THE PREVALENCE OF HUMAN CYTOMEGALOVIRUS AMONG PREGNANT WOMEN ATTENDING THIKA LEVEL 5 DISTRICT HOSPITAL KIAMBU COUNTY, KENYA

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Declaration

This thesis is my original work and has not been presented for a degree in any other University.

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Dedication

I dedicate this work to my dear parents and siblings who have been a supporting pillar throughout my life.

Acknowledgement

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List of Abbreviations and Acronyms

AI	Avidity index
CMV	Cytomegalovirus
CSF	Cerebral Spinal Fluid
DNA	Deoxy Ribonucleic Acid
EDTA	Ethylene Diamine Tetra acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
Fc	Fragment of crystallization
HCMV	Human cytomegalovirus
HLA	Human Leucocyte Antigen
HRP	Horseradish peroxidase
IE	Immediate Early
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ICTV	International Center for Taxonomy of Viruses
ORFs	Open Reading Frame
PCR	Polymerase Chain Reaction
TMB	Tetramethylbenizidine

Abstract

Cytomegalovirus (CMV) is a member of herpesviruses. It is one of the most common causes of congenital and prenatal infections. It is a ubiquitous virus with the ability to establish latency following primary infection, and can be reactivated particularly during episodes of immunosuppression. CMV infection of pregnant women, especially in the first trimester may lead to congenital abnormalities and is often associated with serious complications, such as microcephaly, mental retardation, spastic paralysis, hepatosplenomegaly, anaemia, thrombocytopenia, deafness, and optic nerve atrophy leading to blindness in infants. The prevalence of CMV infection in developed countries is about 40% and in developing countries may be 100%.This study was done to determine the seroprevalence rate of the infection and its associated risk factors in pregnant women at Thika Level 5 District. This cross-sectional study was done in 260 pregnant women. Demographic data were collected by a questionnaire. About 3 ml of blood was taken from each patient. Aliquots of serum samples were stored at -20°C until analyzed. The presence of IgM and IgG CMV specific antibodies was assessed by enzyme linked immunosorbent assay. Data were analyzed by chi square test using EPI Info 2003 software. The prevalence rate was at 85.4% with the majority of women (77.3%) being positive for CMV-IgG in pregnancy while the rate of positive CMV-IgM infection was 8.1%. Samples that tested positive for both IgG and IgM were 20.8% and underwent avidity test to evaluate strength of the IgG antibodies. Those with avidity index >35% were 79.63% showing IgG antibody pre dominancy while those with avidity index <or equal to 35% were 20.37% indicating that IgM was the predominant antibody. Participants with IgG protective antibodies in this study were 60.8% and this confers protection to the foetus from CMV infection while 21.4% were not protected. The findings of our study indicated a prevalence of 85.6% with age, marital status, parity and education being factors that were significant ($p>0.05$). Adoption of CMV screening into the antenatal profile tests, health education of this virus on how it is acquired and contributing factors like personal and community hygiene were recommended as preventive approaches to congenital CMV infections.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Human Cytomegalovirus screening is not among the routine antenatal screening monitoring tests (Okwori *et al.,* 2009). It is only done upon request despite the heavy implication to the health development of infants (Sheevani *et al.,* 2005). This negligence is part of the contributing factors to high intrauterine transmission leading to high recorded cases of irreversible sequeal and mental disorders in foetuses (Ahmad *et al.*, 2010). In this study, prevalence of CMV infections was conducted to assess the magnitude of the problem among expectant mothers.

Human cytomegalovirus (HCMV) is a major public health problem throughout the world (Dowd *et al.,* 2009). Serological surveys have shown HCMV infection in virtually every population tested (Colugnati *et al.*, 2002). In most developed countries, 54% of adults 30-39 years of age and 91% of adults 80 years of age and older are CMV positive and thus harbor the virus (Leila *et al.*, 2012). Lower prevalence rate of CMV antibodies (40-80%) has been reported from developed countries, and higher rate (90 – 100%) from developing countries, depending upon the variability of accessibility of virus and its circulation rate in the community (Tremblay *et al.,* 2009). Worldwide CMV prevalence ranges from 52% to 99% among women of reproductive age (Nishimura *et al.*, 1999). Cytomegalovirus infection in pregnancy can either be acute or chronic (Spano *et al.*, 2004). Infection transmitted in acute infections during pregnancy has an estimated incidence of 25-75% with chronic having an estimation of 0.2-2% (Stagno *et al.,* 1982, Murray *et al.,* 2002). It was first described in 1881 when large cytoplasmic inclusions

(protozoan-like cells) were seen in the kidney of a still-born infant (Leguizamon *et al.*, 1997). The term cytomegalia was introduced in 1921, but the viral aetiology of the disease was confirmed in 1926 (Ivarson *et al.*, 1997).

CMV is transmitted from person-to-person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation and once someone has been infected with CMV they have it for life due to the virus ability to establish latency (Boppana *et al.,* 2001). It is estimated that a third of women who become infected by CMV for the first time during pregnancy will pass the infection on to their unborn baby (Ahlfors *et al.,* 2005). During pregnancy, transmission of the virus to the baby may occur through the placenta (Okwori *et al.,* 2009). The seroprevalence of CMV among women of childbearing age ranges from 35% to 95% in different countries and, as well as increasing with age, may also depend on sexual activity, occupation, particularly occupations involving close contacts with children in a community setting (Liesnard *et al.,* 2000). In the case of parents, contact with the urine or saliva of their children is a major source of infection or a baby may get CMV by coming into contact with infectious secretions or blood during birth or later through infected breast milk (Tremblay *et al.,* 2009). Most babies who contract the virus during birth or from breastfeeding (particularly those who are full-term) develop few or no symptoms or problems later appear from the infection (Pass *et al.,* 1990) thus infected mothers may deliver vaginally and in most cases breastfeed their babies (Picone *et al.,* 2009). It has also been reported that adverse living conditions, poor hygiene and closeness of contact in women of lower socioeconomic class and rural area increases the probability of contracting the infection (Sheevani *et al.,* 2005).

CMV can have protective IgG and harmful IgM antibodies. Protection occurs when the level of CMV specific IgG antibodies are at a high concentration of more than 1U/ml and these antibodies are known to protect infection transmission to foetuses or from person to person (Revello *et al.,* 2006). CMV replication is required for CMV disease to develop, and as long as the CMV load can be kept below this critical level by the immune system, there is protection against retinitis and other end-organ manifestations (O'Sullivan *et al.,* 1999). In a study carried out in Nigeria, 97.85% of pregnant women tested positive for CMV IgG and were termed as having protective antibodies and the age group found to be most at risk was 16-30 years (Akinbami *et al.,* 2010). This is contrary to active or primary infection which is shown by a high concentration of IgM antibodies and thus lacks protective capability (Ahmad *et al.,* 2010). The implication is that those infants born to these women are likely to have acute visceral disease with hepatitis, pneumonia purpura and severe thrombocytopaenia (Stagno *et al.,* 1985). However, where they happen to be asymptomatic at birth, they will have late developmental problems like mental retardation, cerebral palsy, sensorineural hearing loss and vision impairment (Stagno *et al.,* 1982; Istas *et al.,* 1995; Ivarson *et al.,* 1997).

The virus is ubiquitous with the ability to establish latency (dormancy) following primary infection, and can be reactivated particularly during episodes of immunosuppression (Pignatalli *et al.,* 2004, Adler *et al.,* 2004, Ornoy *et al.,* 2006). However, infections in healthy immunocompetent people are usually sub clinical and asymptomatic, and the virus does not generally present a health risk to these individuals.

