SEROPREVALENCE OF CYTOMEGALOVIRUS INFECTION AND ASSOCIATED RISK FACTORS AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED PATIENTS ATTENDING THIKA LEVEL 5 HOSPITAL, KENYA

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A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN INFECTIOUS DISEASE IN THE SCHOOL OF MEDICINE OF KENYATTA UNIVERSITY

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DECLARATION

This thesis is my original work and has never been presented for any degree or any other award in any other University.

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Dedication

I dedicate this work to my dear father, Vincent Mangare and my late mum, Lucia Motongori who have been crucial pillars throughout my life.

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I would like to express my deepest Gratitude to my supervisors, Dr. Margaret Muturi and Dr. George Gachara who throughout the entire process of the thesis writting have dedicated their time to encourage and guide me.

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ABBREVIATION AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
AU	Arbitrary Unit
CMV	Cytomegalovirus
CNS	Central Nervous System
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EODS	End Organ Diseases
GCV	Ganciclovir
GI	Gastrointestinal
HAART	Highly Active Antiretroviral Therapy
HCMV	Human Cytomegalovirus
HIV	Human Immunodeficiency Virus
KNH	Kenyatta National Hospital
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
T	Triangulation Number

USAID	United Agency States for International Development

Abstract

Cytomegalovirus (CMV) is an important pathogen in immunocompromised individuals. In Human Immunodeficiency Virus (HIV) patients, it causes end organ diseases leading to increased morbidity and mortality in the population due to down-regulation of the immune system of the affected individuals. The prevalence of Cytomegalovirus infection is high in the general population. Its prevalence in Kenya has been found to be above 93% (CMV-IgG) in HIV infected children. Despite, a high Cytomegalovirus seroprevalence found in children few studies have documented CMV among adults. This study was done to determine the seroprevalence of CMV infection and its associated risk factors among HIV patients attending Thika level 5 Hospital in Kiambu County, Kenya. The study also evaluated the effect CMV infection on the immunity of HIV infected patients. A cross-sectional study involving 163 HIV positive participants from different age groups were enrolled. Blood samples were collected; ELISA was used to confirm the HIV status of the participants. The CD4+ cell counts were determined immediately after blood collection using BD FACSCount and CMV IgG and IgM specific antibodies were analyzed by ELISA. Demographic and behavioural risk factors were collected by the use of a structured questionnaire. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20. Chi-square test was used to assess the statistical significance of different demographic and behavioural risk factors to CMV serostatus. The seroprevalence was found to be 89% (CMV IgG) while the incidence was 10.4% (CMV IgM). The study found that CMV infection leads to more suppression of the immunity among the HIV infected patients. The study also found out that education, economic status, other sexual transmitted infections, sharing drinks, immune status and blood transfusion were associated with CMV infection ($p<0.05$). Adoption of CMV screening services and education on CMV risk factors are recommended as CMV infection preventive strategies.

1.0 INTRODUCTION

1.1 Background Information

Cytomegalovirus (CMV) is a ubiquitous virus commonly infecting people of all age groups, gender and race. Worldwide, CMV burden is high with exposure rates between 40-100 % (Mocarski *et al*., 2007). In immunocompetent individuals, CMV exists in a symbiotic equilibrium and thus disease manifestations are rarely encountered (Griffiths & Emery, 1997). However, in immunocompromised persons such as patients with Human Immunodeficiency Virus (HIV), neonates or through iatrogenic means following organ transplantation, CMV exerts its full pathogenic potential (Mocarski, 1993). Human Immunodeficiency Virus infects CD4 T-Helper cells that regulate humoral and cellular immunity resulting to a weakened immune system (Cheesbrough, 2005). After primary infection, CMV disperses and become latent in multiple organs and due to immunosuppression for example in HIV patients, CMV reactivation occurs (Hummel & Abecassis, 2002). Human cytomegalovirus has become a major public health problem throughout the world since its discovery. In this study, the prevalence of CMV infection was conducted to assess the magnitude of the problem among the HIV infected patients.

Kenya has the fourth largest HIV epidemic in the world. By 2015 an estimate of 1.8 million Kenyans were living with HIV (WHO, 2016). In 2003, only 6000 people living with HIV were accessing antiretroviral (ARV) therapy but by 2015 the number increased to 900,000. In the absence of highly active antiretroviral therapy (HAART), patients with CD4+ cell counts below 100 cells/µl are at a high risk of CMV associated retinitis, gastrointestinal and neurologic disease (Salmon-Céron *et al*., 2000). In the pre-HAART era CMV end organ diseases among patient with advanced HIV infection were above 40% (Wohl *et al*., 2009).

With the advent of HAART the incidence of CMV end organ diseases (EODs) has reduced by 5 to 10% (Wohl *et al*., 2009).

Currently, information on the prevalence of CMV and associated risk factors are scanty. There are two published studies on CMV infection among HIV patients in Kenya. In a study conducted at Nyumbani Children's Home, Nairobi by Chakraborty *et al* (2003), viral co-infections among children infected with HIV-1 was evaluated. There was 100% CMV seroprevalence found in the 71 children of median age 2.2 years. Another study conducted at Kenyatta National Hospital by Slyker *et al* (2009) aimed at studying the kinetics of CMV replication in HIV infants. At the third month of life CMV DNA was detected in 93% of infants who were HIV infected at birth and 90% in HIV exposed infants (born by HIV infected mother) who were uninfected. These two studies did not evaluate the risk factors associated with CMV acquisition among HIV patients. They also did not factor in the reactivation of CMV among HIV patients and also failed to consider the exposure rates in different age groups. Recently Maingi and Nyamache, (2014) reported a high seroprevalence of CMV among pregnant women attending Thika level 5 Hospital. This current study was conducted to examine different exposures and behaviour associated with CMV infection among HIV patients from different age groups. The study also evaluated the exposure rates; past, present and reactivation and the effect of CMV infection on the immunity of HIV infected patients.

1.2 Statement of the problem

Cytomegalovirus infection leads to heightened morbidity and mortality in immunocompromised persons. With the advent of HAART the mean $CD4^+$ cell count of the population with HIV has generally increased thus reducing the proportion of patients at risk for reactivation of CMV disease. However, despite availability of HAART today, many HIV

patients cannot access treatment and, this will predispose them to opportunistic infections such as CMV. Human Immunodeficiency Virus coupled with CMV infection down-regulates the immune system of the patients leading to more lethal conditions such as EODs and an increase in the mortality. With global HAART treatment coverage at 46 percent at the end of 2015, the treatment target has not yet been met on a global scale. Globally, antiretroviral medication has led to a decline of AIDS-related deaths by 26% between 2010 and 2015. Currently, there is inadequate data on prevalence, incidence and risk factors for CMV infection in HIV patients in Kenya. Two studies on CMV infection among HIV patients have been published in Kenya both of which mainly focused on children. The effect of Cytomegalovirus infection on the immunity of HIV infected patients across different ages has not been evaluated. This study therefore focused on CMV/HIV co-infection across different age groups and established the effect of the co-infection on the immunity of the participants while evaluating different risk factors associated with CMV acquisition among HIV patients.

1.3 Justification of the study

Cytomegalovirus still remains a great concern in patients when CD4+ T-cell counts decline below 100 cells/µl (Gilbert & Boivin, 2005). Information on the prevalence of CMV and associated risk factors among the HIV infected patients in Kenya currently remains scanty. Only two studies have documented CMV infection in HIV patients mainly in children. Therefore there was a need to find conclusive data on the burden and risk factors associated with CMV infection among different HIV infected age groups. CMV has been linked to various end organ diseases in immunocompromised patients. For this reason it was important to examine the prevalence and incidence of CMV among various age groups within diverse socioeconomic conditions. Understanding the burden and the effect of CMV/HIV co-infection on the immunity will offer important information to the health care providers that

will aid in timely initiation of treatment and management of CMV in preventing end organ diseases.

1.4 Research Questions

1. What is the prevalence and incidence of CMV infection among HIV patients across different age groups?

2. What is the effect of CMV infection on the immunity of HIV infected patients?

3. What are the risk factors associated with CMV infection among HIV infected patients of different age groups?

1.5 Objectives

1.5.1 General objective

To determine the seroprevalence of Cytomegalovirus infection among Human Immunodeficiency virus infected patients attending Thika level 5 Hospital and the associated risk factors.

1.5.2 Specific objectives

1. To determine the prevalence and incidence of CMV among HIV patients.

2. To determine the effect of CMV infection on the immunity of HIV infected patients.

3. To identify risk factors associated with CMV infection among HIV infected patients.

1.5.3 Significance of the study

Understanding the seroprevalence of cytomegalovirus and its associated risk factors among HIV infected individuals is important in devising interventions that will allow them to live near normal lives. The current study provides beneficial information not only on the burden of CMV in this population but also identifies risk factors associated with CMV acquisition

2.0 LITERATURE REVIEW

2.1 Taxonomy of Cytomegalovirus

According to Kenneth and Ray, (2004), Cytomegalovirus is also known as Human Herpesvirus 5 (HHV-5). It is commonly referred to as human cytomegalovirus (HCMV) and more commonly abbreviated as CMV. Taxonomically, the International Committee for the Taxonomy of the Viruses (ICTV) classifies CMV in the order *Herpesvirales,* Family *Herpesviridae,* Subfamily *Betaherpesvirinae,* Genus *Cytomegalovirus* and Species *Human Herpesvirus 5*.

