SERO-PREVALENCE OF HUMAN CYTOMEGALOVIRUS INFECTION AND THE ASSOCIATED FACTORS AMONG HIV INFECTED PATIENTS ATTENDING THE COMPREHENSIVE CARE CLINIC AT THE KENYATTA NATIONAL HOSPITAL, KENYA

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Sero-Prevalence of Human Cytomegalovirus Infection and the Associated Factors among HIV Infected Patients Attending the Comprehensive Care clinic at the Kenyatta National Hospital, 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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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my parents (Mr and Mrs Gicho), brothers (Samuel, John and Isaac), husband (Antony) and daughter (Eliana).

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LIST OF ABBREVIATIONS AND ACRONYMS

ACV	Acyclovir
°C	Degree Celsius
CCC	Comprehensive Care Clinic
CI	Confidence interval
CPE	Cytopathic effect
CD4	Cluster of Differential 4
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EOD	End-organ disease
GCV	Gancyclovir
HIV	Human immunodeficiency virus
HEU	HIV Exposed Uninfected
HCMV	Human Cytomegalovirus
HAART	Highly active antiretroviral therapy
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IR	Internal repeats
KNH	Kenyatta National Hospital
KM	Kilometer
KEMRI	Kenya Medical Research Institute
Ml	Milliliters

MoH	Ministry of Health
OR	Odds Ratio
PCR	Polymerase Chain Reaction
p	Probability value
SPSS	Statistical package for social sciences
SD	Standard deviation
TR	Terminal repeat
UoN	University of Nairobi
UL	Unique long
US	Unique short
VGCV	Valgancyclovir

ABSTRACT

Human Cytomegalovirus (hCMV) is a member of Herpes Virus family. During an active infection in HIV infected patients, it is one of the most common causes of pneumonia, retinitis, gastrointestinal disease, and hepatitis. It is a cofactor in HIV disease. The virus is an opportunistic pathogen in immunocompromised individuals. It is associated with HIV disease progression leading to high mortality and morbidity. The magnitude of hCMV in Kenyan HIV patients is not known, especially at the Kenyatta National Hospital. This problem has not been adequately investigated in Kenya and warrants strong preventive measures. Currently there is scanty data on this disease in Kenya leading to lack of its recognition in HIV patients. To determine the seroprevalence of hCMV infection and its predisposing factors among comprehensive care clinic (CCC) attendees at Kenyatta National Hospital, Nairobi County, Kenya, a cross-sectional study was carried out. Ethical approval was obtained from Kenyatta national hospital and university of Nairobi ethics and research review committee.A systematic random sampling method was used to select participants among the target population. Five millilitres of blood was aseptically collected from each participant by venepuncture. The blood was separated and plasma used to test for presence of hCMV IgM and IgG using enzyme linked immunosorbent assay (ELISA). Demographic data was collected using a questionnaire and data analysed using statistical package for statistical parameter test (SPSS). Bivariate analysis was carried out using chi-square and student t-test. Four hundred HIV-infected participants were recruited. Their mean age (SD) was 42.73 (9.5) years. Of these, 246(61.5%) were females and 154(38.5%) were males. Of 400, 398 (99.0%) were hCMV IgG seropositive, 32 (8.0%) were hCMV IgM seropositive. Age group between 19 and 28 years [OR = 4.8 (1.4-16.4) 95% CI; p=0.012], never been married [OR = 4.3 (1.3-14.5) 95% CI; p=0.020], never had children [OR=3.2 (1.2-8.5) 95% CI; p=0.022] and use of highly active antiretroviral therapy (HAART) [OR=3.5 (1.2-10.3) 95% CI; p=0.031] were significantly associated with hCMV sero positivity using bivariate analysis. In multivariate analysis, both CD4 ($p < 0.001$) and HIV viral loads ($p < 0.001$) were significantly associated with hCMV sero positivity. Routine screening for hCMV in HIV patients would be recommended with emphasis on patients with high viral loads and low CD4 counts.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Human cytomegalovirus (hCMV) is a member of the Herpesviridae family and Betaherpesvirinae subfamily. As a herpes virus the virion is characterised by structural features which include: an icosahedral nucleocapsid, surrounding tegument layer, and host derived envelope studded with virally encoded glycoproteins (Mocarski & Courcelle, 2001). Its mode of transmission requires direct contact with infected fluids. The most frequent mode of transmission is through sexual contact or in the context of childcare. Another important route of hCMV exposure is vertical transmission from mother to fetus in utero, during birth, or through breast milk (Pass 2001).

During the primary infection the virus is shed for weeks or months in bodily fluids including saliva, urine, tears, semen, and cervical secretions. This new infection will lead to production of specific IgM antibodies (Pass, 2001). The virus remains latent until when it reactivates and sheds in the immune system of a compromised individual (Crough & Khanna, 2009). Human cytomegalovirus is a widespread virus and does present asymptomatic and persistent infection in immunocompetent people (Landolfo *et al.,* 2003). A secondary infection can be acquired through reinfection with a different strain of virus, or reactivation from latency. The diagnoses can be done if there is a

significant rise in hCMV-specific IgG and/or IgM previously infected persons (Ornoy & Diav-Citrin, 2006).

