdall and Johnson, 2012).

Transcription of the viral genome and viral protein synthesis proceeds in a coordinated and regulated manner in three phases starting from Immediate early proteins (α), which is

consisting of proteins important for the regulation of gene transcription and takeover of the cell, secondly the Early proteins (β), which is consisting of more transcription factors and enzymes, including the DNA polymerase, and last of all the Late proteins (γ), which is consisting mainly of structural proteins, which are generated after viral genome replication has begun (Murray *et al* .,2013).

The viral genome is transcribed by the cellular DNA- dependent ribonucleic acid (RNA) polymerase and is regulated by viral-encoded and cellular nuclear factors. The interplay of these factors determines whether a lytic, persistent, or latent infection occurs. Cells that promote latent infection transcribe a special set of viral genes without genome replication. Progression to early and late gene expression results in cell death and lytic infection. (Murray *et al*., 2013)

The viral-encoded DNA polymerase, which is a target of antiviral drugs, replicates the viral genome. Viral- encoded scavenging enzymes provide de oxy ribonucleotide substrates for the polymerase. These and other viral enzymes facilitate replication of the virus in non-growing cells that lack sufficient de oxy ribonucleotides and enzymes for viral DNA synthesis (e.g., neurons) (Murray *et al.,* 2013).

Other proteins manipulate cellular machinery to optimize replication, inhibit immune responses, and inhibit apoptosis or establish latency (Murray *et al*., 2013).

Pseudo-virion is assembled in the nucleus, are filled with DNA, acquire an envelope at the nuclear or Golgi membrane, and exit the cell by exocytosis or by lysis of the cell. Transcription, protein synthesis, glycoprotein processing, and exocytotic release from the cell are performed by cellular machinery (Murray *et al.,* 2013).

2.1.5. Transmission

The different modes of transmission are: close interpersonal contact (body fluids like urine and saliva), sexual activity, breastfeeding, blood transfusions and organ transplantation. After the initial infections, the virus stays in saliva, tears, semen, urine, cervical secretions, and blood for month to years. Seroconversion occur at mucosal surfaces via infected urine, saliva, or other body fluids, making children an excellent host for the virus, especially in day care settings. However; all transmission modes are not equivalent in terms of risk. The most dangerous transmission mode is the congenital one, in which the pregnant mother transmits the virus via placenta to the fetus (Nassikas and Tsaples, 2013).

There are two possible routes of trans placental transmission of CMV to the fetus across syncytiotrophoblasts with subsequent infection of the underlying cytotrophoblasts, or via invasive cytotrophoblasts within the uterine wall. Mode of perinatal transmission include

ingestion or aspiration of cervicovaginal secretions at the time of delivery and ingestion of breast milk after delivery (Mc Carthy *et al.*, 2009).

Transplacental transmission of CMV may occur even in seropositive women after non primary infection. The non primary infection consists of two different routes, those are reinfection with a newly acquired CMV strain and reactivation of a latent CMV infection (Nagamori *et al*., 2010) Postnatal, CMV is also transmitted from mother to child through breastfeeding and close contact (Cannon *et al.*, 2010).

2.1.6. Prevalence

Human cytomegalovirus (CMV) is an opportunistic infection with global prevalence that can act as a dangerous pathogen in immunocompromised persons. Especially, transplant recipients, patients with acquired immune deficiency syndrome and congenital infected new-borns are at risk of developing CMV disease (Talkhapifard e*t al*., 2017).

CMV 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 new-borns following intrauterine CMV infection (Adler, 2011). Previous studies have confirmed that CMV infection is relatively common among women of reproductive age with seroprevalence ranging from 45% to 100%. African continent like South America and Asia has one of the highest prevalence of CMV From the previous studies conducted in Africa, CMV prevalence rates in Egypt were found to be 96%, 85.7% Tanzania , 97.2% Benin and 86.4% South Africa (Maingi and

Nyamache , 2014).

CMV is the most common congenital viral infection in the United States and a leading cause of congenital hearing loss and neurological disability. CMV can be transmitted to the fetus when a CMV seronegative woman develops a primary CMV infection during pregnancy, or from latent virus reactivation from maternal CMV infection acquired prior to pregnancy or reinfection with a new CMV strain during pregnancy (Leung *et al*., 2012).

Human herpesvirus family consisted of most important human viruses and their presentation is varying according to the type of virus, patient's immune status and weather the infection is primary or reactivated (Ho, 1982).

2.1.7. Epidemiology of CMV infections

CMV is a global infection, with seropositivity in women of reproductive age ranging from 45 to more than 90%. CMV seroprevalence varies between countries and tends to be higher in developing countries (more than 90% in Brazil, 70–80% in Ghana, more than 90% in India, 80–90% in South Africa and 490% in Turkey) and lower in developed countries (40–70% in

Western Europe, 60–70% in Australia, 60–70% in Canada and 50–60% in the United States).Even within countries the rates of CMV seropositivity in women vary by socio-economic status and ethnicity. Seroconversion, representing a primary (first) infection, occurs annually in approximately 1–2% of seronegative pregnant women (Canon *et al.*, 2010; Zuylen *et al*., 2014).

Anytime during pregnancy, primary or non-primary maternal infection (i.e. reactivation of a woman's latent virus or re-infection with a different strain) can lead to CMV crossing the placental barrier and infecting the fetus, resulting in congenital CMV infection (Zuylen *et al*., 2014).

The prevalence of congenital CMV has been reported to occur in 0.2–2% (average of 0.64%) of pregnancies in the US, Canada, Australia and Western Europe.19–22 In addition, the limited studies in developing countries, including Latin America (Chile, Brazil, Mexico and Panama), Africa (Ivory Coast and Gambia) and Asia (Korea, Taiwan, China and India) have reported a birth prevalence of congenital CMV infection ranging from 0.6 to 6.1% of pregnancies (Lanzieri .T *et al* .,2014; Hamilton.S.T *et al* .,(2014). Based on the number of live births per year (Hamilton *et al.,* 2014) and reported congenital CMV prevalence,20–23 this translates to an estimated 0.12 million congenital CMV infections annually in developed regions and 0.7–4.5 million congenital CMV infections per year in those developing countries that report their infection rates. (Alvarado-Esquivel *et al.*, 2014).

CMV is the most common viral cause of congenital defects in the United States, with 0.2–2% of all new-borns infected with CMV. 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).

Transmission of CMV occurs by person-to-person contact, in addition, CMV infection acquired by blood transfusion may lead to significant complications in immunocompromised individuals (Alvarado-Esquivel *et al.*, 2014).

CMV is ubiquitous in human populations. Higher prevalence of infection and younger age at acquisition of CMV infection have been associated with low socioeconomic status, origin in a developing country, non-white race, and geographic region in the United States with higher prevalence in the South (Pass and Brenna, 2014).

Differences in age-related prevalence of CMV infection are probably related to living circumstances, child-rearing practices, and social customs. Transmission of CMV from person to person requires contact with body fluids as occurs with breast-feeding, intimate (sexual) contact, or care of young children. High rates of CMV infection have been noted in

children who attend day-care centres and their parents, in day care workers, sexually active adolescents, and clients of clinics for sexually transmitted infections. In the United States and in Western Europe, a relatively high proportion of women are CMV sero-negative when they reach reproductive age compared with countries in Asia and Africa in which almost all young women are CMV sero-positive (Pass and Brenna,2014).

The prevalence of congenital CMV infection varies between 0.2-2.4 % in different countries 1-4). In developed countries, around 50-60% of pregnant, middle to high class women have antibodies to CMV, compared with around 70-85% of those from lower socioeconomic groups

(Tao.R,Coleman.M.M,Pennington.J.D,Ozden.O,Park.S,Jiang.H,Kim.H,Flynn.C.R,Hill.S,Mc Donald.W.H,Olivier.A.K,Spitz.D.R,and Gius.D, 2010).

Overall, 1-4% of susceptible women are affected with primary CMV infection during pregnancy, and around 30-40% of the fetus are trans placentally infected, and also about 10% infants manifest clinical signs and symptoms at birth1-5). Approximately 1-3% of infants born of women with pre-existing antibody to CMV are infected in utero, but they have rarely symptomatic illnesses at birth (Tao *et al*., 2010).

2.1.8. Pathogenesis of Cytomegalovirus

CMV infections are usually subclinical, It came to medical attention when the characteristic owl's eye inclusions were seen in stillbirths (1910) and again in 1964 among patients undergoing pioneering organ transplantation (Griffiths *et al*., 2015).

The virus infects epithelial cells, macrophages, and T lymphocytes. CMV is highly cell-associated and spreads to coalescing cells. The close cell interaction protects the virus from antibody inactivation. Cell- mediated immunity is required for resolution of symptoms and contributes to symptoms. CMV eventually becomes latent within T lymphocytes, endothelial cells, and monocyte derived macrophages. Suppression of cell-mediated immunity allows recurrence of symptoms and can result in severe disease. The virus has the ability to induce immunosuppression during primary infections and reactivation of latent infections (Chamberlin, 2009).