2.2 Cytomegalovirus virion

Viruses of the *Herpesviridae* family possess a double stranded DNA viral genome enclosed in a capsid surrounded by a protein rich tegument within a viral envelope (Fig.1). Cytomegalovirus has the prototypical Herpesvirus virion structure. The capsid is icosahedral shaped with a 235-kb double-stranded DNA genome. The capsid is surrounded by a proteinaceous tegument and an outer lipid envelope (Mocarski *et al*., 2007). Cytomegalovirus appears to contain the largest genome of any virus infecting human. Multiple strains of the virus circulate within the population and within an individual, and evidence exists for interstrain recombination hence contributing to the virus diversity within the population (Chou, 1992).

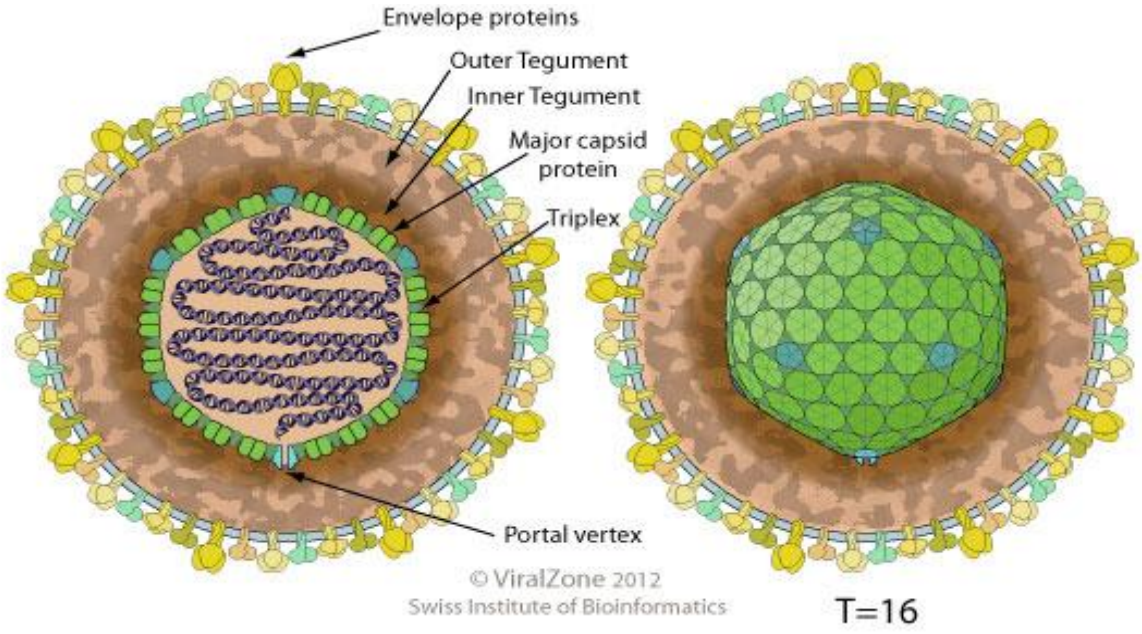

Figure 1: Structure of a Herpesvirus virion

(Source; http://viralzone.expasy.org/all_by_species/180.html; Accessed on 15.04.2016)

2.3 Cytomegalovirus Replication/Life Cycle

During active infection in vivo, endothelial cells, neutrophils and lymphocytes are the predominant cells infected. The major reservoir of CMV in latent infection is CD14 positive monocyte and prolonged allogeneic of this cell stimulation leads to reactivation (Söderberg-Nauclér *et al*., 1997). Cytomegalovirus virion enters the host cell through direct fusion or through the endocytic pathway (Crough & Khanna, 2009). The virion attaches to the cell through viral glycoprotein interaction with the specific cell surface receptor. The initial interaction leads to subsequent disassembly and release of the viral genomic DNA and tegument into the host cell. Several teguments protein mediate the delivery and release of viral DNA to the nucleus (Kalejta, 2008). Usually, CMV uses different receptor binding proteins to mediate entry into the different cell types. Normally, HCMV strains express a complex of five proteins, gH, gL, UL128, UL130, and UL131 (gH/gL/UL128-131), which are necessary for entry into epithelial, endothelial cells, leukocytes, and monocytes (Hahn *et*

al., 2004). Cytomegalovirus also expresses another glycoprotein, gO, that forms distinct complexes with gH/gL that are necessary for HCMV entry into human fibroblasts (Huber & Compton, 1998). The target cells has the Epidermal Growth Factor Receptor (EGFR) which is the site for CMV adsorption, signalling and entry with the CMV envelope glycoprotein gB being the ligand for EGFR (Chern *et al*., 1998). Binding leads to conformational changes and activation of membrane fusion and virus entry.

Upon entry of the viral genome in the nucleus, immediate early genes expression occurs through their activation by the viral pp71 tegument protein, which initiates the lytic stage of the viral life cycle and the subsequent replication of the 235-kb double-stranded CMV DNA genome (Cantrell & Bresnahan, 2006). Usually, there are three kinetic phases of gene expression: immediate early (IE), early (E), and late (L) genes (Mocarski, 1993). Immediately after viral infection, the CMV IE genes code for IE-1 and IE-2 proteins. E gene encodes for enzymes required for viral DNA synthesis but its expression is dependent on IE expression. Viral late genes are also dependant on IE for expression and assists in the assembly and egress of newly formed infectious CMV virions which are then released via exocytosis at the plasma membrane (Crough & Khanna, 2009); (Figure 2).

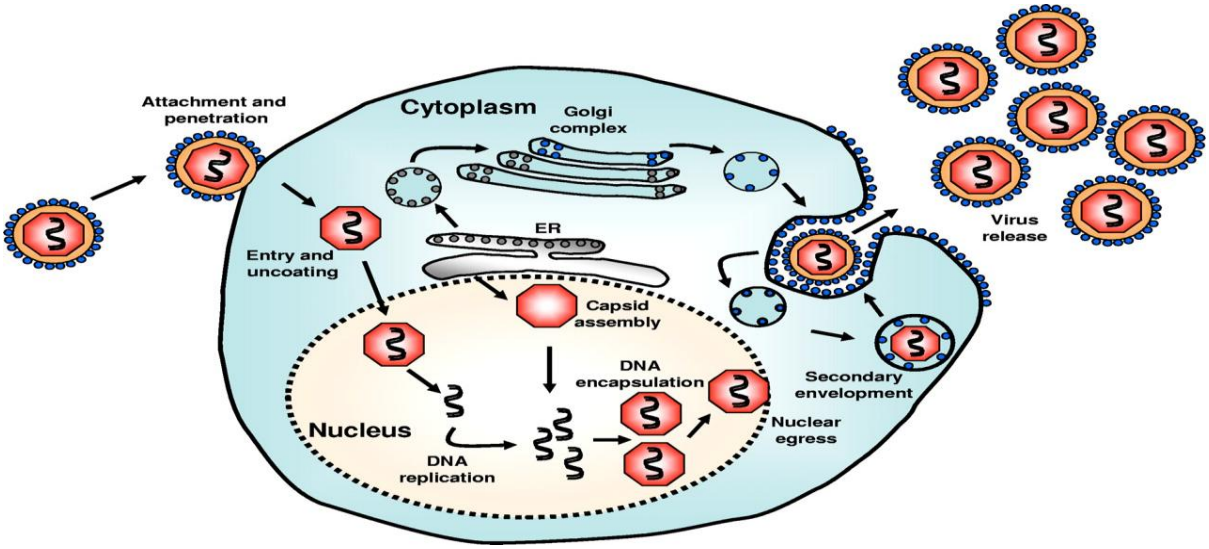

Figure 2: Cytomegalovirus Replication/ life cycle. Source; (T Crough & Khanna, 2009)

2.4 Latency and reactivation of Cytomegalovirus

After primary infection, CMV may persist in latent state in hematopoietic compartment and myeloid progenitor cells (Mendelson *et al*., 1996). Restricted viral gene expression is observed in these latently infected cells (Crough & Khanna, 2009). The infectious virus cannot be detected during latency and viral gene expression is significantly modified (Reeves *et al*., 2005). There are three possible pathways of latency. The virus can enter directly into latency without *de novo* gene expression, initiate a productive infection but prematurely interrupted or by expression of viral genes not associated with productive infection (Goodrum *et al*., 2002).

During CMV pathogenesis, reactivation from latency is key, this can be as a result of immunosuppression, inflammation, infection, or stress (Kutza *et al*., 1998). The exact mechanism of reactivation is unknown, although tumour necrosis factor alpha (TNF-α) is considered to be a key mediator (Fietze *et al*., 1994).

2.5 Human Cytomegalovirus epidemiology

Cytomegalovirus is universally distributed. Majority of the world's population of about 40-100% have been exposed to the virus (Mocarski *et al*., 2007). South America, Asia and Africa record the highest prevalence while Western Europe and United States have the lowest prevalence. This means CMV is more common in developing countries and in areas of low socioeconomic conditions (Drew, 1988). In the United States, at least 60% of the population has been exposed to CMV (Zhang *et al*., 1995). The prevalence is above 90% in high risk groups (Staras *et al*., 2006). According to the analysis of serum samples from the National Health and Nutrition Examination Survey (NHANES) 1999-2004, the CMV seroprevalence in United states was found to be 50.4% (Bate *et al*., 2010).

In a study carried out in Iran, CMV seroprevalence among HIV patients was high. A prevalence of 94% (anti-CMV IgG) was noted among 201 HIV patients aged 3-62 years. The study noted a maximum prevalence of CMV antibody in patients with unsafe sex and injection drug users. However, anti- CMV IgM was negative in all the cases. The prevalence was observed to increase with age. A maximum prevalence of 100% was observed in patients aged 50-60 years and a minimum prevalence of 50% in patients aged 0-10 years (Mehrkhani *et al*., 2011). Another Asian country, India reported a prevalence of 87.9% CMV IgG and 1.6% CMV IgM seropositivity among 431 volunteer blood donors. The subjects were in the age range of 18-59 years. Age was not statistically related to CMV infection (Chaudhari & Bindra, 2009).