On attachment the hCMV virus does penetration and it happens very rapid and efficient. It does involve the broadly distributed hCMV cellular receptors (Mocarski & Courcelle, 2001). Human CMV has two envelope glycoproteins, B (gB) and H (gH) that interact with cellular surface receptors. They do initiate in the attachment and fusion of the virus to the host cell (Compton 2004: Boehme *et al.,* 2006). The replication has three stages namely: immediate early (IE), early, and late. The process is very slow and takes about 48-72 hours for release of a progeny (Mocarski & Courcelle, 2001).

In immunocompetent individuals hCMV is typically self-limiting with very mild flu-like symptoms and do pass without complication. However, in immunocompromised subjects, including patients with congenital and acquired immunodeficiency, hCMV can cause a wide range of clinical syndromes. This might include retinitis (which may cause blindness), pneumonitis, cholangitis, hepatitis, gastrointestinal ulcerations, colitis, abdominal pain, encephalitis, fever and weight loss (Knipe & Howley, 2007).The immune response of an individual to hCMV infection is greatly determined by their immune status (Gandhi & Khanna, 2004). In an immunocompetent individual the hCMV infection triggers establishment of both humoral and cell mediated immunity. This can result in a mild mononucleosis-like illness although rarely (Mocarski *et al.,* 2007). In an immunocompromised individual, hCMV-specific T-cell response is delayed when compared to the immunocompetent population (Lilleri *et al*., 2009). This results to

morbidity and even mortality in immunocompromised individuals (Landolfo *et al.,* 2003).

In Kenya a study reported a strong correlation between peak HIV viral load and peak hCMV viral load (Slyker *et al.*, 2009). This shows that there is an association between the two viruses. It is documented that there is a direct and indirect influence between HIV and hCMV viremia. In the direct it is not clear whether the symptomatic hCMV disease is a cause or an effect to high HIV-p24 antigen concentration. In the indirect the systemic inflammation may fuel the replication of both viruses (Griffiths 2006). The prevalence of hCMV in a given population depends largely on socioeconomic factors. It is endemic in most areas of the world. It does vary in different geographical regions ranging from 30-90% in the developing countries (Crough & Khanna, 2009). In resource-poor settings, examining the occurrence of hCMV disease in HIV-infected individuals has been neglected. The seroprevalence varies greatly with a variety of epidemiological factors such as age, geographical distribution, socioeconomic status, marital status and parity (Basawaraju *et al.,* 2011).

The hCMV disease in the context of HIV is equally well established although interest in the manifestations of coinfection has substantially diminished in the HAART era. However, the complex interplay between these two chronic viral infections continues to be potentially highly significant in both adults and children (King *et al.,* 2013). In studies conducted in developed countries, detectable hCMV DNA in plasma (Wohl *et al.,* 2005) or in whole blood (Deayton *et al.*, 2004; Reus *et al.,* 2004) have been shown to be the cause of death. This is even after low HIV RNA level and normal CD4+ T cell

count. In Tanzania dried blood spots were used to detect hCMV viremia and the findings showed a hazard ratio of 5 for mortality in the presence of hCMV (Brantsæter *et al.,* 2012). In Italy83.3% of HIV infected individuals demonstrated an association between hCMV IgG and time to severe non-AIDS disease (Lichtner *et al.,* 2015). This study importantly demonstrated the potential for enhanced disease burden associated with hCMV in HIV-infected individuals. The coinfection does not necessarily result in AIDS-defining events but instead a more indirect interplay of its pathology resulting from systemic inflammation and immune response (Erlandson *et al.,* 2015).

1.2 Statement of the problem

The hCMV is an important and common cause of mortality and morbidity in immunocompromised patients. Once the immunity is suppressed the virus gets an opportunity to infect the HIV patients. There has been a rise in the number of cancer patients in HIV patients with the knowledge that hCMV causes cancer. This increase in cancer may not be well explained if it is hCMV or something else. The symptomatic hCMV disease in HIV patients can affect almost every organ of the body leading to manifestations that cause death. It is significantly associated with increase in HIV acquisition and disease progression. The most common presentation in HIV-infected patients is hCMV pneumonia, where co-infection with other respiratory pathogens such a tuberculosis and pneumocystis jirovecci, is almost abundant. In primary infection the hCMV becomes latent in multiple organs and can later be reactivated during severe deregulation of the immune system. Currently there is scanty of information as far as the magnitude of hCMV on HIV/AIDS patients is concerned. The unavailability of this data

has led to lack of recognition on the magnitude of hCMV causing its signs and symptoms to be overlooked in HIV patients. This therefore limits the MOH and other health professionals on designing a policy geared towards the control of hCMV in Kenya among HIV infected individuals with active hCMV disease.