Congenital CMV infection is described in 30,000 to 40,000 newborns each year in United

States. Approximately 9,000 of these children have developed permanent neurological sequelae (Colugnati *et al.,* 2007). The death rate of symptomatic congenital human CMV infection is placed at approximately 30% (Neto *et al.,* 2004).

In England, 45% of women in the high economic status did not have antibodies to cytomegalovirus and were therefore susceptible to primary infection compared to 18% in low income status. Congenital infection was seen to occur more often (1.6 vs. 0.6 %) in infants in the low-income group than in the high level income. In this group it was associated with recurrent maternal infection more often (82%) than with primary maternal infection, whereas in the upper-income group, it was associated with primary maternal infection in half the cases (Stagno *et al.,* 2006).

For most healthy persons who acquire CMV after birth, there are few symptoms and no long-term health consequences. Once a person becomes infected, the virus remains alive, but usually dormant within that person's body for life (Daiminger *et al.,* 2005). However, if a person's immune system is seriously weakened, the virus can become active and cause chronic CMV disease. In patients with AIDS, progressive loss of immune function, and, in particular, loss of cell-mediated immunity, permits CMV reactivation and replication to begin; asymptomatic excretion of CMV in urine can be detected in approximately 50% of HIV-infected individuals with a CD4 lymphocyte count <100 cells/µL (Macgregor *et al.,* 1995).

1.2 Statement of the problem

Cytomegalovirus has two kinds of infections namely the active and chronic infection which affects pregnant women and can lead to placental transmission contributing to

pregnancy complications and consequent congenital malformations. It is claimed that the presence of either infection is influenced by various social demographic factors. It has not yet been established which social demographic factors influence the two kinds of infections and which groups are at a high risk of suffering form pregnancy complications with congenital malformations among the newborn babies. (Mustakangas *et al.,* 2000).

1.3 Justification of the study

When CMV is passed on to a fetus during pregnancy the baby will be born with congenital CMV (Ahlfors *et al.,* 2001). Cytomegalovirus infection affecting the human embryo is associated with serious complications, such as microcephaly, mental retardation, spastic paralysis, hepatosplenomegaly, anaemia, thrombocytopenia, deafness, and optic nerve atrophy leading to blindness (Ornoy *et al.,* 2006). Around 15% of babies born with the virus do develop problems either at birth or in later years including hearing difficulties, blindness, learning difficulties, restricted growth, and problems with the lungs, liver, or spleen (Mustakangas *et al.,* 2000). A small percent of babies can be handicapped by CMV and infection during early pregnancy can also lead to stillbirths which is associated with fetal thrombotic vasculopathy (Enders *et al.,* 2001). Primary CMV infection during pregnancy poses a 30% to 40% risk of intrauterine transmission and adverse outcome is more likely when infection occurs within the first half of gestation (Akinbami *et al.,* 2008). Recurrent CMV infection may also affect the foetus causing non- immune foetal hydrops though this is rarely reported (Boppana *et al.,* 2001). Pregnant women with CMV IgG antibodies greater than 1IU/ml are considered to be protected and the implication is that, infant born to these women will not develop any pregnancy complications at birth or consequent late sequelae (Ahmad *et al.,* 2010). There

is no published data concerning CMV seroprevalence in pregnant women in Kenya. The basic data concerning CMV infections during pregnancy is important for health planners and care providers.

1.4 Research question

- What is the prevalence of CMV infection in pregnant women attending Thika Level 5 District Hospital?
- What is the level of active and chronic infection?
- What are the risk factors contributing to CMV infection?

1.5 Hypothesis

1) Cytomegalovirus infection is high among Pregnant women attending Thika Level 5 District Hospital

2) Social demographic factors are not significant in CMV transmission and infection among pregnant women

1.6 Objectives

1.6.1 General objective

To determine the prevalence of CMV infection among pregnant women attending Thika Level 5 District Hospital.

1.6.2 Specific objectives

- To determine the prevalence of CMV among pregnant women attending ante natal clinic at Thika Level 5 District Hospital.
- To determine the number of acute and chronic CMV cases.

- To establish the level of active and protective CMV antibodies in the pregnant women.
- To determine the social demographic characteristics among study participants

1.7 Expected results and applications.

The study was carried out to show the importance of CMV testing in pregnant women and should be included in the regular ante-natal clinic profiles.

CHAPTER TWO

LITERATURE REVIEW

2.1 Structure of Cytomegalovirus

Human cytomegalovirus (HCMV) belongs to the Order *Herpesvirales*, family *Herpesviridae* and genus *Beta-herpesvirinae*. The mature HCMV virion is 150-200nm in diameter, and composed of a 100nm icosahedral capsid that contains a linear 230kbp double-stranded DNA genome with attached proteins, a large tegument component surrounded by an envelope that contains a cellular lipid bilayer with viral glycoproteins (Neto *et al.,* 2004). The inner core (genome) of the CMV virus is a 64-nm linear double-stranded DNA molecule. The capsid is 110 nm in diameter and consists of 162 protein capsomers. The envelope contains lipoproteins and at least 33 structural proteins, some of which are glycosylated (glycoproteins). The glycoproteins determine the strain of CMV, are used for cellular entry of the virus, and are the targets of virus-neutralizing antibody (Chern *et al.,* 1998).

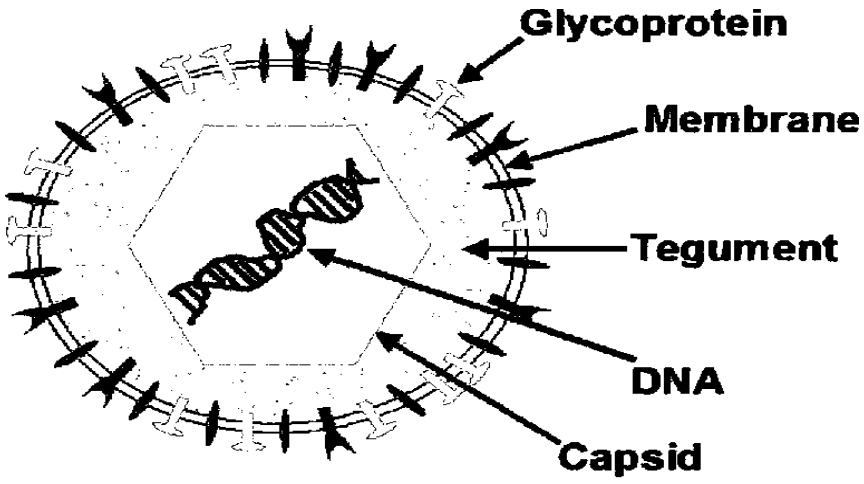


Figure 1: Structure of Cytomegalovirus (Barbi *et al.,* 2001).

CMV is a labile virus and readily inactivated by lipid solvents, pH below 5, heat (56°C for 30 min), and ultraviolet light for 5 min (Kohler *et al.,* 1995). It can survive on

environmental surfaces for several hours. CMV can be stored at 4°C for a few days without loss of infectivity. Storage at -70°C without loss of infectivity is possible for several months (Sever *et al.,* 2002).

A number of the viral and cellular proteins that compose an HCMV virion have been identified by biochemical and immunological approaches (Arabzadeh *et al.,* 2005). They share with other herpesviruses the biological properties of latency and reactivation, which cause recurrent infections in the host. No distinct serotypes of CMV exist; however, strain differences can be detected by molecular analysis of DNA, providing a classification of genotypes (Pignetali *et al.,* 2004). Some areas of the CMV genome are homologous with regions of human chromosomal DNA (Carraro *et al.,* 2003). This means that probes for CMV DNA must be carefully evaluated before being used to diagnose CMV infection in human cells (Spano *et al.,* 2004). The genome also contains a gene with striking homology to class I HLA molecules, although its function remains to be defined (Pass *et al.,* 1990). The CMV DNA can also be digested with restriction endonucleases so that, following electrophoresis, oligonucleotide patterns characteristic of different strains can be produced (Huang *et al.,* 2000). Although this technique cannot totally prove that 2 strains of CMV are identical, it does, however produce useful epidemiological information. The strains are associated with particular clinical diseases (Gaytant *et al.,* 2002).