Similar findings have been reported in African studies including studies in Mali, West Africa, showed a seroprevalence of 89% among AIDS patients, 71% among HIV-infected blood donors and 58% among HIV-uninfected blood donors. The study revealed that there was no statistical relation between age or sex and CMV infection (Izetiégouma Maïga *et al*., 2003).

In a recent study of 140 subjects at Maiduguri teaching hospital, Nigeria on the seroprevalence and risk factors for CMV in HIV patients, an anti-CMV IgG seroprevalence of 100% was observed in 100 HIV patients. Among the 40 control subjects, anti-CMV IgG seroprevalence was 98.6%. The study noted that a number of risk factors such as multiple sexual partners, traditional practices such as tattooing and cupping, and blood transfusion significantly contributed to the acquisition of CMV among the subjects (Kida *et al*,. 2014).

A study in Khartoum, Sudan, carried among 200 pregnant women attending Omdurman Maternity Hospital recorded 97.5% and 6.0%, CMV IgG and CMV IgM positivity respectively. Age was associated with CMV IgM and history of miscarriage was significantly associated with CMV IgG positivity in women, while parity, congenital abnormalities,

educational level, and occupation were not significantly associated with CMV infection (Length, 2013).

Although, most developing countries like Mali, Nigeria, Kenya and Sudan has reported a high CMV prevalence, the findings are contrary to the studies in the West which has reported a lower CMV prevalence of up to less than 50%. In a two year study on Cytomegalovirus infection in a French Hospital among the pregnant women, a low seroprevalence of 46.8% CMV IgG was reported. Primary infection was detected in nine women between 0 and 12 weeks of gestation (0.46%) and seroconversion was diagnosed in five women between 12 and 36 weeks of gestation (0.26%) (Picone *et al*., 2009).

In Kenya several studies on the prevalence of CMV infection in the population have been published. In 2003, a study was conducted on viral co-infections among African children infected with HIV-1. Seventy one (71) Children of median age of 2.2 years from Nyumbani Children's Home, Nairobi Kenya were recruited in the study. All children (100%) were found to be IgG seropositive by ELISA. Cytomegalovirus viruria was detected in 15% of the children by a DNA hybridization assay (Chakraborty *et al*., 2003). In 2009 a study was conducted at the National Blood Transfusion Centre, Nairobi on the seroprevalence of CMV among 395 blood donors. Cytomegalovirus seroprevalence was found to be 97% (anti-CMV IgG) while anti-CMV IgM was 3.6%. It was noted that there were no significant differences in the prevalence with respect to education level, socioeconomic groups, marital status and sexuality. However, there was a higher prevalence in females than males of 99.4 % compared to 95.2% respectively (Njeru *et al*., 2009).

In 2009, another study was conducted at Kenyatta National Hospital on acute cytomegalovirus in Kenyan HIV infected infants. Human Immunodeficiency Virus infected infants (44) were studied over a period of two years and HIV uninfected exposed infants (20) were studied for a year. It was found that by 3 months of age, Cytomegalovirus DNA was detected in 90% of HIV-exposed uninfected infants and in 93% of infants who were HIV infected at birth. At delivery all the women were 100% CMV seropositive but had zero (0%) Cytomegalovirus DNA (Slyker *et al*., 2009). In 2014, Maingi and Nyamache reported on the seroprevalence of CMV among pregnant women in Thika level 5, Kiambu County. Out of 260 patients with a mean age of 28 years, 27 were HIV positive. CMV seroprevalence among pregnant women was found to be 85.2% (IgG). The general prevalence in the study was however 77.3% (IgG) and 8% (IgM). It was noted that marital status, high parity, history of blood transfusion and age were significant risk factors for CMV infection (Maingi & Nyamache, 2014).

2.6 Mode of Cytomegalovirus transmission

Herpesviruses can be transmitted through direct contact with infected blood, tissues, bodily fluids (saliva, urine, and breast milk), faeces, and fomites (Stagno, 2001). Adolescents and adults can be infected with CMV through sexual contact and nonsexual contact (Handsfield *et al*.,1985) including;- close contact with infected individuals, especially children (Pass *et al*., 1987). Cytomegalovirus can also be transmitted through organ transplant; Cytomegalovirus negative transplant recipients can develop primary infection while those with past exposure can lead to recurrence (Ruell *et al*., 2007). Congenital transmission of CMV is very lethal and can cause miscarriage or birth deformities of the baby. Usually transmission from the mother to the fetus is through the placenta and can occur possibly anytime during pregnancy but more commonly in the first six months of pregnancy

(Manicklal *et al.*, 2013). Cytomegalovirus is rarely contagious through casual contact but can be spread through households and among children in the day care

2.7 Risk Factors of Cytomegalovirus infection

Several exposure and behavioural risk factors associated with cytomegalovirus infection has been documented. Saliva sharing behaviour, exposure to children at home, group living habits, circumcision, blood transfusion and sexual transmitted infections are among the risk factors that have been closely associated with CMV acquisition. Sexual activities have been reported to be associated with CMV infection, especially increased number of sexual partners (Sohn *et al*., 1991) and heterosexual contact (Handsfield *et al*., 1985). Stadler *et al*., (2010) in his previous study found that there was an association between CMV infection and exposure to children at home, although he further noted that there was no relationship of CMV infection to saliva sharing behaviour (kissing, sharing toothbrush and sharing drink) and group living habits. In another finding it was reported that there was no correlation of CMV infection and circumcision (Kida *et al*., 2014).

2.8 Pathogenesis of Cytomegalovirus in HIV patients

Cytomegalovirus can infect a broad range of cells within a host including epithelial cells, fibroblasts, smooth muscle cells, endothelial cells and macrophages during acute infection (Ng-bautista & Sedmak, 1995). After primary infection, CMV will disseminated to various cells and become latent. Latent infection is characterized only in cells of the early myeloid lineage, including CD34+ hematopoietic progenitor cells and CD14+ monocytes (Smith *et al*., 2010). There are several viral genes that have been mapped that prevent viral clearance due to interference with the immune system (Miller-Kittrell & Sparer, 2009). The level of damage may vary widely depending on the cell type or host but is directly correlated with viral load (Boppana *et al*., 2001). Cytomegalovirus infection in HIV patients can lead to

CMV end organ disease and death as a consequence of the impaired immunity (Drew, 1992). The frequency of clinical manifestations of CMV infection largely depends on the $CD4^+$ T-cell count. Usually CMV end-organ disease is observed when $CD4^+$ T-cell count drops below 50 cells/mm^3 (Gallant *et al*., 1994). Cytomegalovirus retinitis is the most frequent CMV end organ disease in HIV patients, representing about 80–90% of all CMV end-organ manifestations (Masur *et al*., 1996). Cytomegalovirus retinitis is a late manifestation of AIDS and occurs after reactivation of latent CMV infection (Goldberg *et al*., 2005). Cytomegalovirus retinitis presents with decreased visual acuity, visual field loss, floating black spots, pain or redness in the eyes and sensitivity to light and excessive tearing. Visual loss occurs due to inflammatory edema in the retina. If diagnosed early and treated appropriately these edemas are potentially reversible (Freeman *et al*., 1987).

Cytomegalovirus infection of the gastrointestinal (GI) tract is the second most common CMV end-organ manifestation. GI manifestation in HIV patients can be gastritis and gastroduodenal ulcers (Varsky *et al*., 1998). Cytomegalovirus infection of the stomach results into superficial ulceration and inflammation of the stomach wall. Cytomegalovirus infection has been identified as a possible causative agent of infectious cholecystitis, cholangitis and pancreatitis in HIV-infected patients (Teixidor *et al*., 1987).

Cytomegalovirus is associated with neurological diseases in immunocompetent persons. Central nervous system (CNS) involvement in CMV end organ disease is rare in HIV patients (McCutchan, 1995). Neurological signs of CMV-related CNS disease are not specific for CMV as a pathogen and include drowsiness, confusion, fever, disorientation, impaired short-term memory or dementia, apathy, nystagmus and focal signs such as cranial nerve palsies as signs of ventriculoencephalitis (Salazar *et al*., 1995). When CMV causes pulmonary disease in patients with AIDS, the syndrome is that of an interstitial pneumonia. CMV causes severe pulmonary diseases in HIV infected patients (Miles *et al*., 1990). Patients normally complain

of shortness of breath, dyspenia on exertion and a dry non-productive cough with elevated respiratory and heart rates.

2.9.0 Laboratory Diagnosis of Cytomegalovirus

There are several methods used for the diagnosis of cytomegalovirus;

2.9.1 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction is a highly sensitive and rapid technique. The PCR is based on the principle of selective amplification of specific nucleic acid sequences. Polymerase Chain Reaction can be used to detect Cytomegalovirus DNA in the body fluids such as whole blood, plasma, cerebrospinal fluid, or bronchoalveolar lavage fluid (Wolf & Spector, 1992). The PCR technique is the most versatile since it can be used either qualitatively or quantitatively to measure the viral load. The main disadvantage is it's high sensitivity thus can detect very low levels of CMV that are not usually predictive of the disease (Zipeto *et al*., 1992).