1.3 Justification of the study

Inadequate information on disease burden limits treatment or intervention of hCMV infection. Since hCMV presentation mimics other pathogens, it is easily misdiagnosed. Establishing the burden of the disease is important in prevention of classical cytomegalovirus syndrome. Currently, tissue diagnosis is cumbersome, costly and requires hospitalization especially in HIV/AIDS patients. This shows that the economic impact is big when it comes to hospital bills, medication and manpower that is lost. In Kenya the situation has not been adequately investigated and is still a major health problem, warranting strong preventive measures (Njeru *et al*., 2009). The negative economic impact on families, the health system and the country is high. The information might also enlighten the importance of routine check for hCMV in HIV patients which is not currently available. Determining the sero prevalence of hCMV in HIV patients especially those attending CCC will mitigate its impact. It will also contribute to the millennium development goal number six to combat HIV/AIDS and other diseases. This will form useful reference material for researchers and the HIV/AIDS control council in the management system.

1.4 Research questions

1) What is the prevalence of human cytomegalovirus (hCMV) among HIV infected patients attending the comprehensive care clinic at Kenyatta National Hospital?
2) What are the biological associated factors of human cytomegalovirus (hCMV) infection in HIV infected patients attending the comprehensive care clinic at Kenyatta National Hospital?
3) What are the demographic associated factors of human cytomegalovirus (hCMV) infection in HIV infected patients attending the comprehensive care clinic at Kenyatta National Hospital?

1.5 Objectives

1.5.1 General objective

To determine the sero-prevalence of human cytomegalovirus infection and its associated factors among HIV infected patients attending the comprehensive care clinic at Kenyatta National Hospital.

1.5.2 Specific objectives

1) To determine the sero-prevalence of human cytomegalovirus (hCMV) infection among HIV patients attending the comprehensive care clinic at Kenyatta National Hospital.

2) To determine the biological associated factors of human cytomegalovirus (hCMV) infection in HIV infected patients attending the comprehensive care clinic at Kenyatta National Hospital.
3) To determine the demographic associated factors of human cytomegalovirus (hCMV) infection in HIV infected patients attending the comprehensive care clinic at Kenyatta National Hospital.

CHAPTER TWO

LITERATURE REVIEW

2.1 Human Cytomegalovirus

The hCMV belongs to the Order *Herpesvirales,* family *Herpesviridae* and genus *Beta-herpesvirinae.* It's the largest in the herpes group of viruses with a genome of about 230kb. It has a restricted host range, production of nuclear as well as cytoplasmic inclusions and its long life cycle. It is an enveloped icosahedral symmetry virus containing a large genome of double-stranded DNA. The tegument involves most of the virion protein such as the herpes virus core virion maturation protein pp150, transactivator pp71, the biggest tegument proteins being UL48 gene product, and the UL99-encoded pp28. The mature virions have a diameter of 200-300 nanometer (Crough & Khanna, 2009). It's a double-stranded DNA genome with attached proteins. It has a large tegument component surrounded by an envelope that contains a cellular lipid bilayer with viral glycoproteins figure 2.1.

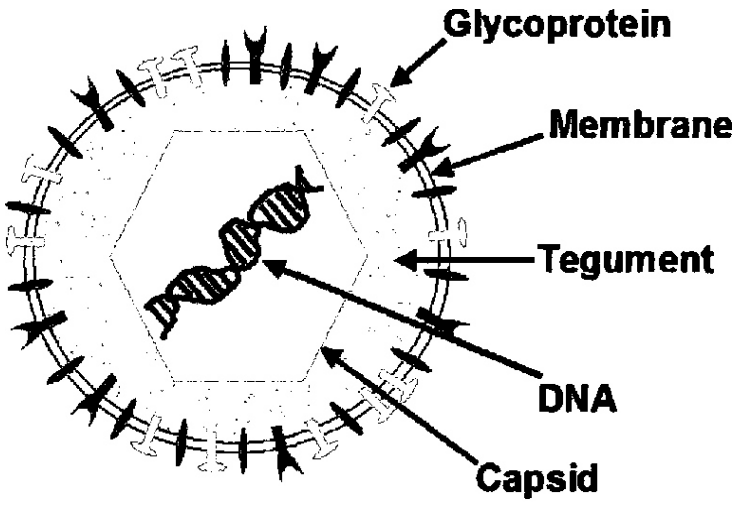


Figure 2.1: Structure of Cytomegalovirus (Barbi *et al.,* 2001).

The hCMV genome is composed of domains the unique long (UL) and unique short (US). They are lined on one end by terminal repeated sequences (TRL and TRS) and on the other end by internal repeats (IRL and IRS). The hCMV DNA has four isoforms that are attributable to recombination. This does result in the US and UL regions existing in both locations figure 2.2 (Murphy *et al.,* 2003).