In the urine and probably other bodily fluids, CMV is physically associated with host ß2-microglobulin, which does appear to have a protective effect on the virus against host immunoglobulins (Daiminger *et al.,* 2005). Since ß2m normally binds to HLA-I molecules, this suggests that CMV may use such cell surface molecules as receptors

(Pignatelli *et al.*, 2004). The only cells fully permissive for CMV infection *in vitro* are human fibroblasts, whereas *in vivo*, cells of all types can be infected (Murray *et al.*, 2002). Later in the infection, CMV induces the appearance in infected fibroblasts of an Fc receptor which has a high affinity for human IgG (Barbi *et al.*, 2001). Human CMV grows only in human cells and replicates best in human fibroblasts. The DNA is replicated by rolling circles and may be categorized into immediate early, delayed early, and late gene expression based on time of synthesis after infection (Okwori *et al.*, 2009).

2.2 Epidemiology and Transmission

CMV is one of the most successful human pathogen; it can be transmitted vertically or horizontally usually with little effect on the host (Picone *et al.*, 2000, Ahmad *et al.*, 2010). Transmission may occur in utero, perinatally or postnatally (Akinsegun *et al.*, 2011).

Once infected, the person carries the virus for life which may be activated from time to time, during which infectious virions appear in the urine and the saliva (Feldman *et al.*, 2010, Murray *et al.*, 2002)). Reactivation can also lead to vertical transmission (Pass *et al.*, 1990). It is also possible for people who have experienced primary infection to be reinfected with another or the same strain of CMV, this reinfection does not differ clinically from reactivation although it may be important epidemiologically to distinguish between reactivation and reinfection (Leila *et al.*, 2012). Maternal sexual behavior and contact with infected young children are the common source of infection. Vertical transmission of HCMV to the fetus can be attributed to either recurrent maternal infection or primary infection (Sheevani *et al.*, 2005).

Intrauterine infection is thought to follow maternal viraemia and placental infection

(Gaytant *et al.*, 2002). It only occurs in a third of women who experience primary infection during pregnancy though congenital infection can also result from recurrent maternal infection (Picone *et al.*, 2009).

Intrauterine transmission can occur at any time during pregnancy which may be surprising in view of the fact that large amounts of anti-CMV antibodies may be in circulation (Enders *et al.,* 2001, Akinsegun *et al.,* 2011). Perinatal infection is acquired mainly through 2 sites; infected genital secretions, or breast milk (Ahlfors *et al.,* 2001, Adler *et al.,* 2001). Once ingested, CMV usually gains access to the neonate by infecting the salivary glands. Overall, 2 - 10% of infants are infected by the age of 6 months worldwide (Siadiati *et al.,* 2002). Perinatal infection is thought to be 10 times more common than congenital infection though the exact mode of transplacental passage is uncertain (Pass *et al.,* 1990, Giovanni *et al.,* 2005). The virus replicates in fetal tissues, producing inflammation, tissue necrosis, and organ dysfunction (Liesnard *et al.,* 2000, Akinbami *et al.,* 2009). Cytomegalovirus hepatitis in the neonate can present with an intense inflammatory response involving the portal triads (Delfan *et al.,* 2001). In these cases, lobular disarray, degeneration of hepatocytes, and cholestasis are also seen (Huang *et al.,* 2000). The cause of ascites in congenital cytomegalovirus infection is not certain. Contributing factors may include low serum protein levels due to hepatic dysfunction and portal obstruction resulting from periportal inflammation (Galia *et al.,* 2001). The routes mostly speculated for transmission is saliva, sexual and transplantation receptors (Ahmad *et al.,* 2010). Saliva is probably the main route through which the virus is transmitted postnatally (Boppana *et al.,* 2001; Ornoy *et al.,* 2006). This is likely to be the route through which the virus is transmitted amongst young children (El-Nawawy *et al.,* 1996).

Sexual transmission is possible where CMV is found in semen and in the cervix (Sever, 2002). However oral-oral contact frequently occurs before intercourse which may well be the route through which the virus has been transmitted after intimate contact (Tremblay *et al.,* 2009). Patients undergoing renal transplantation are particularly at risk (Redwan *et al.,* 2011). Seronegative recipients have a 5% chance of acquiring primary infection from seronegative donors compared to a chance of 70 - 80% of acquiring primary infection from seropositive donors (Logan *et al.,* 2006). Blood is a major route for most viral infections and a decreased incidence of transfusion associated with CMV infection occurred when only blood products tested negative for CMV IgM were used (Akinbami *et al.,* 2009).

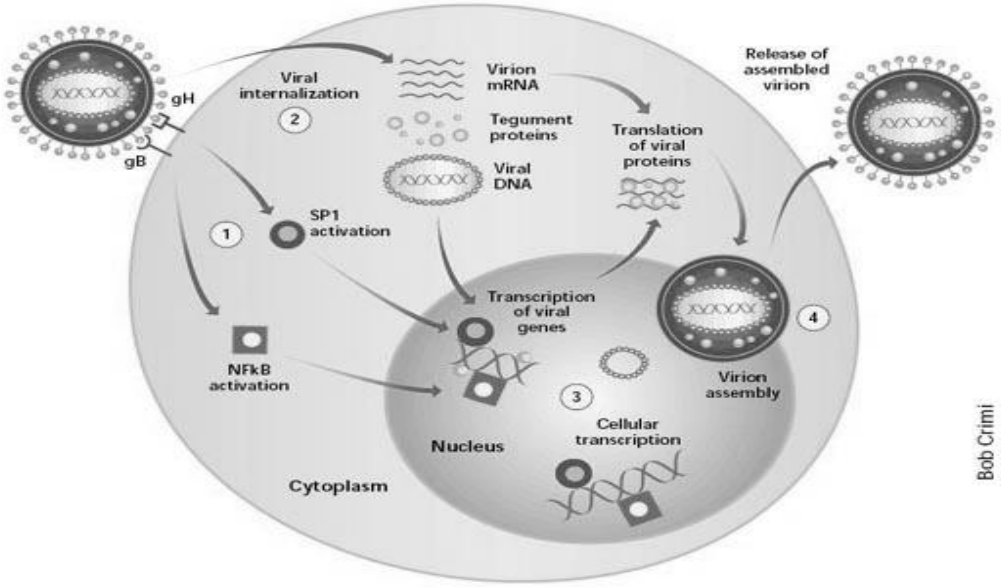
A study carried out in Alabama School evaluated pregnant women from two income groups to determine the incidence of primary CMV infection and its consequences for the offspring. In the high-income group, 64.5% of the women were seronegative for CMV and 1.6% had primary CMV infection. In the low-income group, only 23.4% of the women were seronegative for CMV, but 3.7% experienced a primary infection. The rate of transmission in utero was similar in the two groups (39% and 31%). Congenital infections were seen to be more frequent in the low-income group; however, primary CMV accounted for 25% of the congenital infections in this group, in contrast to 63% of the high-income cases. Infections acquired early and late in gestation had similar rates of transmission in utero,8%) of infants with symptomatic congenital infection and five infants (13.5%) who had developed significant handicaps were exposed in the first half of pregnancy. A study carried out in Khartoum reported 95% seroprevalence among the antenatal women (Dowd *et al.,* 2009).