2.1.8.1. Primary CMV Infection

Primary CMV infection is defined as the first detection of CMV infection in an individual who has no evidence of CMV exposure before transplantation. It is recognized that severely immunocompromised individuals such as transplant recipients might not develop CMV-specific antibodies (Ljungman *et al*., 2017).

2.1.8.2 CMV Latency

HCMV latency is characterized by maintenance of the viral genome as an intranuclear episome (Stern et *al.,* 2019) without replication, but with the potential to reactivate to a productive infection. Awide range of cell types support productive infection (Stern *et al.,* 2019).

But latency appears to be restricted to primitive bone-marrow-resident CD34C cells and CD33Cmyeloid progenitor cells (Stern *et al*., 2019), which retain the latent viral genome as they differentiate into peripheral blood CD14C monocytes and myeloid dendritic cells (mDCs) (Stern *et al.,* 2019).

2.8.3 Recurrent CMV Infection

Recurrent infection is defined as new CMV infection in a patient with previous evidence of CMV infection who has not had virus detected for an interval of at least 4 weeks during active surveillance. Recurrent infection may result from reactivation of latent virus (endogenous) or reinfection (exogenous). It is recognized that CMV-specific antibodies can be passively transferred by blood products or immune globulin administration. For practical purposes, presence or absence of CMV-specific antibodies by serology can be used as acceptable estimates of previous CMV exposure to classify patients for entry into clinical trials (Ljungman *et al*., 2017).

2.1.8.4. Reactivation of CMV infection

Reactivation from latency is responsible for significant morbidity and mortality in immunocompromised and immunosuppressed populations. including solid-organ transplant and allogeneic hematopoietic stem cell transplant (HSCT) recipients, HIV/AIDS patients and the developing fetus HCMV reactivation is the major viral infectious complication after allogeneic HSCT and is associated with an increased risk of non-relapse mortality (Takenaka *et al*., 2015; Green *et al*., 2016; Teira *et al*., 2016), which is not principally attributable to direct HCMV disease (Green *et al.,* 2016).

Uncontrolled HCMV replication following reactivation can lead to life-threatening end-organ disease, Reactivation may also indirectly contribute to detrimental patient outcomes through antiviral drug toxicities and complex impacts on post-transplant immune reconstitution, including links with graft-versus-host disease (GvHD) (Cantoni *et al.,* 2010; Stern *et al.,* 2019) and microbial superinfections (Young *et al.,*2017).

There is no licensed HCMV vaccine and current antiviral agents are limited by their toxic side-effects and the risk of antiviral drug resistance. The cellular sites and mechanisms associated with HCMV latency and reactivation are incompletely understood, in part due to

the high human-specificity of HCMV which precludes the use of animal models to directly study HCMV infection (Stern *et al.,* 2019).

Reactivation may derive from endogenous latent HCMV in the seropositive recipient (Stern *et al.,* 2019) and/or from latently infected cells transferred within the seropositive donor stem cell allograft. Recipient seropositivity alone is an adverse prognostic factor for overall survival (Stern *et al.,* 2019).

Seropositive recipients who receive grafts from HCMV-naïve donors (RC/D-) experience the highest incidence of HCMV reactivation and disease (Webb *et al*., 2018; Stern.L *et al.,* 2019), a likely consequence of delayed HCMV specific immune recovery owing to the lack of pre-existing HCMV-specific memory lymphocytes in the graft (Stern *et al*., 2019).

HCMV pneumonia remains associated with high (up to 70%) mortality (Erard *et al.* 2015). Gastrointestinal HCMV disease often develops without detection of HCMV DNAemia (Cho *et al.,* 2013; Gabanti *et al.,* 2015) and can be difficult to distinguish from gastrointestinal GvHD, often occurring in the same patients (Priori *et al.,* 2013; Bhutani *et al.,* 2015).

2.1.8.5. CMV reinfection

Reinfection is defined as detection of a CMV strain that is distinct from the strain that caused the initial infection (Ljungman *et al*., 2017).

2.1.8.6. Cellular sites of HCMV latency and reactivation

A recent study found that CD14C monocytes expressing the surface marker B7-H4 were a predominant site of latency in peripheral blood of healthy donors (Zhu *et al.,* 2018). It may be that HCMV preferentially infects early myeloid progenitors or promotes the differentiation of infected pluripotent CD34C cells to myeloid-lineage subsets that support latency (Zhu *et al*., 2018) Latently infected cells contain HCMV DNA, but do not support infectious virus production (Zhu *et al*., 2018).

The terminal differentiation to mature mDCs and macrophages is accompanied by chromatin remodelling of the HCMV major immediate-early promoter (Reeves *et al*., 2005), which facilitates reactivation of the lytic gen cascade and the production of infectious virus (Reeves *et al*.,2013; Poole *et al*., 2015). Allogeneic stimulation and pro-inflammatory cytokines such as IFN-g, TNF, and IL-6 are implicated in driving myeloid cell maturation and reactivation (Hargett and Shenk, 2010; Reeves *et al*., 2011; Huang *et al.* 2012; Reeves *et al*.,2013; Forte *et al.,* 2018).

Latently infected cells are present at very low frequencies (0.004–0.01% of mononuclear cells) in GCSF-mobilized peripheral blood or bone-marrow from healthy seropositive donors, but underlie the capacity for iatrogenic transmission of latent HCMV through DC HSCT

allografts. Additionally, the high risk of reactivation in RC/D- patients suggests that pre-transplant conditioning regimens incompletely eradicate latent HCMV reservoirs in the recipient (Wills *et al.,* 2015). It also remains possible there are additional sites of HCMV latency, with conflicting evidence regarding possible latency in aortic endothelial cells Whether HCMV establishes a low-level productive infection in bone-marrow stromal cells (Cordon *et al*., 2017) or in a self-renewing CD34C cell subset ,also remains unclear, yet HCMV DNA has been detected in diverse tissue sites (Cordon *et al*., 2017) and recent RNA-sequnceovered HCMV transcripts at multiple locations, including the ovaries, blood, adipose tissue, and lung (Shnayder *et al*., 2018).

The specific cell types harbouring HCMV in these studies and whether they represent productive, abortive, or latent infection is unknown. The widespread prevalence of HCMV within asymptomatic individuals nonetheless highlights the importance of host immune control in preventing unchecked HCMV replication leading to life threatening disease (Stern *et al.,* 2019).

2.1.9. Clinical significance

2.1.9.1. Congenital and neonatal cytomegalovirus infections

CMV is a ubiquitous herpesvirus spread by close interpersonal contact through saliva, blood, genital secretions, urine, or breast milk that infects up to 90% of the US population by the 8th decade of life and establishes lifelong latency in monocytes and granulocytes (Hargett *et al*., 2010;Swan and Schleiss, 2013) Maternal transmission to the fetus of a new or reactivated latent infection may occur at any gestation, leading to congenital CMV. About 20,000-40,000 infants per year in the United States are born with congenital CMV infection, with a corresponding incidence of 0.6 - 0.7% of all deliveries of the developed world, making CMV the most common congenital viral infection (Dollard *et al.,* 2007; Swan and Schleiss, 2013).

CMV is mostly asymptomatic or mildly symptomatic however can be devastating to immunocompromised hosts including infected new-borns, and results in the greatest long-term neurodevelopment morbidity of all the prenatally acquired viral infections. The most frequent sequel is sensorineural hearing loss (SNHL), of which CMV is the leading nonhereditary cause overall **(**Swan and Schleiss, 2013).

2.1.9.1.2. Congenital infection

Congenital CMV occurs transplacentally and may result in symptomatic or asymptomatic infection in the neonate. The likelihood of fetal transmission and symptomatic disease is much greater during a primary maternal CMV infection. It is estimated that 1-4% of CMV

seronegative mothers will become infected during pregnancy, and 30-40% of these infected women will transmit virus to the fetus (Swan and Schleiss, 2013).

Non-primary maternal CMV infections can also result in fetal transmission. These infections may represent reactivated latent infection or reinfection with a new strain in seropositive women. Currently it is estimated that 10-30% of women with preconception immunity become re-infected, and 1-3% will transmit to the fetus. Symptoms of disease in the newborn and long-term neurodevelopmental sequelae can occur after transmission in the setting of primary or recurrent infection. Symptoms occur in 11% - 12.7% of all neonates with congenital CMV (Swan and Schleiss, 2013).

Symptoms of disease in the new-born and long-term neurodevelopmental sequelae can occur after transmission in the setting of primary or recurrent infection. Symptoms occur in 11% - 12.7% of all neonates with congenital infection (Swan and Schleiss, 2013).