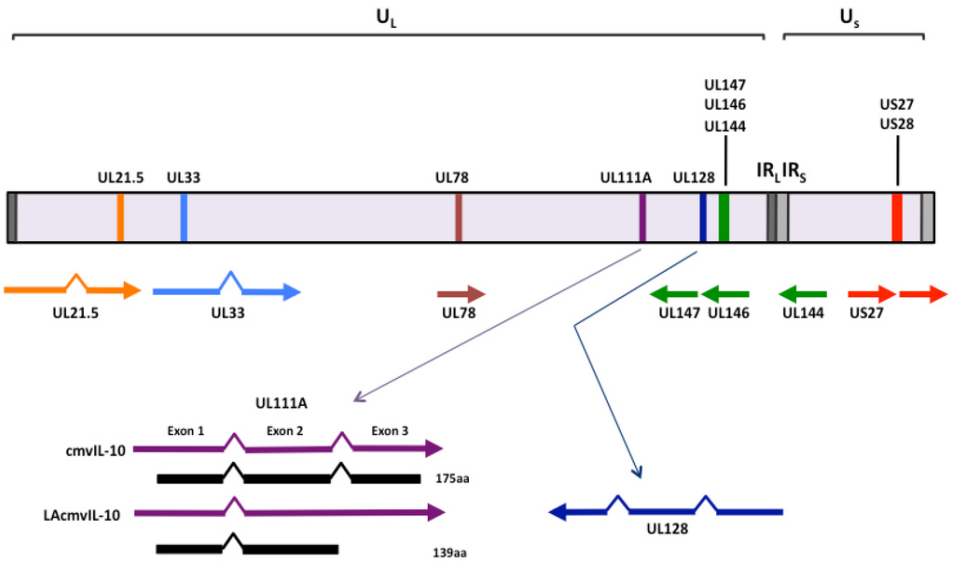


Figure 2.2: Genome organisation of Cytomegalovirus (McSharry *et al*., 2012)**.**

There are several subtypes of hCMV that have been described (Rasmussen *et al.,* 2002). Each strain has a unique biological identity and reinfections may occur with different strains (Rasmussen *et al.,* 2003). The frequency of infections and reinfections with hCMV in different populations is difficult to understand. This is because there is no universal method for their identification. Some studies have analysed clinical isolates for genotyping (Leach *et al.,* 1994; Rasmussen *et al.,* 1997) while some have analysed strain-specific antibody responses to epitopes on different proteins. This are proteins such as envelope proteins gH (AP86, TO86) and gB (AD55, TO55) of different strains such as the AD 169 and Towne strains (Novak *et al.,* 2011).

2.2 Mode of transmission and clinical manifestation of human cytomegalovirus

It is transmitted through person to person contact (Gregory *et al.,* 2003). This may be through sexual contact, saliva, urine, placental transfer, blood transfusion, breast milk and bone marrow transplantation (Ahmad *et al.,* 2010).Human cytomegalovirus can also be transmitted through solid organ transplant which was confirmed by a study in renal and liver transplant patient's analysis. It showed that majority (78%) of the donor and recipient serostatus was hCMV seronegative at time of transplant. They later developed hCMV viremia (primary infection), with hCMV seropositive recipients developing hCMV viremia, 54% of cases been reactivation and re-infection (Atabani *et al.,* 2012). It is also possible for people who have experienced primary infection to be re-infected with another or the same strain of hCMV (Leila *et al.,* 2012). This re-infection does not differ clinically from reactivation although it may be significant epidemiologically to distinguish between reactivation and re-infection (Leila *et al.,* 2012).

The hCMV establishes latency in various types of cells, different from other herpes viruses that mostly remain localized. The most common cell type the virus uses to enhance spreading is the primary fibroblast; a cell type that is not mainly infected in vivo (Emery *et al.,* 2001).The hCMV infects the endothelial cells. Macrophages play a significant role in the establishment of latency and persistence (Figure 2.4). This makes it critical to maintain hCMV within the host (Jarvis & Nelson*,* 2002). The Monocytes, CD 34+ haematopoietic progenitor cells, and endothelial cells are the major sites where the hCMV remains latent and can be easily detected (Crough & Khanna, 2009). The initial infection with hCMV may cause a mild flu like illness and later the virus

remaining dormant if the immune system is fully functional. The Clinical manifestations of hCMV reactivation are rare in immunocompetent individuals but manifest in immunodeficient individuals. It does affect different parts of the body such as mental retardation, deafness and blindness in primary infection in utero. In immunocompromised patients it does cause retinitis, pneumonitis, hepatitis, nephritis and gastroenteritis/ colitis during episodes of primary and reactivation of the hCMV (Knipe & Howley, 2007).

The hCMV genome is highly conserved with minor differentiation between several serotypes (Steininger *et al.,* 2006). They are grounded on the differences in the glycoprotein B, which is part of the virion envelope. The HIV-infected individuals happen to suffer from acquired immunodeficiency syndrome (AIDS). This does lead to the opportunistic infection of hCMV strains due to immunity suppression (Steininger *et al.,* 2006). In patients with AIDS, there is a loss of the immune function, and especially, loss of cell-mediated immunity, which permits hCMV reactivation and replication to begin. There is evidence that the hCMV is linked to a variety of malignancies such as the breast, prostrate, colon, lung and brain (gliomas) (Macgregor *et al.,* 1995). This is as a result of alteration of the regulatory proteins and non-coding RNA which are associated with the malignant phenotype. Asymptomatic excretion of human cytomegalovirus can be detected in urine in approximately 50% of HIV-infected individuals with a CD4 lymphocyte count <100 cells/μL (Macgregor *et al.,* 1995).