In a study carried out in Israel, 42.3% had primary CMV infection; 57.7% had nonprimary CMV infection. Clinical CMV symptoms were seen to be more common with primary (60.2%) than with nonprimary (36.6%) infections (Ahlfors *et al.,* 2001).

2.3 Mechanism of Entry

Human cytomegalovirus infects multiple cell types in vivo and it fuses with plasma membranes of fibroblasts but enters retinal pigmented epithelial cells and umbilical vein epithelial cells via endocytois (Murray *et al.,* 2002). HCMV encodes for two complexes of surface proteins that can help to determine the route of entry. These complexes are the gH/gL/gO complex and gH/gL/pUL128/pUL130/pUL131 complex (Barbi *et al.,* 2011) The first 3 protein complex is sufficient for fibroblast infection, whereas the larger complex is required to infect epithelial cells. (Carraro *et al.,* 2003). Epidermal Growth Factor Receptor (EGFR) on the target cell is the site of HCMV adsorption, signaling and entry with the HCMV envelope glycoprotein gB being the ligand for EGFR (Chern *et al.,* 1998). It has also been shown that presence of alpha-v-beta3 integrin on the target cell is also sufficient for the effect infection of HCMV and they must mediate cell signaling as the presence of both is required for cellular infection (Huang *et al.,* 2000).

After CMV infects a cell the viral capsid is transported to the nucleus, where the viral DNA is released and transcribed by the host cell machinery (Siadiati *et al.,* 2002). However, CMV not only releases viral DNA, it also releases viral mRNA (Macgregor *et al.,* 1995). These mRNAs are delivered to the the cell cytoplasm when the viral envelop fuses with the plasma membrane at the beginning of infection (Chern *et al.,* 1998). These mRNA stay in the cytoplasm and are translated into proteins in the absence of gene products encoded by viral DNA (Neto *et al.,* 2004).



Figur

e 2: Human cytomegalovirus mode of entry and replication process (Murray *et al.,* 2007).

CMV can also affect certain immune cells such as monocytes (Turbadkar *et al.,* 2003). Binding of CMV to monocytes induces immunoregulatory gene expression and up regulates cell cytokine synthesis, indicating that viral absorption can alter cell metabolism even if the infection is nonproductive (Chern *et al.,* 1998, Redwan *et al.,* 2011).

CMV replication produces immediate-early (IE), early, and late CMV antigens. IE antigens appear in the nucleus of CMV-infected cells 1 to 3 h after infection and remain present even in latent infection (Huang *et al.,* 2000). IE antigen gene products direct production of both viral and cellular genes. Early antigens appear in the cytoplasm or membrane approximately 3 h after infection. Early antigen gene products direct viral DNA synthesis. Late antigens appear in the nucleus and cytoplasm within 6 to 24 h after infection. Late antigen gene products direct production of structural nucleocapsid proteins (Carraro *et at.,* 2003). IE and early antigens are virus-induced nonstructural

proteins and appear before DNA synthesis (Giovanni *et al.,* 2005, Fries *et al.,* 1994). This is important because the mechanism of action of ganciclovir, foscarnet, and cidovir (the three most common agents used for treatment of CMV) is through interruption of DNA synthesis (Galia *et al.,* 2007). Late antigens are virally encoded structural proteins and appear after DNA synthesis, so their appearance is sensitive to the common antiviral agents (Fries *et al.,* 1994).

2.4 Pathogenesis and pathology

The extent of damage due to cytomegalovirus varies widely and is correlated with viral load (Boppana *et al.,* 2001) as well as immune-mediated damage for cytotoxic T lymphocytes and consequent hypoxic cerebral damage (Arabzadeh *et al.,* 2007, Delfan *et al.,* 2011, Liesnard *et al.,* 2000). Tumor necrosis factor receptor UL144 polymorphisms have been suggested as a predisposing factor affecting virus load (Adler *et al.,* 2004). Gap junction protein beta-2 (GJB2) mutations are more frequent in patients with cytomegalovirus and hearing loss than those without (Volpe *et al.*, 2001).

Cytomegalovirus can infect many different cell types and all major organs. The cochlea is frequently involved, as is the central nervous system (Staras *et al.,* 2006, Revello *et al.,* 2006, Leila *et al.,* 2012). There is a predilection for periependymal neurons and glia, with focal encephalitis and periependymitis. Necrotic periependymal tissue subsequently calcifies. Calcifications are typically periventricular but may also be scattered throughout the brain (Boppana *et al.,* 2001). Cytomegalovirus produces cytolysis, with focal necrosis and a localized mononuclear inflammatory response. Tissue damage results from direct effects of the inflammatory response as well as an associated vasculopathy resulting in

ischemia and encephalomalacia, immune-mediated reactions, and apoptosis (Volpe *et al.*, 2001).

Typical pathology involves cytomegalic brain cells with intranuclear inclusions. When cytomegalovirus infects developing CNS tissue, it can produce microcephaly with neuronal migration defects (Delfan *et al.*, 2011, Ender *et al.*, 2001 Ahmad *et al.*, 2010). Severe destructive changes lead to more severe brain abnormalities such as porencephalic cysts, cerebellar hypoplasia, aqueductal stenosis, and hydrocephalus (Delfan *et al.*, 2011).

Cytomegalovirus can induce specific chromosomal damage that requires viral entry into the cell, but not de novo viral protein expression, a probable mechanism for damage in the developing fetal brain (Raynor *et al.*, 1993, Volpe *et al.*, 2001). Another mechanism in the pathogenesis might be related to down regulation of the EGF receptor on the cell surface by cytomegalovirus (Galia *et al.*, 2007).

Congenital cytomegalovirus is a persistent chronic infection. Half of infected children show viremia for months and viruria for 6 years or more (Arabzadeh *et al.*, 2007, Ahmad *et al.*, 2010). Cytomegalovirus may be excreted in saliva for 2 to 4 years. Late sequelae reflect this chronic infection of developing tissue. In addition, some abnormalities that are present at birth may not be detectable until the infant is older (Griffiths *et al.*, 1982).

2.5 Prevention and treatment

The primary sources of CMV infection for women of childbearing age are young children and sexual contacts. All preschool-age children should be considered potential sources of infection (Pass *et al.*, 1990). Because transmission of virus appears to require contact

with secretions, it is sensible to stress avoiding contact with body fluids from young children and careful handwashing whenever such contact occurs (Sheevani *et al.,* 2005). It is advisable to inform women who are pregnant to avoid new sex partners. Although it is not certain that infections in pregnant women can be prevented by avoiding exposure, it is important to emphasise on the need of avoiding new sex partners and should also be educated about possible consequences of congenital CMV infection. This education is most important for women who have occupational contact with young children or a history of sexually transmitted infections (Lamberson *et al.,* 1988, Stagno *et al.,* 1985, Leila *et al .,* 2012). Secretion precautions and careful handwashing should be used in the clinical setting to prevent spread of CMV from patients to staff or other patients (Sever *et al.,* 2002). Children known to have CMV infection should not be identified for special infection control procedures; rather, all children should be considered sources of CMV (Staras et al., 2006, Okwori *et al.,*2009). Transfusion acquired CMV infections can be prevented by limiting seronegative patients to blood products from seronegative donors or by removing leukocytes and platelets from whole blood by using special filters (Lamberson *et al.,* 1998).

Passive immunization with CMV immune globulin preparations have been used to prevent congenital infection and in individuals who underwent transplant (Giovani *et al.,* 2005). Hyperimmune globulin presumably reduces maternal systemic or placental viral loads, thus decreasing the likelihood of fetal infection (Volpe *et al.,* 2001). Once the fetus is infected, however, hyperimmune globulin presumably reduces placental or fetal inflammation, or both, resulting in increased fetal blood flow with enhanced fetal nutrition and oxygenation (*Giovani et al.,* 2005).