2.9.2 Antigen Testing

Antigen assays are quick, specific and sensitive. They entail detection of the CMV pp65 antigen in the leucocytes (Ljungman *et al*., 2002). The pp65 assay uses either immunofluorescence assay or messenger RNA amplification to detect messenger matrix protein on the CMV virus usually expressed only during CMV replication. Usually low or moderate CMV in immunocompromised patient such HIV is indicative of reactivation or reinfection (Cunha, 2010). Cytomegalovirus antigenemia can be performed by the procedure described by (Van der Bij *et al*., 1988).

2.9.3 Shell vial culture

This is a modified technique of conventional cell culture technique for rapid viral detection in vitro (Forbes *et al*., 1998). The clinical specimen is inoculated on a permissive cell line for CMV on the cover slip in a shell vial culture tube then centrifuged at low speed followed by incubation. Usually after 24 and 48 hours, the tissue culture medium is removed and the cells are stained using a fluorescein-labelled anti-CMV antibody then read by fluorescent microscope. When using Human Diploid Fibroblast (MRC-5) cells for the rapid identification of CMV in human urine specimen, shell vial culture has proved very effective (Gleaves *et al*., 1985).

2.9.4 Histology/cytology

This technique is used to find out the intranuclear and intracytoplasmic inclusions consistent to herpesvirus in the specimen. Cells with these characteristics could be found in saliva, cervical and tracheal secretions or biopsy and necropsy tissues. The inclusions can be observed with papanicolaou or haematoxylin-eosin stains. However they are best seen with Wright-Giemsa Stains (Shulman *et al*., 1982). However, this technique is not sensitive enough and suffers from high level of false positives.

2.9.5 Antibody testing

This is used to determine whether or not one had a recent or past exposure to cytomegalovirus. Cytomegalovirus specific IgG and IgM antibodies are produced during CMV infection. Immunoglobin M antibodies are produced first after primary infection within 1- 2 weeks, increases for a short period then slowly decline and eventually fall below detectable levels. However during re exposure or reinfection IgM rises again. Immunoglobin G is then produced several weeks after exposure, increases then stabilises as CMV infection

resolves or become dormant. Enzyme-linked Immunosorbent assay technique is available for detection of CMV antibodies. Enzyme-linked Immunosorbent assay is accurate, speedy and efficient (Booth *et al*., 1982).

2.9.6 Cytomegalovirus IgG avidity test

Avidity is the strength with which IgG binds to antigenic epitopes (Hazell, 2007). The avidity slowly matures for several months after primary infection (Lazzarotto *et al*., 2008). Usually the IgG antibodies that are produced after primary infection bind weakly while those produced after (about six months and above post-infection) are of high avidity. The test is carried on the principle that Antigen-bound low-avidity IgG, but not high-avidity IgG, dissociates from the antigen in the presence of mild protein denaturants, such as urea, potassium thiocyanate, and guanidine chloride. Enzyme-linked immunosorbent assay is commonly used that uses urea as a dissociating agent (Chakravarti *et al*., 2007). Diluted sample is added to two microtiter wells coated with CMV antigen, incubation is done then washing of the wells, one with wash buffer and the other buffer with urea. The optical density (OD) values are read and the result calculated to obtain avidity index (AI).

$$AI = 100 \times \frac{\text{OD of urea washed well}}{\text{OD of well washed with normal buffer}}$$

The Avidity index is defined as low (AI value of ≤50%) indicating primary infection and high avidity as an AI value of ≥60% indicating past exposure; AI values of 51% to 59% are considered intermediate avidity (Prince *et al*., 2014).

2.10 Analysis of CD4 by Flow Cytometry

Flow cytometry measures optical and fluorescence characteristics of single cells. Immunofluroscence analysis by flow cytometry is the gold standard for CD4 T lymphocytes measurements and also the method of choice if a large throughput of samples is required. Flow cytometry work on the principle of scattering of light due to different sizes, granularity of the cells passing thorough the laser beam, and also by the fluorescence emitted by the cells after staining with the specific monoclonal antibodies to cell surface markers that are tagged with different fluorescence dyes. The monoclonal antibodies specifically bind different surface receptors like CD4 for T helper cells. Relative percentages of the cells expressing the specific receptor like CD4 on its surface are obtained from the flow cytometer and the absolute counts obtained (WHO, 2007).

2.11 Cytomegalovirus prevention

The primary mechanism of transmission of Cytomegalovirus among adolescents is sexual and non-sexual contact. Education on various method of acquisition is most important since it will help reduce transmission (Lamberson *et al.,* 1988). Secretion precautions and careful hand washing should be used in the clinical setting to prevent spread of CMV from patients to staff or other patients (Sever, 2002). Transmission through blood transfusion can as well be prevented by restricting transfusion of blood and blood products that has been confirmed CMV seronegative only (Lamberson *et al.,* 1988). All children should also be considered as source of CMV and therefore should be monitored keenly to prevent acquisition or transmission (Okwori *et al*., 2009)

2.12 Cytomegalovirus treatment

Ganciclovir was the first anti-CMV drug to be approved. Ganciclovir (GCV) acts as an inhibitor of the CMV DNA polymerase. In 2001 Valganciclovir an oral form of Ganciclovir became available (Brown *et al*., 1999). Foscarnet was the second drug to be approved in 1991 for treatment of CMV retinitis in AIDS patients and it is the preferred drug for patients not responding or failing Valganciclovir therapy due to viral resistance, or those who cannot be treated with GCV due to dose-limiting neutropenia or leucopenia. Other anti-CMV drugs available today include Cidofovir available as an intravenous formulation and Fomivirsen administered by intraocular injection in a four week induction phase.

3.0 MATERIALS AND METHODS

3.1 Study Site

This study was carried out at Thika level 5 Hospital at the comprehensive care clinic (CCC). The Hospital is located in Kiambu County and serves as a referral hospital for dispensaries and Health centres in Kiambu County, Kenya.

3.2 Research Design

The study was a cross sectional study.

3.3 Sampling technique

Systematic random sampling technique was used to sample the study participants. All the patients that met the inclusion criteria and consented to participate in the study had equal probability to be selected. Out of the total estimate of seven hundred patients attending the general clinic on Tuesdays and Thursdays for the three months of data collection, every fourth patient was sampled to obtain the required sample size.

3.4 Participant sensitization

The participants were mobilized for participation through Thika level 5 Hospital comprehensive care clinic (CCC). Through the help of the head of the CCC and other staff at the clinic, participants were sensitized on the ongoing research. Full explanation concerning the study was provided. Participation was on a free and volunteer basis.

3.5 Recruitment of participants

All the patients who met the criteria received full information on the study and were requested to consent by signing a consent form. They had an option to participate, refuse or withdraw from the study at any stage. Patients were assured of confidentiality of the results and that all the data was coded to conceal their identity. Adult patients filled and signed a consent form, while patients aged below 18 years or those mentally challenged; full consent was obtained from their parents or guardians and then signing the consent form. Patients who could not read and write were well explained to in the best language they could understand and consent was sort from them. Once the patients consented to participate in the study, he/she was required to fill in a structured questionnaire on his/her own or by the help of the researcher to determine socio-demographic and risk factors that were associated with CMV infection. After which blood sample was taken by a phlebotomist.

3.6 Eligibility criteria

Before being recruited potential participants were required to meet the following criteria.

Inclusion criteria

1. Agreed to participate in the study by signing a consent form.
2. Willing to provide blood sample for the study.
3. Agreed to be re-tested for HIV and confirmed HIV positive

Exclusion criteria

1. Declined to sign a consent form.
2. Unwillingness to provide blood sample.

3.7 Care and protection of research participants

Care and protection of patients from any harm were observed during the research. During the research all aseptic precautions were observed. All the sharp objects like needles and any disposables used were disposed in the relevant safe boxes and taken for incineration with other hospital wastes as per the Hospital waste disposal protocols. To avoid contamination, a fresh pair of glove was used for each patient and disposed after use. All the samples were de-identified to conceal patient identity. Codes and numbers were used for the purpose of data entry rather than names. No information regarding the individual patients was communicated to any third party. To avoid stigma there was no segregation/isolation of any participants. No special treatment was accorded to any of the participants.

3.8 Ethical clearance

Before commencement of the Research, ethical clearance was granted by the Kenyatta University Ethics Review Committee (KUERC).

3.9 Sample size determination

The sample size was be calculated using the formula by Daniel, 1999

$SS= Z^2P (1-P)/ d^2$

Where:

SS = Desired Sample size

Z = Z statistic for a level of confidence (e.g. 1.96 for 95% confidence level)

P =Current prevalence of Cytomegalovirus in immunocompromised patients (93% determined by Slyker *et al*, (2009).

d = Confidence interval expressed as decimal (the desired level of precision ±5%, (0.05)

$N=1.96^2 X 0.93 (0.07)/0.05^2=100$ samples

For this study **163** samples were collected and analysed

3.10 Sample collection

Four (4) mls of blood was collected through venipuncture technique from each participant into two tubes, each two (2) mls; BD Vacuitainer Serum tubes and an EDTA vacuitainer tube by a phlebotomist. The tubes were labelled using codes rather than patient name to conceal the identity of the patient and taken to the laboratory. CD4+ count was analysed immediately at the hospital. The other sample was centrifuged at 3000 revolution per minute for five minutes and serum obtained. Serum was separated and put into cryovials and stored at -20^0C awaiting laboratory analysis. After analysis serum was decontaminated, disposed into a biohazard safety bin and incinerated.

3.11 Sample Transport

Serum samples for CMV antibodies and HIV confirmation were transported to Nairobi Womens Hospital for laboratory analysis since Thika level 5 Hospital did not have an ELISA Machine. The cryovials were put in a plastic leak proof container and properly labelled. The container was then put in a bag with adequate cotton wool to absorb liquid if any leakage occurred accidentally. The package was put in a thermocool box containing ice packs to maintain proper cold chain system during transit. The box was sealed securely and labelled ‘‘biohazard material’’ and transported.