2.3Human Cytomegalovirus replication

The hCMV infects a wide spectrum of cell types. The main pathway of entry into host cells is mediated through a membrane fusion event. It involves multiple receptor-ligand interactions on the cell surface. The second pathway involves endocytosis of the enveloped capsid within the membrane of the endocytic vesicle (Ryckman *et al.,* 2006). Many different cell surface molecules can serve as receptors for virus entry. Nevertheless only two viral complexes glycoprotein B (gB) and the glycoprotein gH/gL dimer have been shown to be essential for entry. (Vanarsdall & Johnson, 2012). The interaction of gB with heparan sulphate glycoprotein is believed to initiate a signalling cascade. This cascade allows the interaction between cell surface molecules and viral glycoproteins (Compton & Feire, 2007).

The virus nucelocapsid containing the viral genome and tegument is deposited into the cytoplasm. The capsid is transported along cytoplasmic microtubules and is translocated to the nucleus. This is where the viral DNA is released to enter through nuclear pores as a linear molecule (Mocarski *et al.,* 2007). The double stranded DNA undergoes circularisation. The gene expression pathway of hCMV follows a standard template for herpesviruses (Fortunato & Spector, 1999). During the first 4 hours, immediate early (IE) gene expression occur post infection. The early (E) gene expression and the synthesis of proteins key for viral replication do follow. Finally late (L) gene synthesis occurs, producing structural proteins that are used to construct new virions (Mocarski *et al.,* 2007). The hCMV genome replicates using a rolling circular mechanism. This

produces a concatameric structure, which needs to be cleaved into unit genomes lengths prior to encapsulations (Mocarski *et al.,* 2007).

The L and E genes do carry out capsid maturation, encapsulation and release of cells figure 2.3. Viral DNA is encapsulated in the nucleus and matures by moving to the cytoplasm (Mocarski *et al.,* 2007). The nucleocapsids are transported out of the nucleus through a process of envelopment and de-envelopment across the nuclear membranes. This process is called nuclear egress figure 2.3 (Mettenleiter *et al.,* 2013). This process occurs in different subcellular compartments. The first step (envelopment) the viral capsid starts at the inner nuclear membrane where a complex of two proteins is encoded by the genes UL50 and UL53. This facilitates disruption of the nuclear lamina by recruiting cellular protein kinase C (PKC), and/or the viral protein kinase UL97 to phosphorylate lamins (Mettenleiter *et al.,* 2009). This process delivers viral particles to the perinuclear space where they undergo a de-envelopment event. A secondary process (final envelopment) occurs in the cytoplasm in close proximity to the Golgi apparatus figure 2.3. Tegument proteins are added to the nucelocapsid as it travels through the cytoplasm (Mocarski *et al.,* 2007). Once the virions are packaged, they are shed from the host cell through an exocytosis mechanism. The vacuoles containing the enveloped infectious virions are transported to the plasma membrane where they fuse. This does result in the release of mature virions from the infected cell into the extracellular space figure 2.3 (Mettenleiter *et al.,* 2013).