Eating a balanced diet to boost the immune status is also advisable in order to prevent recurrency (Daiminger *et al.,* 2005). Vaccines for CMV are currently under trial. A live vaccine known as the Towne strain has been reported to be effective in conferring protection or in reducing the severity of disease in seronegative recipients given seropositive kidneys (Redwan *et al.,* 2011). It was also well tolerated and immunogenic but could not prevent reinfection of the recipients with a different strain of CMV. There were concerns about latency and reactivation of a live vaccine as well as potential oncogenicity (Tremblay *et al.,* 2009). However, there was no evidence of reactivation in immunosuppressed patients or an excess of malignancies. Because of the possibility of reactivation, a live vaccine would be unacceptable for use in seronegative women. Therefore, subunit vaccines are currently being developed based on the membrane proteins SSK and GC1. One encouraging candidate is live vaccine using adenovirus as the vector (Enders *et al.,* 2001). There are four antivirals that are active against CMV and they include; ganciclovir, valganciclovir, foscarnet, and cidofovir. Ganciclovir is the one highly recommended due to its ability to reduce the viral load within a short period and can also be used in infants (Ornoy *et al.*, 2006).

2. 6 CMV diagnostic methods

CMV diagnostic tests can be grouped into 3 categories: direct detection, indirect examination (virus isolation), and serology (Kohler *et al.,* 1995). In direct examination, the clinical specimen is examined directly for the presence of virus particles, virus antigen or viral nucleic acids (Pignatelli *et al.,* 2004). In indirect examination, the

specimen is inoculated into cell culture, eggs or animals in an attempt to grow the virus: this is called virus isolation (Kohler *et al.,* 1995). Serology actually constitutes by far the bulk of the work of any virology laboratory. A serological diagnosis can be made by the detection of rising titres of antibody between acute and convalescent stages of infection (Engvall *et al.,* 1971).

The commonly used serological test is known as Enzyme Linked Immunosorbent Assay which determines if one has had a chronic or acute infection by detecting the level of IgG and IgM antibodies respectively. The major limitation for IgM antibody is lack of specificity for primary infection leading to false-positive results. The reason as to this is that IgM antibodies can persist for over three months after primary infection and also during reactivation, IgM antibodies are produced thus giving a false infection period. IgG avidity assays are utilized in such situations to help distinguish primary from non-primary CMV infection (Engvall *et al.,* 1971).

Polymerase chain reaction (PCR) is a widely available rapid and sensitive method of CMV detection based on amplification of nucleic acids. The techniques usually target major immediate early and late antigen genes in their well conserved regions.This method can either be qualitative or quantitative, in which the amount of viral DNA in the respective sample is measured. The procedure is very sensitive thus contamination can lead to erroneous diagnosis (Pignatelli *et al.,* 2004, Huang *et al.,* 2000, Robert *et al.,* 2000).

2.6.1 Serological markers

This method of diagnosis is clinically important in establishing the type and period of infection in the body by establishing the antibodies present in circulation. It also

demonstrates the body's level of response by titrating the quantity of antibodies produced towards the causative agent (Kohler *et al.,* 1995, Engvall *et al.,* 1971).

2.6.1.1 CMV Rapid Test

This is a One-step Cytomegalovirus (CMV) Screening Test Device which can be divided into two types, according to the antibody type to be tested, CMV IgM test and CMV IgG test (Pass *et al.,* 1990). The two CMV rapid tests are based on the principle of Gold Immuno-chromatography Assay (GICA), using the antibody-antigen reaction to detect CMV IgM and IgG antibodies in human serum or plasma specimen respectively. These Cytomegalovirus tests are IVD CMV test, and the assay principle are simple (Housman et al., 1995). With a CMV IgM assay, when the serum sample added contains CMV-IgM, the antibody will react with anti-human IgM monoclonal antibody in the membrane strip. These complexes move along the membrane strip chromatographically to the test region (T), where these complexes will be captured by the pre-coated recombinant CMV antigen. Then a red or pink line will appear, indicating a positive result. The unbounded complex moves on to the control region (C), where they are captured by the anti-mouse antibody, and a red line will appear, indicating the assay is a valid one. In this way, the control line provides an inner quality control mechanism for this CMV serum test. For a CMV IgG assay, the story is the same.

2.6.1.2 Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme Linked Immunosorbent Assay is one of the immunoassay tests and it is specially used due to rapidity in experimentation, greater sensitivity and specificity. The reaction is both qualitative and quantitative types. It is widely used in clinical diagnosis and scientific research. Samples, standards or calibrators are first added to the precoated

antibody micro plate. Next, the enzyme conjugate is added and the mixture is incubated at room temperature. During incubation, competition for binding sites on the micro plate is taking place. The plate is then washed removing all unbound material. The bound enzyme conjugate is detected by the addition of a TMB based substrate. Test results may be obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a micro plate reader at 650 nm or 450 nm if acid stop is used. The extent of color development is inversely proportional to the amount of analyte in the sample or standard (Fries *et al.,* 1998). For example, the absence of the analyte in the sample will result in a dark blue color, whereas the presence of the analyte will result in a light blue color or no color as the concentration of the analyte increases. If acid stop is used to halt the assay then the dark blue color will change to a dark yellow color and the light blue color to no color will change to light yellow to no color (Stagno *et al.,* 1985).

2.6.1.3 CMV IgG avidity test

IgG avidity test is defined as the strength with which the IgG attaches to antigen and usually matures with the length of time following primary infection (Drew et al., 1988). Thus, IgG produced within the first few months following primary infection exhibits low avidity, whereas IgG produced several months or years later exhibits high avidity. Several groups of investigators have shown that detection of CMV specific IgG of low avidity is a reliable indicator of infection within the previous 6 to 8 months. However, detection of CMV-specific IgG of high avidity is actually more informative from a

clinical standpoint; the presence of high avidity IgG essentially excludes the possibility that infection occurred within the previous 4 months (Fowler *et al.,* 2000). CMV IgG avidity ELISA utilizes urea, an agent that disrupts hydrogen bonds, to differentiate low avidity from high avidity antibodies. Following attachment to immobilized antigen, low avidity IgG readily dissociates from the antigen in the presence of urea, whereas high avidity IgG does not. Thus, avidity is assessed by performing duplicate sets of the routine ELISA for CMV specific IgG; the only difference is that, after the initial incubation, one set is washed with buffer containing urea, and the other set is washed with buffer lacking urea. The CMV specific IgG OD of the set washed with urea buffer is then divided by the CMV specific IgG signal of the set washed with non-urea buffer, thus providing the AI. AI values ≤ 0.50 indicate low avidity, values of 0.51-0.59 indicate intermediate avidity, and values ≥ 0.60 indicate high avidity (Akinsegun *et al.,* 2011).

2.6.2 Molecular methods

2.6.2.1CMV DNA by PCR

Detection of CMV DNA by polymerase chain reaction (PCR) is a rapid, highly specific and very sensitive method. PCR has been reported to detect the onset of viremia 1 to 2 weeks prior to either culture or antigenemia tests. PCR not only detects CMV DNA in specimens that are positive for antigenemia and/or positive by culture methods, but also detects an additional 30% of specimens as positive (Picone et al.,2009).

In PCR, target CMV DNA sequences are selectively amplified through repeated cycles of denaturation, annealing with CMV-specific primers, and primer extension with DNA

polymerase. Due to the high specificity and sensitivity of the polymerase chain reaction, CMV DNA can be detected in various specimens from patients, including blood, urine, tissues, and CSF. To ensure the absence of a nonspecific PCR inhibition, Focus includes an internal amplification control for each specimen. A sample can be interpreted as negative only if the analysis of the internal control indicates that DNA amplification has occurred (Chern *et al.*, 1998).