The clinical findings include IUGR, hydrops, generalized petechiae, purpur thrombocytopenia, jaundice, hepatosplenomegaly, pneumonitis, microcephaly, periventricular calcifications, seizures, chorioretinitis, sensorineural hearing loss, bone abnormalities, abnormal dentition, and hypo calcified enamel (Swan and Schleiss, 2013).

Approximately 13.5% of the asymptomatic neonates may still go on to develop neurodevelopment injury, which is most commonly manifest as hearing loss. Hearing loss is most common when CMV infection occurs in the first or second trimester. Sensorineural hearing loss following symptomatic or asymptomatic congenital infection is often progressive, can be unilateral or bilateral, and may be absent at birth, only to become clinically manifest later in childhood (Swan and Schleiss, 2013).

About 21% of all hearing loss at birth and 25% of hearing loss at 4 years of age is attributable to congenial CMV infection; therefore, these children require regular hearing evaluations and early intervention (Swan and Schleiss, 2013). Unilateral or bilateral hearing loss and mental retardation are common consequences of congenital CMV infection. The risk for serious birth defects is extremely high for infants born to mothers who had primary CMV infections during their pregnancies (Murray *et al*., 2013).

Congenital cytomegalovirus infection is a leading non genetic cause of permanent congenital or early-onset hearing loss (PCEHL). Although its rates are high, the contribution of Congenital cytomegalovirus infection to PCEHL in the developing world is unclear (Fowler *et al.,* 2017).

2.1.9.3. Intrapartum and perinatally acquired infection

Intrapartum Transmission. CMV MTCT also occurs during birth and is due to presence of virus in the cervix or vagina. Cervicovaginal shedding of virus is common in women who are CMV antibody positive. Study of pregnant women that included testing for vaginal shedding at term and testing the new born for CMV at birth and then again at intervals during the first few months after birth estimated that approximately 50% of babies who were not breastfed and were born to mothers who had positive vaginal CMV cultures at birth acquired the virus (Pass.R.F, and Anderson .B,2014).

These babies usually begin to shed virus at 3–6 weeks of age. Active CMV infection with cervico-vaginal shedding of virus is more common in women infected with HIV, especially those with poor control of HIV infection and low CD4 T cell counts, and CMV infection rates are high in their babies (Pass and Brenna, 2014). CMV is transmitted from mother to fetus in approximately 35% of pregnancies in which a maternal primary infection (Pass and Brenna, 2014). Trans placentally transmission rates are lower, approximately 20%, with infection in the first trimester, and increase with advancing gestational age to approximately 75% with third trimester infection. ((Pass.R.F, and Anderson .B,2014).). Maternal CMV infections are usually clinically unrecognized; studies that used screening for CMV antibody to detect primary infections were instrumental in improve our knowledge of them Congenital CMV infection due to first trimester maternal primary infection is more likely than later gestational infection to cause fetal disease that is apparent in the new-born as signs of congenital infection and results in disability due to central nervous system damage, hearing loss, or impaired vision (Pass.R.F, and Anderson .B,2014).

CMV can also be transmitted from mother to fetus if the mother had CMV infection in the past was immune at the time of conception (recurrent infection). Little is known about the biology of these infections or why maternal immunity from previous infection is unable to prevent all congenital infections (Pass.R.F, and Anderson .B,2014).

CMV infection is chronic; the human host never eliminates the virus, and persistent infection or reactivation of latent virus is possibly involved. There is evidence that reinfection with a CMV strain with slightly different envelope glycoprotein epitopes from the initial strain results in maternal reinfections that can lead to congenital infection(Pass.R.F, and Anderson .B,2014). If reinfection is the major cause for congenital infection in babies of mothers with prior immunity, it would mean that a successful strategy for prevention of primary maternal infection might also prevent reinfections. Rates of congenital MV infection due to recurrent maternal infection vary widely among populations, ranging from approximately 0.1%–1% or

higher; they are generally higher in populations in which the overall prevalence of CMV infection is high, and they are lower in populations with low CMV prevalence rates in women of childbearing age (Pass.R.F, and Anderson .B,2014).

2.1.9.4. Postnatal CMV infection

Post-natal acquisition of CMV has little significance, is not associated with long term disability, and rarely causes clinical signs of any illness in term infants. The exception is low-birth weight premature infants. Premature babies appear to be at particularly high risk for CMV-associated disease. These infants may additionally have symptoms of worsening respiratory status, neutropenia, or septic appearance (with apnea, bradycardia, pallor, and bowel distention) at the onset of infection, regardless of whether the virus was acquired postnatally from human milk or transfusions (Buxnam *et al*.,2009; Swan and Schleiss, 2013). For premature infants acquiring infection postnatally, CMV's ability to cause long-term sequelae independent from prematurity remains unclear, though minor effects on motor development have been suggested. Recent study suggested that postnatal CMV infection of preterm infants did not result in an increased risk of SNHL (Swan and Schleiss, 2013).

2.1.10. Immunocompromised Patients

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 haemodialysis 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*).*

2.1.11. 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).

There are many uncertainties about CMV disease in the critically ill patients. First, while identification of the virus is common in critically ill patients, the actual rate of CMV as a disease in critically ill patients is unclear. Second, the importance of CMV detection in critically ill patients remains questionable, especially in the absence of histologic evidence of

infection. On the other hand, the existing evidence is strong regarding the association between CMV detection and increased mortality and morbidity rates in ICU settings. Still there are no high-quality data to guide the decision regarding when to treat detected CMV in ICU patients if there is no definite clinical confirmation of CMV disease (pre-emptive treatment), and there is no risk assessment tool to guide prophylactic therapy in ICU setting (Al-Omari *et al*., 2016).

2.1.12. Immunity to Cytomegalovirus

HCMV has the potential to spread in the bloodstream to all organs, but only produces overt disease if the viral load increases to high levels. This is normally prevented by a robust immune response, so that the infected individual usually remains asymptomatic. HCMV is a recognized cause of disease in the fetus, the allograft recipient and AIDS patients. More recently, it has been recognized as a pathogen for those admitted to intensive care units, for the elderly and for the general population. (Griffiths *et al*., 2015)

Cell-mediated immunity is essential for resolving and controlling the outgrowth of CMV infection. However, CMV is an expert at immune evasion and has several means for evading innate and immune responses one of these evasion mechanisms conducted by exploit the IL-10 pathway, as part of their infectious cycle, either through their own encoded IL-10 (hcmvIL-10 for HCMV) or manipulation of the cellular IL-10 signalling cascade (Chang and Barry, 2010).

The ability to establish and maintain a persistent infection in the presence of such antiviral immunity requires a commensurate dedication of the HCMV coding capacity to immune evasive/modulating proteins that alter cellular activation, signalling, trafficking, and apoptosis (Chang and Barry, 2010).CMV infection alters the function of lymphocytes and leukocytes. The virus prevents antigen presentation to both CD8 cytotoxic T cells and CD4 T cells by preventing the expression of MHC I molecules on the cell surface and by interfering with cytokine-induced expression of MHC II molecules on antigen-presenting cells (including the infected cells) (Chang and Barry, 2010).

A viral protein also blocks natural-killer-cell attack of CMV-infected cells. Similar to EBV, CMV also encodes an interleukin-10 analogue that would inhibit TH1 protective immune responses (Murray *et al*., 2013).

The CD4+ subset of T lymphocytes are not effective by itself in controlling murine CMV (MCMV) multiplication in tissue or essential for the protective function of the CD8+ CD4- effector cells (Reddehase *et al.,* 1987) The transfer of CMV-specific clones of CD8+ T cells

derived from the bone marrow donor is a safe and effective way to reconstitute cellular immunity against CMV after allogeneic marrow transplantation (Walter *et al.*, 1995).

CD4+ T cell-mediated immunity to CMV in humans is generated in an age-dependent manner, and may have a substantial role in controlling renal viral replication and urinary shedding. CD4+ T cells from children had reduced CMV-specific IL-2 and CD154 (CD40 ligand) expression, suggesting an early blockade in the differentiation of viral-specific CD4+ T cells. Following CMV acquisition, children, but not adults, persistently shed virus in urine, and this was observable for at least 29 months' post-infection (Wizman *et al.*, 2016).

CMV has been assigned the highest priority for vaccine development, but presently there is no licensed CMV vaccine. Although the infants of seropositive women are more protected against CMV complications but the chances in getting the infection among both communities (with either high or low seropositive prevalence) are approximately the same (Manicklal *et al.*, 2013).

2.1.13. 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).

Detection of HCMV specific antibodies is the most common approach used to identify HCMV infected individuals. Many types of assay are available for the determination of the anti HCMV antibody titer in serum with different degree of sensitivity; the most widely used procedure is the ELISA (Hama and Abdurahman, 2013)

The presence of CMV specific Immunoglobulin M (IgM) may not be indicative of primary infection, since it is also produced during reactivation and re-infection (Khalf *et al*., 2012). Serological diagnosis of primary CMV infection during pregnancy can be difficult as CMV immunoglobulin M, while suggestive of recent infection, can remain positive for many months and can also represent reactivation of past infection (McCarthy *et al*., 2009).