3.12 Laboratory analysis

Laboratory analysis of sample was done to determine the CMV antibodies, HIV antibodies and CD4 lymphocyte count among the participants.

3.12.1 CD4 Count by BD FACSCount

Sample analysis for CD4 was done at Thika level 5 Hospital laboratory immediately after collection. BD FACSCount™ was used to enumerate the absolute counts of CD4 T lymphocytes and determine the percentage of lymphocytes that are CD4 T lymphocytes in unlysed whole blood (CD4 counts and CD4 percentages). The reagent tubes were labelled patients identification unique codes as indicated in the EDTA vacuitainer. Fifty (50) µL of whole blood was pipetted into each of the reagent tube labelled with the corresponding patients' code. Fluorochrome-labelled antibodies in the reagents bind specifically to white blood cell surface antigens and a fluorescent nuclear dye binds to the nucleated blood cells. The tube was capped and vortexed upright for 6 seconds. The tubes were incubated for 30 minutes at 25°C. Each sample tube was uncapped and 50 µL of fixative solution (5% formaldehyde in phosphate-buffered saline (PBS) pipetted into each tube. The sample tubes were recapped and vortexed for 6 seconds and then run on BD FACSCount instrument within 48 hours of adding fixative. During sample acquisition, the cells passes through the laser light, which causes the labelled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to identify and count the lymphocytes and CD4 T lymphocytes. In addition, the reagent tubes contain a known number of fluorescent reference beads to which a precise volume of whole blood is added. The software automatically identifies the lymphocyte populations of interest and calculates the CD4 counts (cells/µL) by comparing cellular events to bead events. Results presented include CD4 counts and CD4 percentages.

3.12.2 Testing for CMV antibodies

Samples were analysed at Nairobi Womens Hospital Laboratory. The test method used was ELISA. To assay for CMV antibodies, anti-CMV IgM Human ELISA, and anti-CMV IgG Human ELISA kits (HUMAN Diagnostics Worldwide, Germany) were used. Diluted serum

was prepared in a ratio of 1:100 (1 part of sample into 100 parts of diluent). A 100 µl aliquot of the diluted sample was added to the microwells precoated with purified CMV antigens and incubated for 30 minutes at 25°C. It was then washed four times. Then 100 µl horseradish peroxide (HRP) labelled anti-Human IgM and IgG conjugate was added to the respective wells for detection of IgM and IgG antibodies respectively. Incubation was done for 30 minutes at 25°C and CMV specific antibodies, if present bound to the antigen. Washing was done again five times to remove excess HRP-conjugate. This was followed by addition of 100 µl 3, 3′, 5, 5′-Tetramethylbenzidine (TMB)-reagent as a substrate and incubated at 25°C for 15 minutes. TMB-reagent is catalysed by HRP to produce a blue colour. After incubation, 100 µl of stop solution (0.5 M sulphuric acid) was added to terminate the reaction. This was mixed thoroughly and the blue colour changed to yellow on addition of the acidic stop solution. The microwell reader was set to read the absorbance at a wavelength of 450 nm and the OD of each well measured within 30 minutes. The density of yellow colouration was directly proportional to the amount of CMV IgM and IgG sample captured in the respective plates.

To calculate the cut-off value, the mean absorbance of 10 AU/ml standard was measured at 450nm (**Cut-off**= 10 AU/ml standard mean OD). To determine the positive and positive results the sample/Cut-off index was used (**S/Co**=Sample OD/Cut-off value). Based on the index of each sample, result higher than 1.1 was considered as positive whereas results less than 0.9 were considered negative. For Results between 0.9 and 1.1 CMV IgG avidity test was done.

3.12.3 Cytomegalovirus IgG Avidity Testing

The fourty (40) samples that were IgM and IgG positive at ELISA were also tested for IgG avidity. A set of ELISA test with micro titer for CMV IgG specific was assessed. Procedure for ELISA was followed as mentioned above until after initial incubation where the wells were washed with buffer containing 40% urea. The optical density (OD) values were read and the result calculated to obtain avidity index (AI).

$$AI = 100 \times \frac{\text{OD of urea washed well}}{\text{OD of well washed with normal buffer}}$$

The interpretive criteria used was follows: low avidity, AI of ≤0.50; intermediate avidity, AI of 0.51 to 0.59; high avidity, AI of ≥0.60 (Prince *et al*., 2014).

3.12.4 Confirmation for HIV

To determine and confirm the HIV status of the subjects, ELISA was used. The ELISA HIV 1 and 2 antigen kit used (Pishtaz Teb Zaman Diagnostics, Iran). The microwell precoated with HIV I and 2 antigens were set. The first well was set as Blank, and the next two wells as Positive and Negative controls. A 100 µl of the sample diluent was put in each well except the Blank. A 50 µl of positive and negative controls solution was added to the positive and negative control wells respectively. Then 50 µl of serum sample was added to each of the remaining wells. The plates were sealed with a plate sealer tightly and gently mixed for 15 seconds. The wells were incubated for 30 minutes at 37°C. Rinsing was then done 5 times. Then 100 µl of horseradish peroxidase labelled anti-HIV-1 and 2 was added to each well except the blank. The incubation was done for 30 minutes at 37°C followed by washing 5 times. After incubation, 100 µl of substrate was then added to each well and then incubated at room temperature in the dark for 15 minutes. Finally, 100 µl of the stop solution (0.5 M

sulphuric acid) was added to the wells to stop the reaction. The absorbance was read at 450 nm by an ELISA reader. To obtain the cut off value of the test, blanks OD was subtracted from the OD of the controls (positive and negative) and samples and the following formulae was used to calculate the cut off values (**Cut off**=Negative control mean OD (450nm) +0.15). To determine positive and Negative results S/Co index (**S/Co**= Sample OD/Cut-off). Based on the index, results equal or higher than 1.0 was considered as positive while results less than 1.0 was considered Negative.

3.13 Data presentation and analysis

The data collected from laboratory analysis was presented in tables. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20 (IBM, Chicago). Chi-square test was used to test associations between demographic and behavioral risk factors and the CMV serostatus of study participants.

4.0 RESULTS

4.1 Participants demographic variables

The total participants in this study were 163. Females constituted a majority of the study population (53.4%) while male were 46.6%. Out of the total participants 33 were below the age of 12 years while 130 were above 12 years old. The participants had a mean age of 35.5 years. Participants were in the following age groups; below 2 years were 9 (5.5 %), 2-12 years were 24 (14.7%), 13-24 years were 23 (14.1%), 25-34 years were 17 (10.4%), 35-44 years were 19 (11.7%), 45-54 were 36 (22.1%), 55-64 years were 24 (14.7%), 65 years and above were 11 (6.7%). Majority (54.6%) of the participants were married while widowed were the minority (9.2%) with participants who were single and divorced being 26.2% and 10% respectively. The highest (62.3%) number of participants had attained only primary education while 3.1% having no any formal education, 23.8% with secondary education and 10.8% having attained post-secondary education. Most (46.9%) participants were casual workers with only a few (20%) having formal employment and 33.1% being unemployed. Participants who had low income were higher (65.4%) than middle income earners (34.6%) (Table 4.1)

Table 4.1. Demographic factors of the HIV infected participants

Variables	Number of participants (n)	Percentage (%)
Gender		
Male	76	46.6
Female	87	53.4
Total	163	100
Age Group		
Below 2 year	9	5.5
2-12	24	14.7
13-24	23	14.1
25-34	17	10.4
35-44	19	11.7
45-54	36	22.1
55-64	24	14.7
65 and above	11	6.7
Total	163	100
Marital status		
Single	34	26.2
Married	71	54.6
Divorced	13	10
Widowed	12	9.2
Total	130*	100
Education		
None	4	3.1
Primary	81	62.3
Secondary	31	23.8
Post-Secondary	14	10.8
Total	130*	100
Occupation		
Unemployed	43	33.1
Casual	61	46.9
Formal	26	20
Total	130*	100
Economic status		
Low income earner	85	65.4
Middle income earner	45	34.6
Upper income earner	0	0
Total	130*	100

* Total participants excluding children below the age of 12 years

4.2 Demographic variables of subjects in relation to Cytomegalovirus serostatus

In this study, all the participants within the age group of 2 years and below were positive for CMV IgG (seroprevalence 100%) while within the age group 55-64 years, less number of participants were positive for CMV IgG (seroprevalence of 83.3%) as compared to other age groups. However, age was not statistically associated with CMV serostatus (p=0.443). Educational background showed significant statistical relationship with the CMV serostatus of the participants (P=0.000). HIV Patients without any form of education had 100% exposure to CMV (IgG) while those with primary, secondary and post-secondary education had 88.9%, 80.6% and 85.7% seroprevalence respectively. A higher number of HIV participants (19.4%) who had attained secondary education had primary infection/reactivation as compared to those with primary education (11.1%), post-secondary education (7.1%) and those without any form of education (0%).

Marital status showed no statistically significant association with CMV serostatus (P=0.148). Single, married, divorced and widowed had CMV exposure (anti-CMV IgG) of 82.4%, 90.1%, 84.6% and 83.3% respectively. Anti-CMV IgM seroprevalence was as follows; Single (17.6%), married (8.5%), divorced (15.4%) and widowed (16.7%). Occupation was not statistically associated with CMV serostatus (P=0.074). Casual workers constituted the majority of the participants with 46.9% while subject with formal employment being the minority with 20% among the participants. The casual workers had the highest exposure to CMV of 90.2%, followed by the unemployed (86%) and formally employed (80.8%) with new infection/reactivation (anti-CMV IgM) being at 8.2%, 14% and 19.2% respectively among the HIV patients (Table 4.2).