Diagnosis of CMV infection should not rely solely upon the result of a PCR assay. A positive PCR result cannot differentiate between latent CMV infection and active CMV disease. Therefore, patients with a positive PCR result should be evaluated with other tests to establish the diagnosis of the disease. A negative PCR result indicates only the absence of CMV DNA in the sample tested and does not exclude the diagnosis of the disease.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

This was a cross sectional study.

3.2 Location of the study

The study was conducted in Thika Level 5 District Hospital.

3.3 Study population

Thika Level 5 District hospital serves a population from both urban and rural setting. Urban setting is mainly from the estates which are: - Starehe, Makongeni, section nine, Ofafa Jericho, Umoja Happy valley. Rural population came from Makuyu, gatundu and workers in the surrounding pineapple and tea plantations.

3.4 Target population

Eligible pregnant women at the ante natal clinic who accented their inclusion in the study by signing a consent form filled the provided questionnaire then proceeded to the laboratory for sample collection.

3.5 Eligibility criteria

All 260 selected subjects were in their first or second trimester of pregnancy. They were informed about the aims of the study. Informed written consent was also obtained from all women. In order to increase the quality of data collection, the questionnaires were completed by trained interviewers.

Inclusion criteria:

- Pregnant women attending the ante natal clinic of Thika Level 5 District Hospital.
- Pregnant women in their first or second trimester of pregnancy.

- Pregnant women in the age group of 16-45 years

Exclusion criteria:

- Pregnant women in their third trimester
- Pregnant women not sure of their gestational period.

3.6 Sample Size and Sampling Techniques

3.6.1 Sample Size

The sample size is obtained through calculation using Fisher, *et al.*, 1998 equation using extrapolation prevalence of 34% (Ahmad *et al.*, 2010).

$$N = \frac{Z^2 P (1-P) D}{d^2}$$

Where; N - Minimum number of sample required

Z - Standard error (1.96)

P - Estimated prevalence (34%)

D- Design effect (1)

d - Precision required (95%, 0.05)

$$\frac{1.96^2 x 0.34(1-0.34)1}{0.05^2}$$

=260

3.6.2 Sampling Techniques

3.6.2.1 Sample collection and storage

The participants were informed of the method to be used to obtain the samples for evaluation. Blood was collected asceptically from each of the 260 participants by tying a tourniquet on the upper part of the arm, locating the median cubital vein, cleaning the site of injection with cotton wool soaked in 70% alcohol then carefully inserting a sterile needle in the vein and pulling the syringe to obtain five milliliters of blood then transferring to a plain vacutainer.. The vacutainers were given a reference number which was also indicated on the request and consent form of the respective participants. The samples were then centrifuged at 3000 revolution per minute for five minutes and serum was obtained. The serum was separated and put into cryovials and stored at -20^0c awaiting analysis.

3.6.2.2 ELISA Analysis

The frozen samples were thawed by placing them on the working bench for two hours at room temperature. ELISA was the preffered method of analysis mostly because of its high sensitivity, specificity (Engvall *et al.,* 1975) and availability of the test kits. Three packs of 96 wells each were used for both IgG and IgM analysis. . Six controls and calibrator determinations containing one blank, one negative control, three calibrators and one positive control per run were pipetted into the respective microwells. A dilution of 1:21 of the negative control, calibrator, positive control and each patient serum were

made using the provided diluent and a colour change confirmed a proper mixture. To individual wells, 100µL of each diluted control, calibrator and sample were added using different pipette tips and gentle shaking of the microtitre plate after covering with an inert synthetic cover ensured that the samples were properly mixed. 100µL of the diluent was added to the first well as a reagent blank. Plate incubation at room temperature (20-25°c) for 30 minutes was undertaken after which the microwells were washed manually. The washing involved vigorously shaking out the liquid from the wells, each microwell was filled with wash buffer making sure no air bubbles were trapped in the wells and this was done for five times. The wash solution was then shaken from all the wells. The plate was inverted over a paper towel and tapped firmly to remove any residual wash solution from the wells.

100µL of TMB was added to each well including the reagent blank well, this was done at the same rate and in the same order as the specimens were added. Incubation of the plate at room temperature for 10 to 15 minutes followed, and then the reaction was stopped by adding 50µL of stop solution to each well. Positive samples for IgM/IgG (depending on the test) turned from blue to yellow. After adding the Stop Solution, the plate was tapped several times to ensure thorough mixing of samples. The microwell reader was set to read at a wavelength of 450nm and the OD of each well was measured against the reagent blank and this was done 20 minutes after addition of the stop solution. Results were obtained by calculating the index value then interpretation done according to the manufacturer's specifications.

The cutoff value used to determine IgM/ IgG was above 0.4 IU/mL and borderline when it was between 0.39 IU/mL and 0.41 IU/mL, and negative when their index was >0.39

IU/mL. As IgM anti-CMV antibodies may test positive for more than 12 months and may be produced duringreactivation or reinfection.

3.6.2.3 Avidity testing

The samples that were IgM and IgG positive at ELISA were also tested for IgG avidity. Avidity was assessed by performing another set of the ELISA test with micro titer wells for CMV specific IgG. The procedure as mentioned above was followed until after initial incubation where the wells were washed with buffer containing 40% urea. The CMV specific IgG OD was then divided by the CMV specific IgG OD of the previous results then multiplied by a hundred and this provided the AI. Interpretation of results was if the index was <35% it was considered low, and high if it was > or equal to 35%. Low IgG avidity levels strongly suggest an infection contracted less than three months before, whereas a high avidity tends to exclude this.

3.7 Data management/analysis

Data collected was analyzed using EPI info statistical tool at 0.95 confidence level and variables analyzed using chi square.

Variables that were analysed included; age group, marital status, parity,trimester of pregnancy, location, literacy level, occupation, blood transfusion history and HIV status.

CHAPTER FOUR

RESULTS

4.1 The participant's social demographic variables

A total of 260 pregnant women in the age group of 16-45 years and in their first or second trimesters were examined. The mean age of the participants was 28 years. Subjects in age bracket 26-30 years were 36% , 21% were in the age group of 31-35 years, 20%)were in the age group of 21-25 years, 12% were in the age group of 16-20 years, 7% were in the age group of 36-40 years and 4% in the age group of 41-45 years. Marital status was also considered with 64.61% of the respondents being married, 25.39%were single and 10.39%were divorced. Respondents with 1-4 children were 53.08% and62.9%were married. The respondents who were pregnant for the first time were 45%, >4 children were 2.30%. Subjects in their first trimester of pregnancy were 50.4% and, 49.6% were in their second trimester with 40.31% being of age group 26-30 years. Majority of the participants resided in the urban centres of Thika town. However, majority (90%)of the study participants were HIV negative (Table 4.1).