Detection of increasing HCMV IgG levels over time is an unreliable approach for distinguishing primary from non-primary HCMV infection (Hama and Abdurahman, 2013). Another method of determining the timing of maternal CMV infections is to measure antibody avidity, which refers to strength of antibody binding to a target antigen. As the immune response to a particular antigen mature over time, avidity increases (Carlson *et al*., 2010).

The IgG avidity assay can help distinguish primary infection from past or recurrent infection and can assist in determining when infection occurred (Yinon *et al*., 2012).Cytomegalovirus specific immunoglobulin G avidity may assist in timing the infection but it is only performed in reference laboratories and it is often unreliable (McCarthy *et al*., 2009) .

Patients who are acutely infected also will usually test positive by culture or PCR for virus in the blood and urine (Duff, 2010)

The single best test for the diagnosis of congenital infection is detection of virus in the amniotic fluid by culture or PCR (Duff, 2010)

2.1.13.1 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).

2.1.13.2. Cell culture

Viral culture of the urine and saliva obtained within the first and two weeks of life continues to be the gold standard for diagnosis of congenitally infected infants (Ross *et al*., 2011) .the virus can be recovered most readily from throat washings and urine (Brooks *et al*; 2010). In culture, 2 to 3 weeks are usually needed for the appearance of cytologic changes, consisting of small foci of swollen, translucent cells with large intra nuclear inclusion, the virus stays cell- associated (Brooks *et al.,* 2010). Cell culture methods of viral isolation are too slow to be useful in guiding therapy, particularly in immunocompromised patients (Brooks *et al*., 2010).

2.1.13.3. Electron microscope

Studies in virology go hand in hand with the development of microscopy techniques. Among them, electron microscopy (EM) has played a major role due to the small size of virus particles that, with very few exceptions, cannot be visualized by conventional light microscopy. Prior to the invention of the electron microscope in 1931 by the German engineers Ernst Ruska and Max Knoll, viruses were detected indirectly e.g., by means of the cytopathic effect they cause in infected cells or through clinical manifestations. However, the availability of EM enabled us to visualize and identify many infectious agents causing diseases or living “in symbiosis” with other organisms. Thus, during the 20th century EM has been a standard technique for virus diagnosis. Since they are simpler and faster, molecular biology techniques like PCR or ELISA with higher throughput are more commonly used.

2.1.13.4. Antgenemia assay

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 (Cunha, 2010).

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).

2.1.13.5. 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 low speed and placed in an incubator, after24-48 hours, the tissue culture medium is removed and the cells are stained using a fluorescein – labelled anti CMV antibody.The cell are observed using a florescent microscope, alternatively, the cells are stained with an antibody against CMV, followed by a fluorescein – labelled anti-immune globulin. This test has been found to be as sensitive as traditional tissue culture, probably because of the enhancement of the infectivity provided by configuration (Jahan, 2010).

2.1.10.6. Histopathology

Histopathology is the most specific method for diagnosing CMV, especially by detecting organ-specific manifestations related to CMV. However, this method is invasive and has a limited sensitivity that depends on the sampling site and pathologist's skills. suggest using CMV PCR or CMV antigen detection assays to diagnose CMV disease not earlier than 3 days and up to 2 weeks after the onset of critical illness, especially when the pre-test probability of the disease is high **(**Al-Omari *et al*., 2016).

2.1.13.7. Viral load

The term "viral load" first appeared in the literature in 1987 in a report by Jonas Salk proposing that viral load in HIV-1–infected individuals could be reduced by boosting the immune response, leading to reduced morbidity, mortality, and disease transmission (Poole.E, *et al*., 2015). Viral load assays assess the overall virus replicative activity that reflects the underlying disease process, usually by quantification of the viral nucleic acid in the blood. Although viral load testing in HIV-1 infection is an early example of how testing has increased our understanding of a disease process and improved patient care, viral load

testing has had a similar impact on patients with many other viral infections (Poole.E *et al*., 2015).

PCR assay have replaced virus isolation for routine detection of CMV infections. The PCR assays are designed to detect replicating virus, not latent viral genomes. Blood and urine are most commonly tested, PCR assays can provide viral load data, which appears to be important in predicting CMV disease (Brooks *et al*, 2010).

Diagnosis of acute maternal CMV infection by the presence of IgM and low avidity IgG require confirmation of fetal infection, which is typically performed using of PCR assays for CMV on amniotic fluid. PCR assays of dried blood spots from new-born have been shown to lack sufficient sensitivity for the identification of most neonate with congenital CMV infection for universal screening purpose. However, saliva PCR assays are currently being assessed as a useful screening method for congenital CMV infection (Poole.E *et al*., 2015).

2.1.13.8. Serological tests

2.1.13.8.1 Enzyme –linked immunosorbent Assay

The enzyme-linked immunosorbent assay (ELISA) is the most commonly available serologic test for measuring antibody to CMV (CDC, 2010).

Based on indirect ELISA principle for qualitative detection of IgG and IgM antibodies to CMV in human serum or plasma. Various fluorescence assays and indirect hem agglutination 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

2.1.13.8.2. Epitope-specific IgG immunoblots

Epitope-specific IgG immunoblotting was investigated by Eggers and *et al*. to explore the potentially different patterns in primary versus non primary infections. Compared an in-house-developed anti-glycoprotein B and anti -glycoprotein H epitope immunoblot assay to a standard micro neutralization method (Saldan *et al.,* 2017).

2.1.13.8.3. Interferon gamma release assays (IGRA)

Done by use of maternal CMV-specific T-cell immunity as a marker for intra uterine infection. are widely used to detect patients' cell mediated immunities (CMIs). Enzyme-linked immunosorbent spot (ELISPOT) and QuantiFERON (QFT) assays are the most standardized and employed platforms and were recently used to detect CMV-specific CMI in solid organ and allogeneic hematopoietic stem cell transplant recipients . Although their performances for CMV CMI were very similar, intraassay differences were found (Saldan *et al.*, 2017).

Recently, several studies assessed the role of CMV CMI in pregnant women.

Bialas and colleagues showed that CD4 T-cell depletion in infected pregnant rhesus macaques led to dismal and more severe outcomes compared with those of immunocompetent pregnant primates, thus suggesting a protective role of CD4 T cells with respect to cCMV. A previous study of primary-infected pregnant women had already identified CMV-specific CD4 T cells as key players in maternal CMV infection, and a low lymphoproliferative response associated significantly with an increased risk of congenital transmission (Saldan *et al* .,2017).

2.1.13.8.4. CMV IgG Avidity Test

CMV IgG avidity assay seems to be one of the most accessible tools to differentiate between primary from non-primary CMV infection (Maigi and Nyamache, 2014).

This technique is less expensive and it could be used to confirming CMV primary infections without the use of sophiscated polymerase chain reactions.

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 past (Zhang *et al*., 2009).

Antibody avidity, which is an indirect measure of the tightness of antibody binding to its target antigen, increases in the first weeks after a primary infection, low avidity IgG antibodies to CMV persist for up to 20 weeks after primary CMV infection, these low avidity antibodies are then replaced by high avidity antibodies . Currently, the combination of the presence of CMV IgM antibodies and low avidity CMV IgG antibodies along with maternal or fatal symptoms are used for the diagnosis of a primary maternal infection, (Alder, 2011).

2.1.13.8.5. Molecular methods for CMV diagnosis.

Molecular assays have revolutionized the diagnosis of CMV infection, overcoming almost completely the previous cited methods. Several systems are available, including quantitative assays (nucleic acid sequence-based amplification (NASBA), the hybrid capture assay, and PCR) and qualitative PCR, nowadays rarely employed (Akinbami *et al*., 2011; Saldan *et al.,* 2017).

Real-time PCR is the most commonly used method for the molecular diagnosis of CMV infections, due to its excellent sensitivity and specificity and to the availability of commercial kits and automated platforms. Moreover, PCR performance is less affected by specimen transport and storage (Ross *et al*.,2011; Saldan *et al*., 2017). An important limitation of quantitative PCR lies in the inter assay and inter laboratory variability of copy number determination, which might impact patient management and clinical decisions. Recently, the WHO released a CMV international standard to overcome the inter assay and inter laboratory issues with the aim of reaching an inter laboratory variability of 0.5 log10 UI/ml (Preiksaitis *et al.,* 2016). However, the multicentric study conducted with the WHO CMV standard showed inter laboratory variability higher than the expected target of 0.5 log10 UI/ml, so the route to CMV harmonization and standardization still requires further improvements (Saldan *et al.,* 2017).

2.1.1.13.8.6. Quantitative nucleic acid amplification tests

A variety of methods are used to quantify the amount of viral RNA or DNA in a clinical sample. The most commonly used methods in clinical laboratories include PCR, nucleic acid sequence– based amplification (NASBA), and branched DNA (bDNA) assays (Jonathan *et al*., 2015).