Economic status was statistically associated with CMV serostatus (P=0.042). Among the participants low, middle and upper income earners had anti-CMV IgG seroprevalence of

88.1%, 86.7% and 0% respectively while anti-CMV IgM seroprevalence was 11.9%, 13.3%, 0% respectively. The study did not capture any participants who was an upper income earner. Gender was not statistically significant to the anti-CMV serostatus (P=0.494). Males had an anti-CMV IgG seroprevalence of 88.2% while females had a seroprevalence was 89.7%. Anti-CMV IgM seroprevalance were 10.5% and 10.4% in males and females respectively.

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Table 4.2. The percentage distribution of Cytomegalovirus antibodies (IgG and IgM) and associated risk factors among different HIV infected participants attending Thika level 5 Hospital.

Variables	Participants	CMV IgG/IgM (positive)		CMV IgG/IgM (Negative)	p values
		IgG (n, %)	IgM (n,%)	n (%)	
Gender					.494
Male	76	67 (88.2)	8 (10.5)	1 (1.3)	
Female	87	78 (89.7)	9 (10.4)	0 (0)	
Total	163	145	17	1	
Age Group					.443
Below 2 years	9	9 (100)	0 (0)	0 (0)	
2-12	24	23 (95.8)	1 (4.2)	0 (0)	
13-24	23	19 (82.6)	4 (17.4)	0 (0)	
25-34	17	15 (88.2)	1 (5.9)	1 (5.9)	
35-44	19	18 (94.7)	1 (5.3)	0 (0)	
45-54	36	31 (86.1)	5 (13.9)	0 (0)	
55-64	24	20 (83.3)	4 (16.7)	0 (0)	
65 and above	11	10 (90.9)	1 (0.1)	0 (0)	
Total	163	145	17	1	
Marital status					.148
Single	34	28 (82.4)	6 (17.6)	0 (0)	
Married	71	64 (90.1)	6 (8.5)	1 (1.4)	
Divorced	13	11 (84.6)	2 (15.4)	0 (0)	
Widowed	12	10 (83.3)	2 (16.7)	0 (0)	
Total	130[<]	113	16	1	
Education					.000**
None	4	4 (100)	0 (0)	0 (0)	
Primary	81	72 (88.9)	9 (11.1)	0 (0)	
Secondary	31	25 (80.6)	6 (19.4)	0 (0)	
Post-Secondary	14	12 (85.7)	1 (7.1)	1 (7.1)	
Total	130[<]	113	16	1	
Occupation					.074
Unemployed	43	37 (86)	6 (14)	0	
Casual	61	55 (90.2)	5 (8.2)	1(1.6)	
Formal	26	21 (80.8)	5 (19.2)	0(0)	
Total	130[<]	113	16	1	
Economic status					.042*
Low income earner	84	74 (88.1)	10 (11.9)	0 (0)	
Middle income earner	45	39 (86.7)	6 (13.3)	0 (0)	
Upper income earner	1	0 (0)	0 (0.0)	1 (100)	
Total	130[<]	113	16	1	

** P< 0.01

* P < 0.05

[y] indicates the total samples of both the children and adults while [w] indicates the total sample size for adults

4.3 Participants' immunity characterisation

The mean CD4+ count of participants above 12 years was 401.3cells/mm^3 while those below the age of 12 years was 597.4 cells/mm^3. Immune status for the participants was categorized into three categories to determine severity of immunosuppression and was as follows; severe immune suppression (participants below 12 years <15% and adults above 12 years <200 cells/μL), moderate immune suppression (children below 12 years (14-24) % and adults above 12 years (200-499) cells/μL) and no immune suppression (children below 12 years >25% and adults above 12 years >500 cells/μL) as per Schneider *et al*., (2008) categorisation. (Table 4.3).

Table 4.3. The immune status (%) across different age groups of HIV infected participants. Moderate and severe immunosuppression was found in 45.4% and 18.4 respectively. There was no immune suppression in 36.2%

	Immune category			
Age group	**Participants (n)**	**No suppression (n, %)**	**Moderate suppression (n, %)**	**Severe suppression (n, %)**
Below 2 years	9	1 (11.1)	6 (66.7)	2 (22.2)
2-12	24	11 (45.8)	10 (41.7)	3 (12.5)
13-24	23	9 (39.1)	10 (43.5)	4 (17.4)
25-34	17	8 (47.1)	7 (41.2)	2 (11.8)
35-44	19	10 (52.6)	6 (31.6)	3(15.8)
45-54	36	13 (36.1)	18 (50)	5(13.9)
55-64	24	6 (25)	11 (45.8)	7 (29.2)
65 and Above	11	1 (9.1)	6 (54.5)	4 (36.4)
Total	**163**	**59 (36.2)**	**74 (45.4)**	**30 (18.4)**

Participants above the age 12 years who had severe immune suppression were 19.2%, moderate immune suppression 44.6% and no immune suppression 36.2%. Out of the 87% seroprevalence (CMV IgG) among participants above 12 years, participants with moderate immune suppression (200-499 cells/μL) had 38.5% while those with no immune suppression (>500 cells/μL) and severe immune suppression (<200 cells/μL) had 33.1% and 15.4% respectively. There was only one (1) participant who tested negative for both the CMV antibodies (IgG and IgM) (Table 4.3.1).

Table 4.3.1. Immune status characterisation and distribution of CMV antibodies (IgG and IgM) among HIV infected participants above 12 years

Immune status CD4+ Count (cells/μL	Participants	CMV Anti-IgG (n, %)	CMV Anti-IgM (n, %)	Both (IgG/IgM) Negative (n, %)
>500	47 (36.2)	43 (33.1)	3 (2.3)	1 (0.8)
200-499	58 (44.6)	50 (38.5)	8 (6.2)	0 (0)
>200	25 (19.2)	20 (15.4)	5 (3.8)	0 (0)
	130 (100)	113 (87)	16 (12.3)	1 (0.8)

Children below the age of 12 years had a higher CMV exposure of 97% than participants above 12 years (87%) (Measured by anti-CMV IgG). Out of the 97% seroprevalence (CMV IgG) among participants below 12 years, participants with moderate immune suppression (CD4 14-24%) had 48.5% while those with no immune suppression (CD4 above 25%) and severe immune suppression (CD4 below 15%) had 33.3% and 15.2% respectively. There was no participant who tested negative for both the CMV antibodies (IgG and IgM) (Table 4.3.2).

Table 4.3.2. Immune status characterisation and distribution of CMV antibodies (IgG and IgM) among HIV infected participants' children below 12 years

Immune status CD4 (%)	Participants n , %	IgG n,%	IgM n,%
>25%	12 (36.4)	11 (33.3)	1 (3)
14-24%	16 (48.5)	16 (48.5)	0 (0)
<15%	5 (15.2)	5 (15.2)	0 (0)
	33 (100)	32 (97)	1 (3)

4.4 Prevalence and incidence of Human Cytomegalovirus among HIV patients attending Thika level 5 District Hospital

Out of the total of 163 HIV seropositive participants under the study, 117 (71.8%) of them tested positive for Cytomegalovirus IgG antibodies while 5 (3.1%) tested positive for cytomegalovirus IgM positive and 1 (0.6%) was negative for both (IgG/IgM) antibodies. Nevertheless, 40 (24.5%) tested positive for both cytomegalovirus IgG and IgM antibodies, out of which 28 demonstrated a high avidity (indicating positive for Cytomegalovirus IgG) and 12 low avidity (indicating positive for Cytomegalovirus IgM). The total HIV patients who tested positive for Cytomegalovirus IgG were 145 indicating a high CMV prevalence of 89% while those who tested positive for Cytomegalovirus IgM were 17 indicating an incidence of 10.4%.The total HIV positive patients who had either been exposed or had current/reinfection were 162 leading to a high overall seroprevalence of 99.4%.

4.5 Cytomegalovirus Avidity index

Cytomegalovirus avidity test was conducted among the 40 participants that tested positive for both anti-CMV IgG and IgM to evaluate the strength of IgG antibodies based on its binding affinity to the well bound antigen. The samples that were evaluated were classified as either

high avidity or low avidity in relation to the avidity index. The AI cut-off point for defining low avidity was typically 35% to 50%, whereas the AI cut-off point for defining high avidity was typically 50% to 65%. Majority 28 (70%) of the subjects demonstrated a high avidity index (>50) while 12 (30%) demonstrated low avidity index (≤50%). High avidity was indicative of past exposure to CMV while low avidity was indicative of primary infection of CMV (Table 4.5).

Table 4.5 Avidity index among participants who tested positive for both anti-CMV IgG and anti- CMV IgM

Avidity index	**n**	**%**
>50%	28	70
<50%	12	30
Total	**40**	**100**

4.6 CMV Risk factors

Different risk factors were analysed to assess association with CMV serostatus among participants. Saliva sharing behaviour which include, kissing, sharing drinks, toothbrush and lip balm were among the variables analysed. Sharing drinks showed a statistical association with CMV serostatus (P=0.042) while sharing lip balm (P=0.674), sharing toothbrushes (P=0.054) and kissing (P=0.285) were not associated with seropositivity. Other sexually transmitted infections showed a statistically significant association with CMV serostatus (P=0.041). Blood transfusion also showed a significant statistical relationship with CMV seropositivity (P=0.007). Circumcision methods, exposure to children and group living habits were not associated with CMV serostatus (P= 0.072, 0.180, 0.918) respectively. Engaging in unprotected sex was a constant variable since 100% of the subjects were engaging in unprotected sex hence determination of the significance was impossible (Table 7). The study

did not capture any subjects exposed to the following variables organ transplant, pregnancy, breast feeding, participants with other immunocompromised diseases and drug injection use hence these factors could not be analysed. All (100%) of the HIV patients were under HAART.