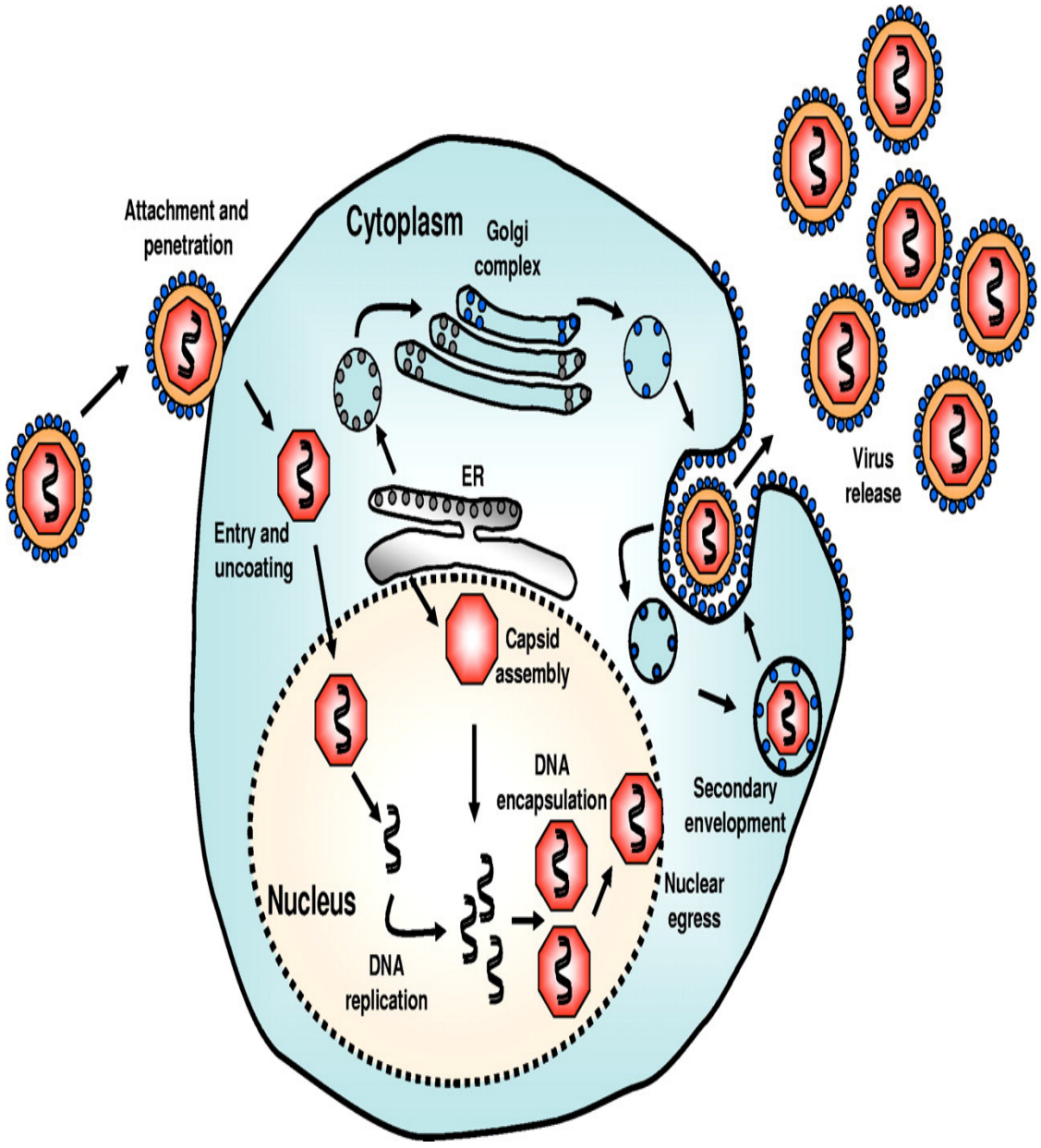


Figure 2.3: Replication cycle of cytomegalovirus (Crough & Khanna, 2009).

2.4 Immune Response to Cytomegalovirus Infection

The initial infection the human immune system does contribute a large and continuous portion of its response to limit hCMV replication (Elkington *et al.,* 2003). Due to the strict host specificity of hCMV, the investigation of functional immune responses in human disease is restricted. The human immune response to hCMV infection is generated by cells of both the innate and adaptive immune systems.

The innate immune system is the first line of defence against viral infection. This response is essential for the early detection of hCMV as it enters the host cell to establish infection. The host recognises the virus as foreign and activates several mechanisms and pathways of innate immune response. These include inflammatory cytokines and interferon which help establish an antiviral state (Marshall & Geballe, 2009). It does lead to up regulation of co-stimulatory molecules that are crucial for priming the adaptive immune response and also includes recruitment of professional antigen presenting cells (APCs), phagocytes and NK cells (Figure 2.4) (Isaacson *et al.,* 2008).

The innate immunity in the perinatal period is an important host defence against hCMV infection, due to the immaturity of the adaptive immune response (Gibson *et al.,* 2004). The hCMV recognition by the innate response is mediated by TLR-2 which recognises gB and gH, leading to activation of the NF-κB-dependent signal transduction pathway (Boehme *et al.,* 2006).Production of the inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8) and interferons (IFNs) are generated in response to nuclear factor kappa B (NF- κB)

activation, function to activate natural killer (NK) cells (Figure 2.4). The natural killer cells function is to limit the replication and spread of the hCMV virus (Juckem *et al.,* 2008).

This innate response is the primary line of defense and is backed by development of adaptive immunity. Specific adaptive immunity via cytotoxic T-lymphocytes is responsible for the final clearance of the virus in the host (Mocarski *et al.,* 2007).

Human CMV infection in immunocompetent individual does trigger the establishment of humoral immunity. The humoral immunity is important during the viremic phase of infection or reinfection. Antibodies are produced directly against the viral proteins to participate in blocking infection of other cells (Jarvis & Nelson, 2002).This proteins of hCMV include structural tegument proteins (e.g., pp65 and pp150), envelope glycoproteins (predominantly gB and gH and gH/gL multimeric complexes), and non-structural proteins such as the IE1 protein (Scrivano *et al* 2011).