Quantitative nucleic acid amplification tests provide important information that can be used to predict disease progression, distinguish symptomatic from asymptomatic infection, and assess the efficacy of antiviral therapy. Despite the advances in technology, large challenges remain for viral testing related to accuracy, precision, and standardization. Digital PCR, a direct method of quantification of nucleic acids that does not rely on rate-based measurements or calibration curves, may address many of the current challenges (Poole.E *et al*., 2015).

2.1.14. Congenital CMV infection as an immune mediated disease:

Serological and immunological studies found that congenital CMV occurred in the presence of high TH1 (Inter feron Enzyme Linked Immunosorbent Spot) and low and delayed TH2 (CMV IgG avidity) maternal responses. These findings suggest that an imbalanced maternal

TH1/TH2 immune response may lead to congenital CMV. An altered cytokines pattern was previously reported at the placental level in congenital CMV cases. Many types of assays can detect CMV IgG antibodies, indicative of past infection (and the potential to undergo reactivation). Detection of viral IgM antibodies suggest a current infection (Brooks *et al*., 2010).

2.1.15. Prevention of CMV infection

CMV is usually not very contagious, and its horizontal transmission requires direct contact with infected materials, such as different secretions that contain the virus, and less likely, formites. So, adherence to standard precautions with good hand hygiene is effective to prevent CMV infection (Kim *et al*., 2010). 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 hyper immune globulin in mothers with primary CMV infection has been shown to be effective in preventing congenital disease in several studies (Nigro *et al.*, 2012*).* Pasteurization (72 ℃ for 5 seconds) of breast milk can inactivate CMV completely without affecting nutritional and immunologic properties of milk, although freezing (-20 ℃ for 3-7 days) will significantly decrease viral titers (Kim *et al*., 2010).

CMV vaccine ultimately may be an important preventive measure, but are not yet successful (Tao *et al*., 2010).

2.1.16. 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. 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 (CDC, 2010).

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 hyper immune 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)

2.2. 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).

In study done By Neiruk *et al*.,(2013) in Palestine ,the Seroprevalence of Cytomegalovirus among pregnant women and hospitalized children were detected. HCMV IgG was positive in 96.6% of pregnant women, in 88% of hospitalized children and in 98.4% of hospitalized new-borns.

Another study was done by Akinbami *et al*., (2011) in Nigeria to estimate seroprevalence of CMV IgG antibodies among normal pregnant women, shows that there are high prevalence rate (50.8%) among pregnant women between the ages of 25–30 years.

Study in Jiangsu, China conducted by Zhang *et al.,* (2014), aimed to determine the seroprevalence in pregnant women and to associate of maternal CMV infection status and adverse pregnancy/neonatal/growth outcomes in. 98.7% was positive to CMV (IgG) antibodies.

The first sero-epidemiology study of CMV infection in pregnant women in Mexico, determined the seroprevalence association with socio-demographic, clinical and behavioural characteristics of pregnant women. Out of 343 pregnant women, 225 (65.6%) were CMV-IgG positive (Roman *et al.*, 2014).

Another study in Central Mexican City of Aguascalientes by Alvarado-Esquivel *et al.*, 2018 To determine sero-epidemiology of CMV among pregnant women Anti-CMV IgG antibodies were detected in 259 (89.6%) of the 289 pregnant women studied. None of the 289 pregnant women were positive for anti-CMV IgM antibodies. Seroprevalence of CMV infection was significantly lower (P = 0.03) in pregnant women with reflex impairment (5/8: 62.5%) than in those without this clinical feature (246/272: 90.4%). Seroprevalence of CMV infection was significantly higher (*P* value = 0.03) in pregnant women with 2 - 9 pregnancies (140/150: 93.3%) than in those with only one pregnancy. Behavioural variables showed that seropositivity to CMV was associated with contact with children, whereas high (>150 AU/mL) anti-CMV antibody levels were negatively associated with washing hands before eating (OR = 0.17; 95% CI: 0.05 - 0.63; P = 0.007).

In study conducted in Thika, Kenya by Maingi and Nyamache (2014). showed high prevalence rate of (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%).

In study was conducted in EL Damazin Hospital for Obstetrics and Gynaecology by El Bushra *et al* (2019) to detect the seroprevalence of CMV among pregnant women who had undergone abortion(s). Participants were categorized into three age groups: 15-25 years (33.7%; 91/270); 26-40 years (62.2%; 168/270); and >41 years (4.1%;11/270). The majority of the participants had IgG antibodies to HCMV (74.8%; 202/270), while only 13.3% (36/270) had IgM antibodies to HCMV. There was an association between HCMV IgG level and first trimester abortion and low socioeconomic status among the studied women.

In study conducted for detection of Anti CMV IgG and IgM among women with history of abortion in Khartoum State Hospitals by Abd Elkareem *et al* (2015). They observed that 67 (74.4 %) were positive for CMV IgG, while 13 (14.4%) positive for CMV IgM.

Another study conducted at Omdurman Maternity Hospital by Khairi *et al* (2013) in pregnant women for detection of the seroprevalence of CMV infection Out of the 200 pregnant women tested, 195 (97.5%) and 12 (6.0%) were CMV IgG and CMV IgM positive, respectively. The age was associated with CMV IgM and history of miscarriage was significantly associated with CMV IgG positive women, while parity, congenital abnormalities, educational level, and occupation were not significantly ($P > 0.05$) associated with CMV infection.

In study in Ibrahim Malik Teaching Hospital, Khartoum, Sudan by Ibrahim *et al* (2017) the seropositivity of anti-CMV IgG was found more frequent (40/88.9%) among women with past history of abortion (test group) than among those (38/84.4%) without past history of abortion (control group). This difference was insignificant (p = 0. 27). Regarding the seropositivity of anti-CMV IgM, only one positive case (2.2%) was found among women with past history of abortion (test group) and none was detected among those without past history of abortion (control group). This difference was insignificant (p = 0. 17 trimesters, yet this difference was statistically insignificant.

Study in Wad Medani Maternity Teaching Hospital, Gezira State, Sudan by Awad alkareem (2017). including 90 participated women with and without history of miscarriage were enrolled in this study. Results showed positive result for all pregnant women when checked for IgG CMV (100%). Cytomegalovirus IgM present in 5 pregnant women (5.5%) the result show that CMV IgG is highly correlated with race, education , rate of miscarriage(100%) CMV IgM is low correlation (5.5%), (P value< 0.5).

Study was done in Sudan and Libya by Babiker (2018) to determine the seroprevalence and molecular detection of cytomegalovirus among Sudanese and Libyan pregnant women in association with socio-demographic data and maternal risk factors. A total of 445 blood samples were collected from pregnant women with different age groups presented to the outpatient clinic of Obstetrics and Gynecology,340 from Benghazi- Libya and 110 samples from Wad-Medani. All samples were tested for the presence of both IgG and IgM using Enzyme-Linked Immuno-Sorbent Assay (ELISA), IgM positive samples with ELISA were examined with Polymerase Chain Reaction (PCR) along with other randomly selected negative samples to determine the sensitivity and specificity of ELISA. The seroprevalence of CMV IgG was 100% in Sudanese and 99.7% in Libyans, while the positive IgM were 0.6% and 6.4% in Libyans and Sudanese respectively. The sensitivity of ELISA compared to PCR was 37.5% while specificity was 100%, risk analysis showed that both Poverty and Rural residence were significant risk factors for CMV infections detected by PCR. While illiteracy and history of abortion were insignificant for CMV infection.

CHAPTER THREE
3. MATERIALS AND METHODS

3.1. Study design

This is a descriptive, cross- sectional study.

3.2. Study area

This study was conducted in El Damer Teaching Hospital, El Damer locality is the capital of River Nile State in North Sudan.

3.3. Study duration

This study was conducted during the period from May 2019 to January 2020.

3.4. Study population

All Pregnant women (with and without history of miscarriage) whom attending El Damer Teaching Hospital were included.

3.5. Ethical consideration

This study was approved by Ethical committee of college of Medical Laboratory Science, Sudan University of Science and Technology. Permission from hospital administration was applied and verbal consent was taken from participants prior to enrolment into the study.

3.6. Inclusion criteria

All pregnant women that attending El Damer Teaching Hospital from May 2019 to January 2020 with different ages and trimesters.

3.7. Exclusion criteria

Non pregnant women whom attending the Gynaecology Department of El Damer Teaching Hospital.

3.8. Sample size

A total of ninety three (n=93) pregnant women were participated in this study.

3.9. Data collection

Data were collected through non self-administrated questionnaire (Appendix 1).

3.10. Sampling technique

This study based on nonprobability, convenience sampling technique.

3.11 Laboratory methods

3.11.1 Collection of blood specimens

A volume of 5 ml blood were collected from each particiant through venepunctures technique then displaced into plain container.