Table 4.6 Risk factors associated with Cytomegalovirus infection among HIV infected participants. Circumcision, group living habits, sharing toothbrushes, lip balm and kissing and exposure to children were not significant. However, Sharing drinks, history of sexual transmitted infection and blood transfusion were statistically significant (Chi square)

Variables		**Participants (n, %)**	**CMV IgG (n, %)**	**CMV IgM (n, %)**	**P values**
STI		53 (40.7)	48 (90.6)	5 (9.4)	.041*
Circumcision	Traditional. Circumcision	16 (25.4)	11 (68.8)	3 (18.8)	.072
	Hospital circumcision	39 (68.4)	35(89.7)	3(7.7)	
Group living Habits	Camps, street, inmates and Homecare	4 (3.1)	4 (100)	0 (0)	.918
Saliva Sharing behaviour	Sharing drinks	125 (76.7)	110 (88)	15(12)	.042*
	Toothbrushes	41 (26.6)	35 (85.4)	6 (14.6)	.054
	Lip balm	14 (19.2)	13 (92.9)	1 (0.8)	.674
	Kissing	100 (76.9)	85 (85)	15 (15)	.285
Exposure to children		58 (35.6)	51 (87.9)	6 (10.3)	.180
Blood transfusion		51 (31.2)	46 (90.2)	4 (7.8)	.007**

** P<0.01
* P<0.05

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Cytomegalovirus infection often goes unnoticed in most clinical settings yet it can cause more devastating conditions. Having understood the lethality of cytomegalovirus mostly in immunocompromised patients, this study sought to find out the current burden of CMV among the HIV patients attending Thika level 5 Hospital. In so doing the study also sought to establish some of the risk factors that may lead to acquisition of cytomegalovirus. Cytomegalovirus coupled with HIV infection down-regulates the immune system, therefore assessing the effect on the immune system by the co-infection is vital.

5.1 Prevalence and incidence of Human Cytomegalovirus among Human Immunodeficiency syndrome patients

This study found out that there was a high CMV seroprevalence of 89 % and incidence of 10.4% among HIV patients attending Thika level 5 District Hospital. This finding is consistent with other investigations that have been previously reported. In Nigeria, 100% CMV seroprevalence among HIV patients has been reported (Akinbami *et al*., 2010). This study finding was also in agreement with other studies conducted in Kenya; 100% among HIV-1 infected street children at Nyumbani Children Home (Chakraborty *et al*., 2003), 97% among healthy blood donors at the National blood transfusion centre and 85% among pregnant women (Njeru *et al*., 2009 ; Maingi & Nyamache, 2014) at Thika level 5 hospital respectively. These high rates could probably be associated with high rates of blood transfusion and lack of proper hygienic practices like saliva sharing behaviours especially sharing of drinks using same vessels. These were commonly among participants from low socioeconomic status. The high seroprevalence observed from this current study could also be attributed to lack of adequate awareness on prevention measures and hygiene among individuals which may pray a primary role to the high CMV transmission and infection rates as also noted by Colugnati *et al*, (2007).

However, seroprevalence results from this study was higher than those obtained in France; 46.8% in pregnant women (Picone *et al*., 2009). This may be ascribed to high hygiene standards observed (Dowd *et al*., 2009). Israel and eight European countries (France, Belgium, Spain, Italy, Germany, Austria, Portugal, and the Netherlands) undertake routine CMV serological screening of pregnant women (Forsgren, 2009) this could be the reason for the low prevalence observed in this countries.

This study reported that 10.4% of the HIV seropositive patients were anti-CMV IgM seropositive (Current infection or reinfection/reactivation) to cytomegalovirus. This is consistent with a study by Fowotade *et al* (2015) that found 11.1% anti-CMV IgM seroprevalence among HIV patients in Nigeria. In another study in Brazil, anti-CMV IgM seroprevalence of 11.36% among HIV infected prison inmates was reported (Osti *et al*., 1998). A high current infection observed in this study could be associated to reactivation of CMV among HIV infected patients due to down-regulation of the immune system (Hummel & Abecassis, 2002)

5.2 Effect of Cytomegalovirus infection on the immunity of participants

In this study, the immune status was categorized into three categories, no immune suppression, moderate immune suppression, and severe immune suppression; - for uniformity across the age groups. For infants and children below 12 years immune status was assessed by their CD4+ percentage based on the recommendation by Schneider *et al*., (2008) while for those above 12 years immunity was assessed by the absolute CD4+ count. Generally, this study observed that there was relatively high number (63.8%) of participants who had immune suppression (severe and moderate), as compared to the participants who had no immune suppression 36.2% (Tables 4.3, 4.3.1, 4.3.2) Majority of the participants having low immunity in this study could be attributed to the synergistic effect of CMV and HIV which could have worsened the immunological profile of the patients (Chakravarti *et al*., 2009).

Despite majority of the participants having immune suppression, this study reported a high (401.3cells/mm^3) mean CD4+ count among the participants above 12 years and (597.4 cells/mm^3) among those below the age of 12 years. This could be as a result of easy access to HAART. This is in agreement with a study by Lifson *et al*., (2011) which found out that HIV immune suppressed patients have shown positive increase in CD4+ count when initiated on HAART for longer duration. Other studies has also showed that HAART has led to a recovery of patients immunity shown by a rise in $CD4^+$ T-lymphocytes count which leads to a reduction in AIDS related deaths (Mermin *et al*., 2008). This therefore has reduced the risk of end organ diseases.

5.3 Demographic and risk factors of cytomegalovirus in HIV patients

Education on basic hygiene measures such as; hand washing techniques with detergents after contact with young children or their diapers or oral secretion is important. Education on safe and careful disposal of items, education on the importance of avoiding sharing drinks and education on safe sex all play a major role in prevention of CMV acquisition. This study found out that education level is associated with CMV serostatus (P=0.000). This is in agreement with the finding by Maingi and Nyamache, (2014) among pregnant women in the same study area. Extreme (100%) exposure observed among participants who had not attended any form education in this study indicates lack of awareness on basic risk factors and prevention mechanism due to lack of exposure to any form of education. This could probably be the reason for education being strongly associated with CMV serostatus in this study.

Economic status was statistically associated with anti-CMV serostatus (p=0.042). In this study economic status was strongly linked to CMV infection with low income earners being at higher risk of CMV infection. The study population did not however capture any upper

income earner. Only the low and middle income earners were used to assess the association. Low income earners are highly exposed to more risk factors that have been found to be more linked to CMV serostatus in this study such as sharing drinks using same vessels without being cleaned and low education background, this could be as a result of financial inability to access better education. Staras *et al*, (2006) in his study also found out that economic status was highly linked to the CMV serostatus.

This study showed that the prevalence of anti-CMV IgG and IgM antibodies was not sex dependant (P=0.494). Male subjects had anti-CMV IgG prevalence of 41.1%, and females had a 47.2% seroprevalence. Anti-CMV IgM seroprevalence was 4.9% and 5.5% among males and females respectively. This finding is similar to that of Fowotade *et al*, (2015) among HIV-1 seropositive patients. This could be so because transmission of CMV infection is not attributed to any given sex. All patients have equal chance of infection provided they are exposed to CMV.

Marital status was not statistically associated with CMV seropositivity among the various marital groups (P=0.148). From the results it was found that 90.1% of the married study subjects had anti-CMV IgG and only 8.5% had CMV anti-IgM. The divorced, widowed and single subjects had 84.6%, 83.3% and 82.4% anti-CMV IgG and 15.4%, 16.7% and 17.6% anti-CMV IgM respectively. From the study findings, all the participants had equal chances of being infected with CMV despite their marital status. Irrespective of the participants' marital status, exposure to CMV led to transmission of CMV infection. This finding however contradict a finding by (Staras *et al*., 2006)

Casual workers constituted the majority 61 (46.9%) of the participants, unemployed 43 (33.1%) while the formally employed were the lowest 26 (20%) among the study subjects. CMV antibodies distribution among the groups was as follows, casual workers, unemployed

and formal employed 42.3%, 28.5% and 16.2% anti-CMV IgG and 3.8%, 4.6% and 3.8% anti-CMV IgM respectively. Occupation was not statistically associated with CMV serostatus (P=0.074). This finding is supported by the fact that an individual job description does not relate to any mode of CMV transmission. This was in agreement with other previous finding (Fowotade *et al*., 2015). This shows that individuals were prone to CMV infection irrespective of their occupation.

This study also observed that there was a significant difference between participants who had history of blood transfusion and those who had no history of blood transfusion (P=0.007). Most (90.2%) of the HIV positive patients who had a history of blood transfusion had been exposed to CMV infection with 7.8% patients having new infection or reactivation/reinfection. Usually new seropositive blood donors have a higher periphery blood concentration of CMV DNA and therefore a high chance of CMV transmission. In regard to that, blood transfusion of seronegative blood products and blood products that are negative of CMV DNA would help reduce CMV transmission from the donor to the recipients. Although this will reduce the rate of CMV transmission the cost of maintaining supply of CMV-negative blood may be high. The high CMV prevalence observed among the patients with history of blood transfusion in this study may be attributed to lack of routine CMV screening among the donor. This is in agreement with Njeru *et al*., (2009).