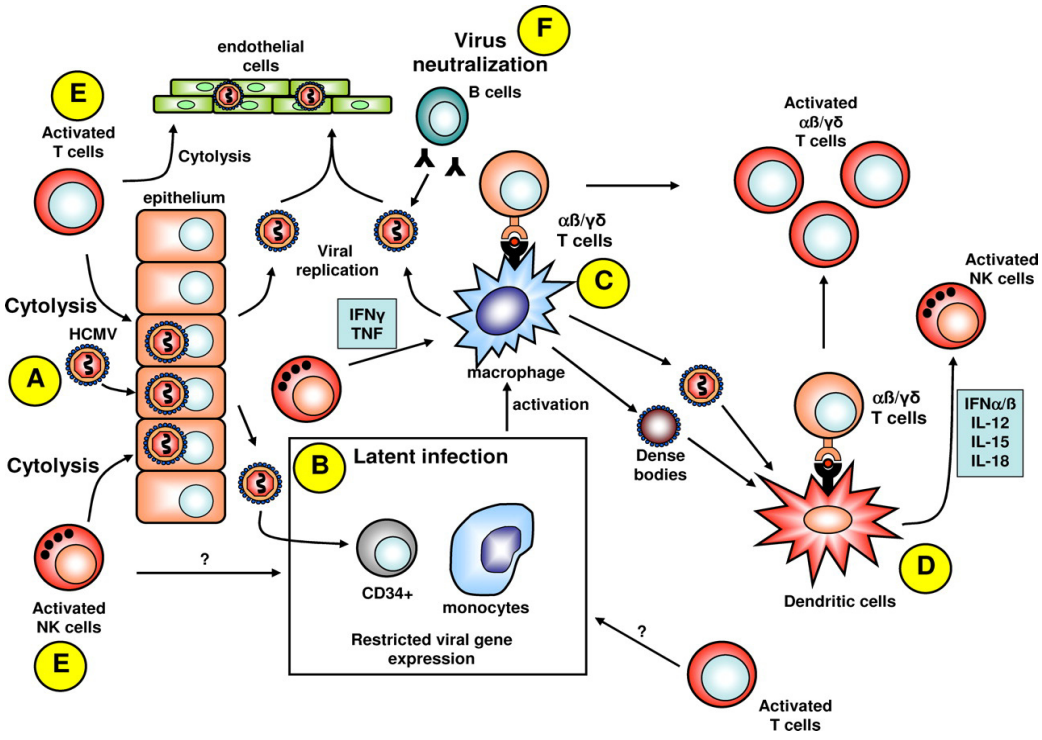


Figure 2.4: Immune response to cytomegalovirus (Crough & Khanna, 2009).

2.5 Pathogenesis and pathology of Human cytomegalovirus coinfection

The hCMV is an opportunistic pathogen, linked to HIV disease progression. The HIV pandemic combined with over 80% of primary hCMV infections occur during infancy(Matthew *et al.,* 2013).A damaged immune system permits the reactivation and replication of hCMV. A synergistic effect may deteriorate the progress of a HIV infected persons (Chakravarti *et al*., 2009). The hCMV extent of damage to the host differs widely and is associated with its 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).

The reactivation of hCMV in AIDS patients is due to the tumour necrosis factor (alpha-TNF) mediated stimulation of the host cells. This leads to intranuclear accumulation and the activation of the hCMV DNA replication (Docke *et al*., 1994). The hCMV can infect several different cell types and all major organs. The cochlea is often involved, as is the central nervous system. There is a preference for periependymal neurons and glia, with focal encephalitis and periependymitis (Leila *et al.,* 2012).

In host embryo it causes serious complications, such as microcephaly, mental retardation, spastic paralysis, hepatosplenomegaly, anaemia, thrombocytopenia, deafness, and optic nerve atrophy leading to blindness (Ornoy & Diav-Citrin,2006).About 15% of babies born with the virus they may develop problems at birth or later on in life. This do include hearing difficulties, blindness, learning difficulties, restricted growth, and problems with the lungs, liver, or spleen (Mustakangas *et al.,* 2000).

A small percentage of babies can be handicapped by hCMV infection during early pregnancy. It can also lead to stillbirths which is associated with fetal thrombotic vasculopathy (Enders *et al.,* 2001).The hCMV infection is most common in the gastrointestinal tract (primarily colitis), followed by the central nervous and then haematological abnormalities. The cytomegalovirus disease of the eye, liver, lung and vasculature are also documented with other conditions (Rafailidis *et al.,* 2008).Ina HIV

patient when the immunity gets compromised cardiovascular disease can occur from hCMV reactivation. The increased replication of the hCMV does cause thymic atrophy and T-cell respond poorly to hCMV. The body tries to boost the immune response triggering T- cell senescence that results also to poor T-cell response. Due to increased T-cell senescence cardiovascular disease later ensues (Patrica, 2013).

2.6 Human Cytomegalovirus disease in HIV individuals

Human immunodeficiency Virus patients have impaired immune system leading to reactivation of hCMV. A synergistic effect may worsen the immunological profile and could potentially translate into a more rapid disease progression in HIV infected persons (Chakravarti *et al.,* 2009). The immune status of the immunocompromised host is certainly linked to the pathogenesis of this virus. Morbidity may result due to the delay in response resulting from hCMV infection (Boehme *et al.,* 2006). The hCMV is common in immunocompromised individuals that may result in primary infection, reactivation and reinfection. The hCMV causes severe disease with a high case fatality (Legendre & Pascual, 2008).