3.11.2 Sample processing

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

3.11.3 Analysis of specimens

The specimens were analysed for qualitative detection of CMV IgM and IgG antibodies by commercially available enzyme–linked immunosorbent assay ʻCMV (IgM and IgG) ELISAʼ kit(Bios USA)

The assays were performed following the instructions of the manufacturer,the immunoassay used has 98.0% sensitivity and 98.3% specificity.

3.11.3.1. Procedure

For CMV IGM antibody

Place the desired number of coated strips into the holder, prepare 1:40 dilution by adding 5µl of the test samples ,negative , positive control and calibrator to 200 µl of absorbent solution and mix well ,dispense 100 µl of diluted sera ,calibrators and controls into the appropriate wells. For the reagent blank dispense 100 µl absorbent solution in A1 well position, tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.

Remove liquid from all wells, repeat washing three times with washing buffer then dispense100 µl of TMB chromogenic substrate to each well and incubate for 15 minutes at room temperature lastly add 100 µl of stop solution to stop reaction and read the optical density at 450nm with a micro well reader.

For CMV IgG antibody

Place the desired number of coated strips into the holder, prepare 1:40 dilution by adding 5µl of the test samples ,negative , positive control and calibrator to 200 µl of sample diluent mix well, dispense 100 µl of diluted sera ,calibrators and controls into the appropriate wells. For the reagent blank dispense 100 µl sample diluent in A1 well position, tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature. Remove liquid from all wells, repeat washing three times with washing buffer then dispense 100 µl

of enzyme conjugate to each well and incubate for 30 minutes at room temperature.

Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer. Dispense 100 µl of TMB chromogenic substrate to each well and incubate for 15 minutes at room temperature lastly add 100 µl of stop solution to stop reaction and read the optical density at 450nm with a micro well reader.

3.11.3.2. Measurement

ELISA reader micro plate was adjusted to zero using the substrate blank in the first well, and the absorbance of all wells measured by ultra violet light at 450 nm.

3.11.3.3. Calculation of control values and cut-off

Mean absorbance values of Negative Control (MNC), mean absorbance value of Cut-off Calibrator (COC), and mean absorbance of Positive Control (PC) were calculated.

The result run was validated according to the manufacture's criteria for validity as following. Absorbance of substrate blank at 450 nm > 0.100, and the absorbance of MNC after subtraction of blank absorbance < 0.150. Also mean Absorbance of COC after subtraction of blank absorbance < 0.400 and mean Absorbance of positive control (PC) after subtraction of blank absorbance > 0.500.

The Index Value was calculated to obtain qualitative specimen results as following: Cut-Off Value was obtained by this equation:

Cut-Off Value = Mean Absorbance of Cut-Off Calibrator – Blank Absorbance

The Index Value was calculated by dividing the specimen Absorbance by the Cut-off Value.

3.11.3.4. Interpretation of the result

To determine the presence or absence of CMV-IgM and IgG antibodies , the Index Value was obtained to each specimen ,if index value < 0.9 the sample was negative , if value ≥ 0.9 and ≤ 1.1 the sample was equivocal , and if the value >1.1 interpret positive sample.

3.11.3.5. Data analysis

The data were analysed by Statistical Package for Social Sciences (SPSS) version 20.0 computerized program.

Frequencies, mean, and standard deviation were calculated. Chi square test was used to compare between qualitative variables. A *p* value of <0.05 was considered as significant for the results of this study.

CHAPTER FOUR

RESULTS

A total of ninety-three pregnant women (n=93) were included in this study, their age between 21 to 38 years with mean age of (28.5 ± 3.5 SD). obtained from pregnant women in El Damer teaching Hospital. The participated women Age were grouped as following:From 20 to 30 years were 69(74.2%) and from 31 to 40 years 24 (25.8%) as shown in table (4.1).

According to gestational stage 37(39.8%) of pregnant women in the first trimester33 (35.5%) in the second trimester and 23(24.7%) in the third trimester as illustrated in table (4.1).

Regarding past history of miscarriage there were 58(62.4%) with history of miscarriage and 35(37.6%) without history of miscarriage. As explained in table (4.1).

According to educational level there were 26(28%) in the primary level and 44(47.3%) in the secondary level,14(15.1%) were graduated and 9(9.7%) were non literate. As explained in table (4.1). All specimens were examined for the presence of CMV IgM and CMV IgG antibodies using ELISA kits. The results showed that out of 93 blood specimens investigated, 29(31.2%) were positive for CMV IgM, and 64(68.8%) were negative as in figure (4.1), while 81 (87%) were positive for CMV IgG and12 (13%) were negative. as in figure (4.2).

According to age of participants the positive CMV IgM were 22(23.6%) within the age group 20–30 years,07(7.5%) in age group 31–40 years, while positive CMV (IgG) were 62(66.6%) within age group 20-30years, and 19(20.4%) within age group31-40 years, also there was statistically insignificant association between age and CMV IgM and IgG (*P* value =0.80 and 0.18) respectively, as presented in table (4.2) and (4.3).

Out of 93 of the pregnant women with history of miscarriage 29(31.2%) were positive for CMV (IgM), while the rest 64(68.8%) were negative. Moreover 56 (60.2%) were positive for CMV (IgG) and 2(2.3%) were negative. Pregnant women without history of miscarriage 1(1.0%) that were positive for CMV (IgM), while the rest 34 (36.6%) were negative as in table (4.2). Also 25(26.8%) were positive CMV (IgG), while the rest 10(10.7%) were negative as in table (4.3). There was strong statistically significant difference between CMV IgM and IgG and past history of abortion (*P* value= 0.00 and 0.00) respectively, as in table (4.2) and (4.3).

According to gestational stages of pregnancy positive CMV (IgM) were 8(8.6%) in the first trimester, 16 (17.2%) in the second trimester and 5(5.4%) in third trimester, while positive CMV (IgG) specimens were 30(32.2%) in the first trimester, 30(32.3%) in the second

trimester and 21(22.5%) in the third trimester and there was statistically insignificant difference between CMV (IgG) and gestational stages (*P* value=0.37), as in table (4.3).

there was statistically significant difference between CMV (IgM) and gestational stages (*P* value=0.028) as in table (4.2).

According to the level of education of each participant CMV IgM were 12(12.9%) in the primary level, 10(10.7%) in the secondary level, 3(3.2%) were graduated and 4(4.3%) were non literate. A positive CMV (IgG) were 24(25.8%) in the primary level, 36(38.7%) in the secondary level, 12(12.9%) were graduated and 9(9.6%) were non literate. And there was statistically insignificant correlation between CMV IgM and CMV IgG and educational level (*P* value =0.14 and 0.38) respectively. As in table (4. 2) and (4.3).

Table (4.1): Demographic data of the study population

Variable	Frequency	Percentage
Age groups/years		
20 – 30 year	**69**	**74.2%**
31 – 40 year	**24**	**25.8%**
Total	**93**	**100%**
Gestational stage		
First trimester	**37**	**39.8%**
Second trimester	**33**	**35.5%**
Third trimester	**23**	**24.7%**
Total	**93**	**100%**
History of miscarriage		
Yes	**58**	**62.4%**
No	**35**	**37.6%**
Total	**93**	**100%**
Education level		
Primary	**26**	**28.0%**
Secondary	**44**	**47.3%**
Graduated	**14**	**15.1%**
Non Literate	**09**	**9.70%**
Total	**93**	**100%**