Having a current or history of a sexually transmitted infection was statistically associated with CMV serostatus (P=0.041). Anti-CMV IgG and IgM antibody was 90.6% and 9.4% among those with and without STI respectively. Usually STI are mainly transmitted through unprotected sexual contact (oral, vaginal or vaginal), direct genital contact through lesion without sex or from a pregnant/breasting feeding mother. High CMV prevalence observed in this study among the participants who had history of STI could be attributed to the fact that 100% of the same participants had unprotected sex leading to transmission of CMV.

In this study it was found out that sharing drinks showed a significant statistical relationship to serostatus of the participants (P=0.042). Cytomegalovirus is usually transmitted by saliva and any mouth contact with the infected saliva will lead to CMV transmission. Kissing, sharing tooth brushes and sharing lip balm showed no statistical significance to CMV antibodies (P>0.05). This finding was however inconsistent to the finding by (Stadler *et al*., 2010). While kissing and sharing drinks would means sharing of saliva, sharing drinks was strongly linked to CMV infection unlike kissing, the findings is unclear though this could have been due to a relatively small sample size to capture this minor differences. It's therefore, my opinion that further study with a larger sample size on this variable would be useful in clearing this the inconsistence.

The study found out that circumcision method, group living habits, exposure to children at home and pregnancy were not statistically associated with CMV serostatus (p>0.05).

Some of the variables like organ transplant, breastfeeding, exposure to injectable drug abuse were not assessed as earlier planned since there were no subjects exposed to these variables. Non protected sex was also a constant hence not assessed since all the subjects eligible for the variable had been exposed to non-protected sex. All the HIV patients were under HAART.

5.4 Limitations of the study

1. Less sample size that might not have captured small differences among the groups and to evaluate differences in frequency amount of specific behaviours.
2. Some variables like non protected sex were constant in all the participants hence unable to assess its significance.

5.6 Conclusions

1. This study shows that Cytomegalovirus is wide spread among HIV infected patients attending Thika level 5 Hospital with a seroprevalence of 89% (Anti-CMV IgG) and an of incidence (active/reactivation) 10.4% (Anti-CMV IgM).
2. CMV leads to more suppression of the immune system among HIV infected patients
3. High CMV seroprevalence was associated with various exposure mechanisms including sharing drinks, blood transfusion, and other sexual transmitted infections among the group which demonstrated significant relationship
4. Education and economic status were significantly associated with CMV serostatus

5.6 Recommendations

1. Based on the high prevalence recorded in this study, there is a need to adopt a CMV screening among the HIV patients to prevent end organ disease
2. The health education to be offered on the risk factors and acquisition of Cytomegalovirus and on methods of improving the immunity among HIV infected individuals.

5.7 Future studies

- Future studies should be done with a larger sample size to evaluate exposure and individual behaviours in preparation for a CMV vaccine.

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APPENDIX I: CONSENT FORM

Title of the study: Determination of seroprevalence, risk factors and immunity for cytomegalovirus infection in human immunodeficiency virus patients at Thika level 5 hospital.

Institution: Kenyatta University.

Principle Researcher: Nchagwa Edward Mangare, Department of Medical Laboratory Science, Kenyatta University, Kenya.

Supervisor (1): Margaret Muturi (PhD), Department of Medical Laboratory Science, Kenyatta University, Kenya.

Supervisor (2): George Gachara (PhD), Department of Medical Laboratory Science, Kenyatta University, Kenya,

Researchers' statement

I am a postgraduate student at Kenyatta University, in the department of Medical Laboratory Science pursuing Master of Science in Infectious Diseases (Immunology). This is to inform you that this is an educational research that is to be carried out in Thika level 5 Hospital. This consent form will provide you with information to enable you to decide whether to participate in the study or not. The researcher will administer it to you. You may ask any question concerning the purpose of the research, procedures that will be followed, your rights as a participant in the study, risks, benefits and any other question of concern about the study.

Purpose of the study

The purpose of the study is to determine the seroprevalence, risk factors and immunity for cytomegalovirus infection in human immunodeficiency virus patients. Therefore, blood samples will be collected for antibodies analysis after administration of a questionnaire.

Questionnaire

The aim of the questionnaire is to asses demographic and risk factors that may lead to Cytomegalovirus acquisition. If you agree to take part in the study, the age, education level, marital status, monthly income and other risk factors that could lead to acquisition of cytomegalovirus will be recorded. Blood sample will be collected for laboratory analysis of Cytomegalovirus antibodies.

Risks

During blood collection, you may experience temporary local pain or bruising when the needle is inserted into your vein to get venous blood.

Benefits

There will be no direct benefit to you; however data obtained from the study will help Health care providers in understanding the burden and risk factors associated with CMV/HIV coinfection, this will enhance timely initiation of treatment to the patients and help in establishing CMV preventive strategies.

Assurance of confidentiality

Any information in relation to your participation in this study will remain private. Only codes and numbers will be used, your name or any other means of person’s identification will not

be used in any of the reports resulting from this study. The consent paper will be safely kept and laboratory specimen will only have a study number.

Further inquiries

If you have any question regarding the study, feel free to contact Kenyatta University Ethical Review Committee on the emails: kuerc.chairman@ku.ac.ke or kuerc.secretary@ku.ac.ke or ercku2008@gmail.com

Subject statement and signature

The study has been explained to me. I volunteer to take part in this study. I have had a chance to ask questions. If I have more questions, I can ask any of the supervisors listed here or contact the Kenyatta University Ethical Review Committee.

Name of the participant ……..

Participant's code.....

Signature or fingerprint of participant/guardian/parent……..

APPENDIX II: QUESTIONNAIRE

This questionnaire is aimed at assessing the demographic and risk factors that can lead to cytomegalovirus infection.

My name is, Nchagwa Edward, an MSc student in the department of Medical Laboratory Science, School of Medicine. I am investigating the demographic and risk factors that can lead to acquisition of cytomegalovirus. I kindly request you to fill the questionnaire below to the best of your knowledge.

Respondent code _____ Gender: male ( ) female ( )

Date of interview_____

Classification of Subjects: Case ( ) Control ( )

SECTION A: DEMOGRAPHIC INFORMATION

1. Age: Below 2 years ( ) ; 2-12 years ( ); 13-24 years( ); 25-44 years ( )
   45-54 years ( ); 55-64years ( ); 65 years and above ( )

2. Marital status: Single ( ); Married ( ); Widowed ( ); Divorced ( ) others ( )
   If others, Specify_____

3. Education level: None ( );Primary ( ); Secondary ( ); Post-secondary ( )

4. Current occupation: Unemployed ( ); Casual ( ); Formal ( )

5. Economic status

(Monthly income < Kshs.23670 low, >Kshs.23671-120000 middle class,>120000 upper)

i. Low income earner ( )

ii. Middle income earners ( )

iii. Upper income earners( )

SECTION B: RISK FACTORS

A. Sexual behaviour

i) Have you ever had any sexual contact; Yes ( ) No ( )

If **yes** classify; protected sex i.e. use of condom ( ) non protected sex ( )

B. Sexual transmitted infection

i) Do you have or ever had any other sexual Transmitted disease? Yes ( ) No ( )

C. Circumcision (males)

i) Are you circumcised? Yes ( ) No ( )

If Yes, specify Traditional ( ) Hospital ( )

D. Group living situation and sharing of injections

i) Do you live in any one of the following places?

Camps, Home care, street; Yes ( ) No ( )

ii) Are you an injection drug user? Yes ( ) No ( )

If, **yes** do you share the needles? Yes ( ) No ( )

E. Saliva -sharing behaviours

i) Do you share the following?

Drinks; Yes ( ) No ( )

Toothbrushes; Yes ( ) No ( )

Lip balm; Yes ( ) No ( )

ii) Do you engage in kissing activity? Yes ( ) No ( )

F. Exposure to young Children at Home

i) Do you care for the young one at home (Changing diapers, feeding children, and looking after young children); Yes ( ) No ( )

G.Breast feeding (Below 18 Months)

i) Is the child breast feeding Yes ( ) No ( )

ii) Do you wash hands after changing diapers? Yes ( ) No ( )

H.Immunocompetence

i) Do you have or ever had any of the following disease conditions (Diabetes, cancer, leukaemia, lymphoma or any known autoimmune disease); Yes ( ) No ( )

ii) Have you ever had any organ transplant; Yes () No ( )

I. Female

i) Are you pregnant? Yes ( ) No ( )

J. Blood Transfusion

i) Have you ever received any blood or blood product before? Yes ( ) No ( )

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 8710901/12
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Our Ref: KU/R/COMM/51/615 Date: 4th February, 2016

Nchangwa Edward Mangare,
Kenyatta University,
P.O Box 43844,
Nairobi

Dear Mangare,

RE APPLICATION NUMBER PKU/416/1385– "INCIDENCE OF CYTOMEGALOVIRUS INFECTION AND ASSOCIATED RISK FACTORS AMONG HUMAN IMMUNODIFICIENCY VIRUS INFECTED PATIENTS AT THIKA DISTRICT HOSPITAL, KENYA."

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic "Incidence of cytomegalovirus infection and associated risk factors among human immunodificiency virus infected patients at Thika District Hospital, Kenya."

2. APPLICANT

Nchangwa Edward Mangare

3. STUDY SITE

Thika District Hospital, Kenya

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 4th February,2016.

5. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

4 FEB 2016
KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE P.O. BOX 43844 - 00100 NAIROBI

DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I Nchagwa Edward accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of 5/2/16 2016.

cc. Vice-Chancellor
DVC-Research Innovation and outreach