Table4.1: Distribution of participants according to social demographic variables

variable	participants (n,%)	IgM (n,%)	IgG (n,%)	p value
agegroup:				
16-20	31(12.0)	6(19.4)	22(70.9)	
21-25	52(20)	3(12)	37(22.26)	
26-30	95 (36)	5(13.68)	77(81.05)	0.001*
31-35	54(21)	2(3.7)	46(85.19)	
36-40	18(7)	4(22.22)	10(55.56)	
41-45	10(4)	1(10)	9(90)	
marital status:				
single	66(24.39)	9(13.6)	38(57.6)	
married	168(64.61)	12(7.2)	137(82)	0.001*
divorced	27(10.39)	0(0)	21(96.3)	
parity:				
0	117(45)	12(10.35)	76(65.5)	
01-Apr	138(53.08)	8(5.8)	121(87.7)	0.0006*
>4	5(13.59)	0(0)	4(66.7)	
trimester of pregnancy:				
First	131(50.4)	9(6.9)	101(77.1)	0.657
Second	129(49.6)	12(9.3)	100(77.5)	
location				
rural	111(42.7)	9(8.1)	84(75.7)	
Urban	149(57.3)	12(8.1)	117(78.5)	0.816
education				
none	3(1.2)	0(0)	3(100)	
primary	12(4.6)	4(33.33)	8(66.7)	
secondary	79(330.4)	9(11.4)	58(73.4)	0.014*
tertiary	166(63.8)	8(4.8)	132(79.5)	
occupation:				
business	68(26.2)	5(7.4)	55(80.9)	
employed	70(26.9)	3(4.3)	58(82.9)	
housewife	33(12.7)	5(15.2)	26(78.8)	0.0839
farmer	43(16.5)	3(7.0)	34(79.1)	
student	46(17.7)	5(10.9)	28(60.9)	
history of blood transfusion:				
No	243(93.5)	20(8.2)	20(8.2)	0.54
Yes	17(6.5)	1(5.9)	12(70.6)	
HIV status:				
positive	27(10.3)	1(3.7)	23(85.2)	0.546
negative	234(89.7)	20(8.6)	178(76.4)	

4.2 Prevalence of Human Cytomegalovirus (HCMV) in pregnant women attending Thika level 5 District Hospital

Out of a total of 260 pregnant women under the study, 85.4% tested positive for cytomegalovirus antibodies and 14.6% were negative.

4.2.1 The distribution of Human Cytomegalovirus antibodies among the respondents

In a total of 260 study subjects, 158 (60.8%) of them tested positive for IgG antibodies while 10 (3.8%) were IgM positive. However, 54 (20.8%) were positive for both IgG and IgM of which CMV avidity test was conducted to evaluate strength of IgG antibody based on its binding affinity to the well bound antigen (Table 4.3). Only 14.6% of the test samples were negative (Table4.2)

Table 4.2: Distribution of Cytomegalovirus antibodies among the respondents

serostatus	***f***	***%***
IgM/IgG	54	20.8%
IgG	158	60.8%
IgM	10	3.8%
none	38	14.6%
total	260	100.0%

4.2.2 CMV avidity index

The samples that tested positive for both IgG and IgM were evaluated for the avidity of IgG antibodies. This test was carried out to evaluate strength of IgG and IgM antibodies inorder for the infection can be classified as acute or chronic. Those with avidity index >35% were 43 (79.63%) showing IgG antibody pre dominancy while those with avidity index <or equal to 35% were 11 (20.37%) indicated that IgM was a predominant antibody (Table 4.3).

Table 4.3: Avidity index among participants with both IgM and IgG antibodies

avidity index	***f***	***%***
<35%	11	20.37
>35%	43	79.63
Total	54	100.0

Table 4.4: Distribution of active and protective antibodies among the participants

	active antibodies (n,%)	**protective antibodies (n,%)**
age group:		
16-20	2(28.6%)	10(58.8%)
21-25	2(100%)	32(76.2%)
26-30	3(60%)	61(79.2%)
31-35	1(50%)	38(82.6%)
36-40	2(50%)	9(90%)
41-45	0(0)	8(88.9%)
marital status:		
single	4(30%)	33(86.8%)
married	6(50%)	105(76.45%)
divorced	0(0)	20(76.86%)
parity:		
none	7(58.3%)	56(73.67 %)
one to four	3(37.5%)	108(89.2%)
>four	0(0)	2(50%)
trimester of pregnancy:		
first	4(44.44%)	87(86.14%)
second	6(50%)	71(71%)
location:		
rural	3(33.33%)	57(67.86%)
urban	7(58.5%)	101(86.3%)
education:		
none	0(0)	0(0)
primary	2(50%)	4(50%)
secondary	5(55.56%)	43(74.1%)
tertiary	3(37.5%)	111(84.1%)
occupation:		
business	2(40%)	43(78.6%)
employed	0(0)	48(82.8%)
housewife	3(60%)	20(76.9%)
farmer	2(66.7%)	25(73.5%)
student	3(60%)	22(78.%)
history of blood transfusion:		
no	9(45%)	147(77.8%)
yes	1(8.3%)	11(5%)
HIV status:		
positive	1(100%)	9(39.1%)
negative	9(45%)	149(83.7%)

4.2.3 Distribution of CMV antibodies according to the social demographic variables

The age group 31-35 years had IgG at 85.19% with 16-20 years age group having the lowest at 54.8% (Table 4.1. Marital status showed significant statistical relationship to serostatus (p=0.001) with married subjects recording 64.61%. Trimester of pregnancy recorded 50.4% and 49.6% in first and second trimester respectively. There was no statistical significance between trimester of pregnancy and serostatus (p=0.657). The parity of the participants was also considered and statistical significance was established in parity and serostatus (p=0.0006). Education also showed statistical significance and the majority of the participants having acquired tertiary level of education (p=0.014). Geographical location, occupation, history of blood transfusion and HIV status showed no statistical significance (p>0.05) (Table 4.1). .

Primary infections were also demonstrated and those with primary education had 33.33% and were the highest to be recorded.. Active infections were highest at 100% in 21-25 years age group and majority of cases with protective antibodies were in the age group of 36-40 years at 90% Those with between 1-4 children recorded the highest rate of IgG and protective antibodies. HIV positive subjects had only one acute case which was a primary infection. The participants who had no history of blood transfusion had equal rates of acute and chronic infections and recorded a high level of antibodies. Equal rates of acute infections were demonstrated among participants in rural and urban areas **(**Table 4.4)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Prevalence of Human Cytomegalovirus antibodies in the pregnant women

Findings in this study indicate high prevalence of CMV (85%) among women in Thika Level 5 District Hospital. It agrees with previous investigation reported in Nigeria at 87%, 84.2% and 88% respectively (Delfan *et al.,* 2011; Okwori *et al.,* 2005; Ahmad *et al*., 2011), 72.1% in Iran (Leila *et al.,* 2011) and 77.5% in Japan (Nishimura *et al.,* 1999). However, results from this study were higher to those obtained in developed countries; 56.9% in Australia and 46.8% in France (Picone *et al.,* 2009). The low rates recorded in developed countries are probably due to inclusion of routine CMV screening among the antenatal profiletest in Israel (Robert., 2000) and observation of improved hygienic standards (Dowd *et al.,* 2009). The results from this study and similar ones conducted in Africa and other developing countries reflect the nature of HCMV hyperendemicity among subjects in more or less similar socioeconomic situations (Leila *et al.,* 2011). These high rates were probably associated with lack of proper hygienic practices. This was demonstrated more particularly in subjects residing in the rural areas. These results showed that lack of adequate awareness on prevention measures and hygiene among individuals plays a major role to the high CMV transmission and infection rates (Colugnati *et al.,* 2007).

5.2 The acute and chronic HCMV cases among the pregnant women.

Immunoglobulin G (IgG) was the most common antibody (77.3%) indicating an earlier infection (Barbi *et al.,* 2001). There was a steady increase in IgG positivity rate up to the age group of 26-30 years at 88.7% then it declined at a slower rate with the age group 36-40 years at 55.6% having the lowest (Table 1). The steady rise could probably be associated with increased sexual promiscuity due to realization of sexual maturity leading to high transmission rates (Griffiths *et al.,* 2001). Divorced subjects are presumed to have multiple sexual partners (Leila *et al.,* 2011) and this was observed when IgG was the dominant antibody. Akinsegun reported a similar trend with a steady increase upto 25-30 years at 49.7% and 1.8% in participants with above 41 years of age (Akinsegun *et al.,* 2011). The current findings however contradict an earlier report that demonstrated a decline in CMV seropositivity with increase in age (Sajed *et al.,* 2011). The high IgG prevalence cuts across all communities irrespective of the developmental status with Egypt having a prevalence of 96% (El-Nawawy *et al.,* 1996), Saudi Arabia with 91% (Arabzadeh *et al.,* 2007) and India 91.05% (Turbadkar *et al.,* 2003).