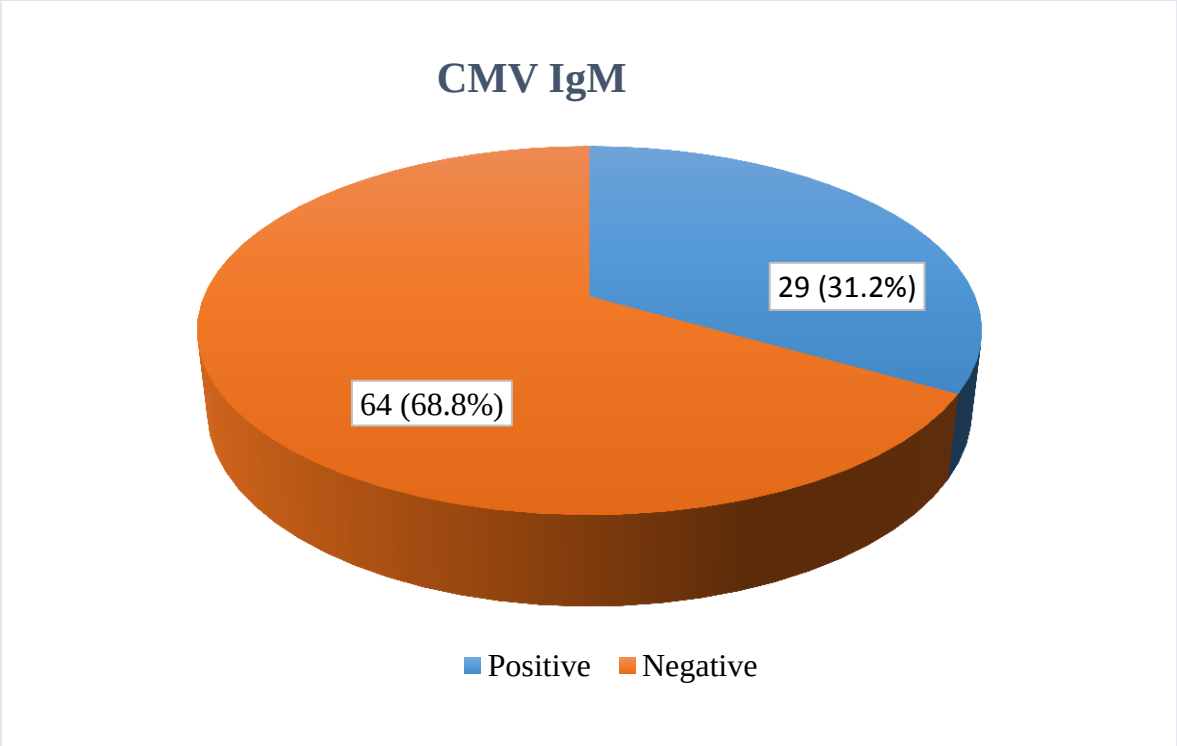

Figure (4.1): The frequency of CMV IgM among pregnant women

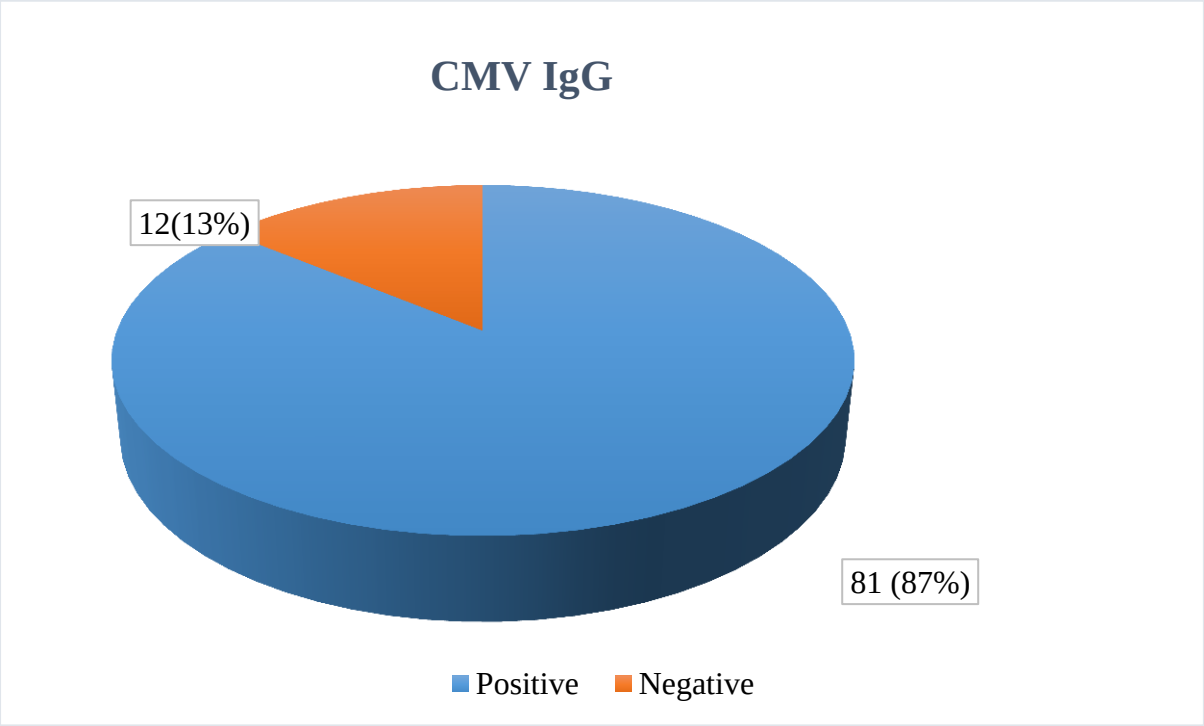

Figure (4. 2): The frequency of CMV IgG among pregnant women

Table (4.2): Association between CMV (IgM) and Socio-demographic data

Character		CMV (IgM)		Total	*P*. value
		Positive (%)	Negative(%)		
Age group/ years	20-30	22(23.6%)	47(50.5%)	69(74.2%)	0.80
	31-40	07(7.5%)	17(18.3%)	24(25.8%)	
	Total	**29 (31.2%)**	**64(68.8%)**	**93(100%)**	
History of miscarriage	Yes	28 (30.1%)	30 (32.2%)	58(62.3%)	0.00
	No	01 (1.0%)	34(36.6)%	35(37.6%)	
	Total	**29(31.1%)**	**64 (68.8%)**	**93(100%)**	
Gestational stage	First	08 (8.6%)	29 (31.1%)	37(39.7%)	0.028
	Second	16(17.2%)	17(18.3%)	33(35.6%)	
	Third	05(5.4%)	18(19.3%)	23(24.7%)	
	Total	**29(31.1%)**	**64(68.8%)**	**93(100%)**	
Educational level	Primary	12 (12.9%)	14(15.0%)	26(28.0%)	0.14
	Secondary	10(10.7%)	34(36.6%)	44(47.3%)	
	Graduated	03(3.2%)	11(11.8%)	14(15.0%)	
	Illiterate	04(4.3%)	05(5.4%)	09(9.7%)	
	Total	**29(31.1%)**	**64(68.8%)**	**93(100%)**	

Table (4.3): Association between CMV (IgG) and Socio-demographic data

Character		CMV (IgG)		Total	*P*. value
		Positive (%)	Negative(%)		
Age group/ years	20-30	62 (66.6%)	07(7.6%)	69 (74.2%)	0.18
	31-40	19(20.4%)	05(5.4%)	24(25.8%)	
	Total	**81 (87%)**	**12(13%)**	**93(100%)**	
History of miscarriage	Yes	56 (60.2%)	02 (2.3%)	58(62.5%)	0.00
	No	25 (26.8%)	10 (10.7)%	35(37.5%)	
	Total	**81(87%)**	**12 (13%)**	**93(100%)**	
Gestational stage	First	30 (32.2%)	7 (7.5%)	37(39.7%)	0.37
	Second	30(32.3%)	3 (3.3%)	33(35.6%)	
	Third	21 (22.5%)	2(2.2%)	23(24.7%)	
	Total	**81(87%)**	**12(13%)**	**93(100%)**	
Educational level	Primary	24 (25.8%)	2(2.2%)	26(28%)	0.38
	Secondary	36(38.7%)	8(8.6%)	44(47.3%)	
	Graduated	12(12.9%)	2(2.2%)	14(15.1%)	
	Illliterate	09(9.6%)	0(00%)	09(9.6%)	
	Total	**81(87%)**	**12(13%)**	**93(100%)**	

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

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 CMV for the first time during pregnancy may have a higher risk of miscarriage.

Out of 93 blood specimens investigated, 29(31.2%) were positive CMV (IgM), similar results obtained by Hassan *et al* (2014) (32.6%). This result was higher than those obtained by Alvarado-Esquivel *et al* (2014) 0% in Durango city Mexico, Alvarado-Esquivel *et al*., (2018) 00% in central Mexican city of Aguascalientes, Mohammed (2015) 0% in Khartoum state, also Ibrahim *et al.,* (2017) 0% in pregnant without past history of abortion and 2.2% in pregnant with past history of abortion) in Ibrahim Malik Teaching Hospital Khartoum State Sudan, Sherkat *et al.,* (2014) 2.3% in Isfahan Iran, Bagheri *et al*., (2012) (2.5%) in Gonabad eastern Iran ,Elamin and Omer, (2015)(3.2%) among Sudanese women with recurrent miscarriage,

Jahromi *et al* .,(2010)(5.25%) in South of Iran , Awad ElKareem *et al* .,(2015) 5.5% at Wad Madani city ,Gizera State Sudan, Khairi *et al.,* (2013) (6%) at Omdurman Maternity Hospital Sudan, Hama and Abdulrahman, (2013) (9.1%) at Sulimani City Irag ,Neiurk *et al.,* (2013) (11.5%) ,at Nigeria, Maingi and Nyamache, (2014) (8.1%) ,at Thika Kenya ElBushrra *et al.,* (2019) 13.3% at El Damazin City Blue Nile State Sudan, Abd Elkareem *et al*.,(2015) (14.4%) at Khartoum State Sudan, Mamuye *et al.,* (2016) (15.5%) at Ethiopia, Khalf *et al*.,(2012) (15.7%)Baghdad Irag, Falahi *et al., (*2010) (28.6%).

The finding of this study was lower than that obtained by Kafi *et al.,* (2013) (38.3%) in Khartoum State Sudan, the differnces in weather condition and economic status may lead to that differences.

Out of 93 blood specimens investigated 81(87%) were positive for anti CMV IgG. This result was slightly similar to that obtained in Ethiopia by Mamuye *et al*., (2016), who reported that (88.5%) of participated women were CMV-IgG positive.

The results of present study were higher than Falahii *et al*., (2010) (14.3%), Akinbami *et al*., (2011) (50.8%) in Nigeria, Elamin and Omer (2015) (55.3%) among Sudanese women with recurrent pregnancy loss**,** Luis *et al*., (2014) (65.6%) at Mexico, Paschale *et al*., (2009) (68.3%**)** in urban areas of northern Italy, Bagheri *et al*., (2012) (72.1%) at Gonabad Eastern Iran, Hamdan *et al*., (2011) (72.2%) in Western Sudan, Abd Elkareem *et al*., (2015) (74.4%)

at Khartoum State, El Bushra *et al*., (2019) (74.8%) in Blue Nile State, and Hassan *et al*., (2014) (80.4%) .

The results in this study disagreed with the results obtained by Falahi *et al*., (2010) IgG was found in 6 (14.28%) and IgM in 12 (28.58%) and the results showed that a high seroprevalence of CMV IgM than IgG and that may be due to the long study period from 2007 to 2008.

In present study pregnant women within age group 20-30 years were highly infected (23.6%) were positive for CMV IgM and (66.6%) positive for CMV IgG, and there was no association between CMV IgM and IgG infection and age (*p* value =0.80 and 0.18) respectively.

These findings in harmony with many studies conducted by Maingi and Nyamache (2014) at Thika Kenya pregnant women within age 31-35 years were highly infected. Also the results of El Bushra *et al*., (2019) at Blue Nile State, who observed that pregnant women within age group 26-40 years had a high rate of infection (62.2%). As well as study conducted by Akinbami in Nigeria (2011) who observed that (50.8%) of infected women within 25-30 years.

Severak studies showed there was no association between age and CMV IgG infection as study of Mohammed (2015) and AbdElkareem *et al*., (2015 at Khartoum State ,also Results of Khalf *et al.,* (2012) in Iraq showed insignificant association between CMV infection and age (*P* value >0.01) all these findings agree with this study.

In a disagree studies conducted by Kafi and his colleges (2013) at Khartoum State observed that CMV IgM and IgG were significantly associated with age (*P* value <0.05). also results of Khairi *et al*., (2013) at Omdurman Maternity Hospital showed a significant association between CMV IgM and age (*P* value <0.05).and these differences may be due to sample size or the characteristics of the study population.

In this study pregnant women with history of miscarriage (31.2%) positive for CMV IgM and (60.2%) positive for IgG, out of 93 pregnant women without history of miscarriage (1%) positive for CMV IgM, and (26.8%) positive for IgG. with strong association between CMV IgM and IgG and past history of miscarriage (*P* value=0.00 and 0.00) respectively.

These results in harmony with finding of Kafi and his colleges (2013) which found CMV IgM and IgG antibodies were significantly associated with frequency of abortion (*P* value <0.05). Another study in Gezira State conducted by Awad Elkareem(2017) who observed IgG highly associated with rate of miscarriage (*P* value<0.05). As well as study of Khairi and his college at Omdurman Maternity Hospital (2013) CMV infection associated with history of miscarriage (*P* value<0.05),Another study in Sulimani City done by Hama and Abdurhman (2012) .

lastly study in Durango City, Mexico by Alvarado-Esquivel and his colleges (2014) showed that past exposure to CMV were associated with infection (*P* value =0.040).

Different results were observed by Hama and Abdurhman,(2013) there were insignificant association between IgG and abortion (*P* value>0.05). also, findings of AbdElkareem et *al*., (2015) in Khartoum State showed insignificant association between IgM and IgG and abortion (P value>0.5). Another study was conducted in Ibrahim Malik Teaching Hospital by Ibrahim *et al*., (2017) whom observed that the difference between the present or absent of past history of miscarriage was insignificant (*P* value =0.17). These findings were disagree with our results may be due to endemicity variation between these different geographical regions.

According to the gestational stage of pregnancy, positive IgM and IgG as follow (8.6), (32.2%) in the first trimester, (17.2%) ,(32.3%) in the second and (5.4%),(22.5%) in the third trimester respectively. There was significant association between IgM and gestational stages (*P* value = 0.028) and insignificant association between IgG and gestational stages (*P* value=0.37). These agreed with a finding of AbdElkareem *et al*., (2015), and Akinbami *et al*., (2011) who showed insignificant association between IgG and gestational stage (P value>0.05)

Another different finding obtained in Blue Nile State conducted by El Bushra and his colleges (2019) showed that there were a significant association between IgG and the first trimester (*P* value= 0.003). Hamid *et al*., (2014) in Nigeria who observed that pregnant women in the first trimester most commonly infected (100%) and these differ from the above, may be due to sample size, and the race.

Regarding educational level positive IgM and IgG was (12.9% and 25.8%) in primary level, (10.7% and 38.7%) in the secondary level (3.2% and 12.9%) were graduated and (4.3% and 9.6%) were illiterate respectively. There were no association between CMV (IgM and IgG) and educational level. (*P* value =0.14 and 0.38) respectively. These findings constituent with that of Akinbami *et al*., (2011), ElBushra *et al*., (2019) and Esquivel *et al*., (2014) showed that there was no releation between IgM and IgG and education (*P* value>.0.05). Substationally different results were observed by Maingi and Nyamache (2014) in Kenya in which (39.4%) of infected women were illiterate and there was a significant association between infection and educational level (*P* value<0.0001).

As well as indicated by Awad Elkareem (2017)in Wad Madani city, Giezera State study who found that CMV IgG highly associated with educational level (*P* value<0.05).

These differences in funding may be due to characteristics of the study population and the method used for detection of the virus.

5.2. Conclusion

This study concluded that there was high frequency rate of HCMV infections among pregnant women in El Damer city.

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.

This study showed that the participated women within the age group (20– 30)years were highly infected with CMV IgM and IgG.

CMV IgM and IgG were highly associated with history of miscarriage.

CMV IgM associated with stage of gestation.

There was no association between age ,educational level and CMV infections.

5.3. Recommendations

Pregnancy health care centres should be improved and routine CMV screening for each pregnant women must be done with high sensitive and specific approach.

Development of vaccine and applying of vaccination program to reduce the risk for CMV infection.

Health educational programs must be improved to facilitate in prevention and control of CMV infections.

Further studies in different geographical locations with large samples size and more advanced techniques are required to confirm the results of the present study. and to provide data base of the epidemiological data about the disease for other cities of Sudan.

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Appendices

Appendix (1)

Sudan university of Science and Technology

College of Graduated studies

Questionnaire in sero-epidemiology of CMV among pregnant women in El Damer city, River Nile State, Sudan

Lab ID : …… **Age:**…….

Previous history of miscarriage:

Yes (.....) No (.....)

Number of abortion:

(…….) once (…...) twice (…….) tertiary

Education level:

(……) Primary (…….) secondary (……) graduated (…….) non literated

Gestational stage:

(……...) First trimester (……..) Second trimester (…….) Third trimester

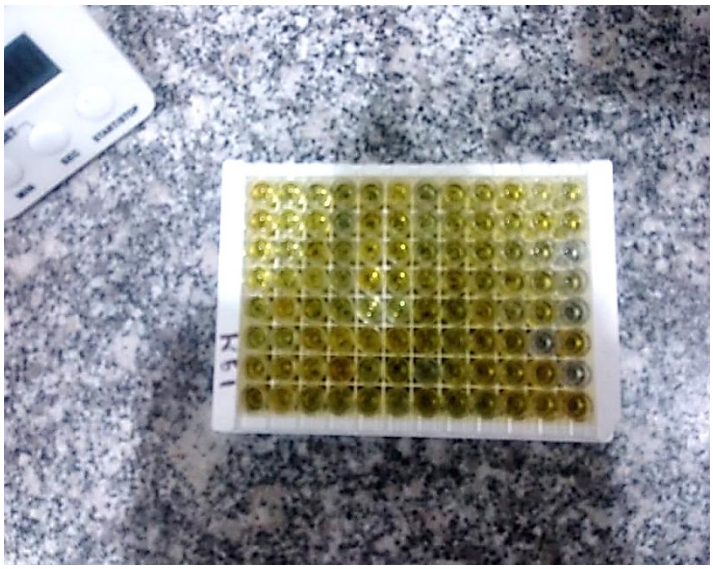
Investigation results of CMV among participated women:

CMV (IgM) (……) Positive (…..) Negative

CMV(IgG) (……) Positive (…..) Negative

Appendix (2)

Color plate (1): Micro titer Plate CMV (IgG)



Color plate (2): Micro titer Plate CMV (IgM)

Appendix (3)

Color plate (3): ELISA Reader