IgM antibodies were demonstrated in 8.1% of the participants and thisn indicated acute infection which could have occurred three months prior to the assay (El-Nawawy *et al.,* 1996). HCMV IgM has been shown to peak during the first three months after primary infection in pregnant women and then persist at a low level for 18 to 39 weeks (Colugnati *et al.,* 2007). In this study, acutely infected HCMV was demonstrated to be highest in the single participants at 13.6% and this can probably be related to the fact that most of the single participants were <20 years of age and were being exposed to new infections through sexual exploitation (Tremblay *et al.,* 2009). It was further noted that the age

group 31-35 years had the highest IgG (85.19) cases and also reported lowest IgM (39.7%). Pregnant women may also have a higher risk of acquiring infections because of pregnancy-induced immune depression (Ornoy *et al.,* 2006).

Children in day care centres have close contacts with one another and this leads to transmission of CMV to uninfected children. The newly infected children later transmit the infection to their mothers which might be of a different strain from what the mother has thus leading to reinfection (Akinbami *et al.,* 2009).

5.3 The level of protective and active HCMV antibodies in the pregnant women

The samples that tested for both of these antibodies had a recurrent infection (Arabpour *et al.,* 2005). Participants with IgG protective antibodies in this study were 60.8% while 21.4% were not protected and their foetuses had a high likelihood of suffering from acute visceral disease with hepatitis, pneumonia purpura or severe sequeal (Stagno *et al.,* 1982). IgG protective antibodies are termed so because of their specific binding affinity to cytomegalovirus and neutralizing the virus ability of causing an infection (Ahmad *et al.,* 2010).

A primary HCMV infection is an active infection and has a high rate of transmission especially in immunocompromised situations (Ahmad *et al.,* 2010). In this study, those with IgM levels above 0.4 IU/mL were considered to having an active infection and were 10 (3.8%). HIV cases are known to be associated with immunosuppression (Macgregor *et al.,* 1995) and in this study, one subject with HIV had an acute HCMV infection. Primary infection posed a higher risk of intrauterine transmission with 1.83 times higher than recurrent infection (Galia *et al.,* 2007). Generally, the average transmission of

HCMV infection from mother to foetus is greater during primary maternal infection with a reported range of 24-75% (Ahlfors *et al.,* 2001). Increasing rate of foetal cell differentiation is observed during early gestation and a primary infection in this stage poses a high risk of developmental sequeal due to destruction of foetal brain cells (Delfan *et al.,* 2011). Children born with these conditions will require extra care which comes at an extra cost and will have a heavy bearing on the economic status of the parents (Revello *et al.,* 2006). The children will also likely suffer from low self esteem which will greatly affect their productiveness in the community (Pass *et al.,* 1990).

5.4 Conclusion

- The present study shows that CMV infection is widespread among women of child bearing age in Kiambu County with the prevalence being at 85.6%.
- The high prevalence can be attributable to lack of proper hygiene practices, lack of adequate preventive measures and lack of screening services.
- Protective IgG antibodies were at 60.8% conferring protection to most unborn babies from developmental sequeal.
- Active Cytomegalovirus infection was demonstrated by IgM antibodies at 3.8% posing a great risk for gestational infection.
- Significant association of CMV with age, marital status, parity and education level suggest that women of child bearing age are exposed to this infection.

5.5 Recommendation

a. Based on the recorded prevalence rates and number of children with health disorders resulting from CMV, there is a need to adopt screening of CMV into the antenatal profile tests as a preventive approach to CMV infections.
b. The study recommends health education of this virus on how it is acquired and contributing factors like personal and community hygiene.
c. A follow up on the pregnant women with CMV infection should be carried out to determine the clinical outcome of both infection(Acute and chronic)

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APPENDICES

Appendix i

CONSENT FORM

DECLARATION OF INFORMED CONSENT TO CYTOMEGALOVIRUS TEST IN PREGNANT WOMEN.

I freely consent to the withdrawal of my blood. I freely consent to the testing of that blood.

I understand that the results of my tests will be kept confidential, except for the disclosure of any reactive result to the attending physician.

I understand that I have the right to request and receive a copy of this form.

Serial number of person being tested Signature

Pre-counseling done by:

_____

Post-counseling Required Yes/ No

If yes done by _____

Appendix ii

QUESTIONNAIRE

Participant's serial number:

Age:

Fundal height:

a) Where is your area of residence?.....

b) How long have you stayed in that area?.....

c) Are you married?.....

d) If yes, how long?.....

e) What is your occupation?.....

f) What is your household average income?.....

g) What is your highest level of education?.....

h) Have you ever been screened for Cytomegalovirus?.....

i) If yes, when was the last time you got tested for Cytomegalovirus?.....

j) Were the results positive or negative?.....

k) If positive, were you put on any treatment?.....

l) If yes, what medication were you given?.....

m) What was your age during your first pregnancy?.....

n) How many children have you had?.....

o) How many are still alive?.....

p) Have you had a child with a disability?.....

q) If yes, what kind of a disability was it?.....

r) Was it inborn or through an accident?.....

s) If inborn, what year was the baby born?.....

t) Do you have any form of immunosuppressive syndrome?.....

u) If yes, what is the cause?.....

v) If the cause is known, are you on any treatment for the particular condition?.....

w) If yes, what medication are you taking?.....

x) Have you ever received donated blood?.....

y) If yes, when was the last transfusion done?.....

Appendix iii **Approval of Research**

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School **DATE:** 12th November, 2012

TO: Mwangi Z. Maingi **REF:** P150/20957/2010
C/o Department of Medical Laboratory Sciences

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 8th November, 2012, approved your Research Proposal for the M.Sc Degree **Subject to, Adding the word "Kenya" on the title Prevalence of Cytomegalovirus in Pregnant Women Attending Thika Level 5 District Hospital, Kiambu County, Kenya.**

Thank you.

DAVID NJOROGE
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman Department of Medical Laboratory Sciences

Supervisors:

3. Dr. Anthony Kebira
C/o Department of Medical Laboratory Sciences
Kenyatta University

4. Dr. Steven Runo
C/o Department of Biochemistry and Biotechnology
Kenyatta University

DNN/cao

Appendix iv **Recommendation**

KENYATTA UNIVERSITY
SCHOOL OF HEALTH SCIENCES
Department of Medical Laboratory Science

New Arts Complex Rooms 2005 & 2006 **P.O. Box 43844,00100 GPO Nairobi, Kenya**

Tel. 020-8710901-19 Ext. 3630 **e-mail: chairman-medical@ku.ac.ke**

January 20, 2012

The Clinical Officer In-Charge
Ngoliba Health Centre
P.O Box 227
THIKA

Dear Sir/Madam,

RE: MWANGI ZAKAYO MAINGI –P57/20957/2010

This is to confirm that the above named is a Master of Science student at Kenyatta University pursuing M.Sc. (Infectious Diseases) programme. His research project entitled "*Prevalence of Cytomegalovirus in Pregnant Women attending Thika Level 5 District Hospital, Kiambu County*".

Any assistance accorded to him will be highly appreciated.

Yours faithfully,

KENYATTA UNIVERSITY DEPARTMENT OF MEDICAL 20 JAN 2012 RATORY SCIENCES 344-00100, NAIROBI

Mrs. Scholastica Mathenge
Ag. CHAIRMAN, DEPARTMENT OF MEDICAL LABORATORY SCIENCE