Prior to the introduction of HAART hCMV disease was the most common serious opportunistic viral disease in patients with HIV infection. The hCMV disease is rare in patients with CD4+ T cell counts >100 cells/mm3. The risk of hCMV disease increases as CD4+ T cell counts drop <100 cells/mm3, and increases dramatically <50 cells/mm3. Previously to the HAART era, the most common manifestation of hCMV disease in patient with AIDS was retinitis, usually progressing to blindness within months unless

appropriate therapy was given. Other commonly affected organs were the gastrointestinal tract, the nervous system, and the adrenal glands. The hCMV seropositive individuals with sexual exposure as a risk factor for acquisition of HIV are reported to have higher risk of hCMV disease (Arne *et al*., 2012). This is in comparison to other modes of transmission, such as injecting drug use and transfusion of blood products.

In resource-poor settings, there are relatively few studies that have examined the occurrence of hCMV disease in HIV-infected individuals (Heiden *et al.,* 2007). As hCMV disease primarily occurs in patients with immunodeficiency, it has been suggested that patients in developing countries generally die from opportunistic infections such as tuberculosis and pneumocystosis that present at less advanced stages of HIV infection (Maartens *et al.,* 2002). However, hCMV disease may represent a significant cause of morbidity in hCMV seropositive patients with advanced HIV infection even in resource- poor countries (Nissapatorn *et al.,* 2008). During 9advanced AIDS, hCMV can produce debilitating end-organ disease (EOD) including retinitis, colitis, and pneumonitis. During the pre-HAART era, increasing incidence rates for hCMV disease was observed in patients with HIV. Later, after the introduction of HAART, a dramatic fall in the incidence of hCMV disease was observed (Arne *et al*., 2012). Previous to the highly active antiretroviral therapy (HAART) era, some studies also observed that the rates of the hCMV EOD among the patients with advanced HIV infection were approximately 40% or greater. With the advent of HAART, the incidence of the hCMV EOD has reduced (Basawaraju *et al.,* 2011).

2.7 Epidemiology of human cytomegalovirus infection

Human cytomegalovirus is known to infect 45 to 100% of the population worldwide, depending on geographical location, sex, age and socio-economic status (Cannon *et al.,* 2010). Its epidemiology also does vary depending on the regions of the world as shown in figure 2.5 (Lopo *et al*., 2011) and with age its sero-prevalence increases (Crough & Khanna, 2009). The sub-Saharan Africa is the epicentre of the HIV pandemic. It had 1,900,000 new infections in 2010, and a total of 23.2 million individuals living with HIV. The hCMV is a climax opportunistic pathogen, linked with HIV disease progression. Over 80% of primary hCMV infections are seen in the HIV pandemic areas, and it occurs during infancy. Active hCMV infections are frequent and exist just as complex co-infections with other viral, bacterial and fungal infections (Matthew *et al.,* 2013). The prevalence of hCMV infections ranges between 50% and 85% in adults in the United States (Lopo *et al*., 2011). A study has confirmed that African continent, South America and Asia has one of the uppermost prevalence of hCMV (Cannon *et al.,* 2010).The seroprevalence of hCMV is 80% in Western Europe and the United States and 78% -100% in South America, Africa and Asia (Anne *et al*., 2008).

It is one of the most successful human pathogens. In new born baby, hCMV infection is the most common cause of congenital abnormalities, occurring in 0.2% to 2.5% of all births, most commonly it causes hearing loss, but mental retardation and visual impairment are also observed (Syggelou *et al.,* 2010). In Kenya, the hCMV prevalence is at 88.4% among pregnant women (Maingi & Nyamache, 2014). In blood donors it was at done at 97.0% IgG and 3.6% IgM respectively (Njeru *et al.,* 2009). In Kenya, the

hCMV prevalence among pregnant women attending the Thika District level 5 hospital was at 88.4% (Maingi & Nyamache, 2014), while a study done at the Kenyatta National Hospital (KNH) showed that the hCMV prevalence of Anti- CMV IgG and IgM positivity in blood donors was 97.0%, (95% CI 96.45-97.53%), and 3.6% (95% CI 1.7-5.2%) respectively (Njeru *et al*., 2009). In another study carried out in Kenya hCMV DNA was detected in 90% of HIV-exposed uninfected infants and 93% of infants who had acquired HIV-1 in utero (Slyker *et al.,* 2009).

The prevalence of hCMV is high in developing countries given the knowledge that HIV and hCMV share several modes of transmission. The seroprevalence of hCMV is extremely high in HIV-infected populations. Almost 90% of heterosexual who were HIV positive in Ghana were positive for hCMV IgG (Compston *et al*., 2009). In resource-poor settings a large proportion of children undergo simultaneous primary HIV and hCMV infection (Miles *et al.,* 2007; Slyker *et al.,* 2009). The hCMV infection is more common in neonates with HIV infection compared with HEU infants (Tembo *et al.,* 2015).

In women of childbearing age hCMV seroprevalence in the UK has been found to be 54% (Tookey, 1992) similar rates were observed in the USA (Staras *et al.,* 2006). This is in contrast in developing countries where the seroprevalence in women of childbearing age is almost 100% (Kenneson & Cannon, 2007).

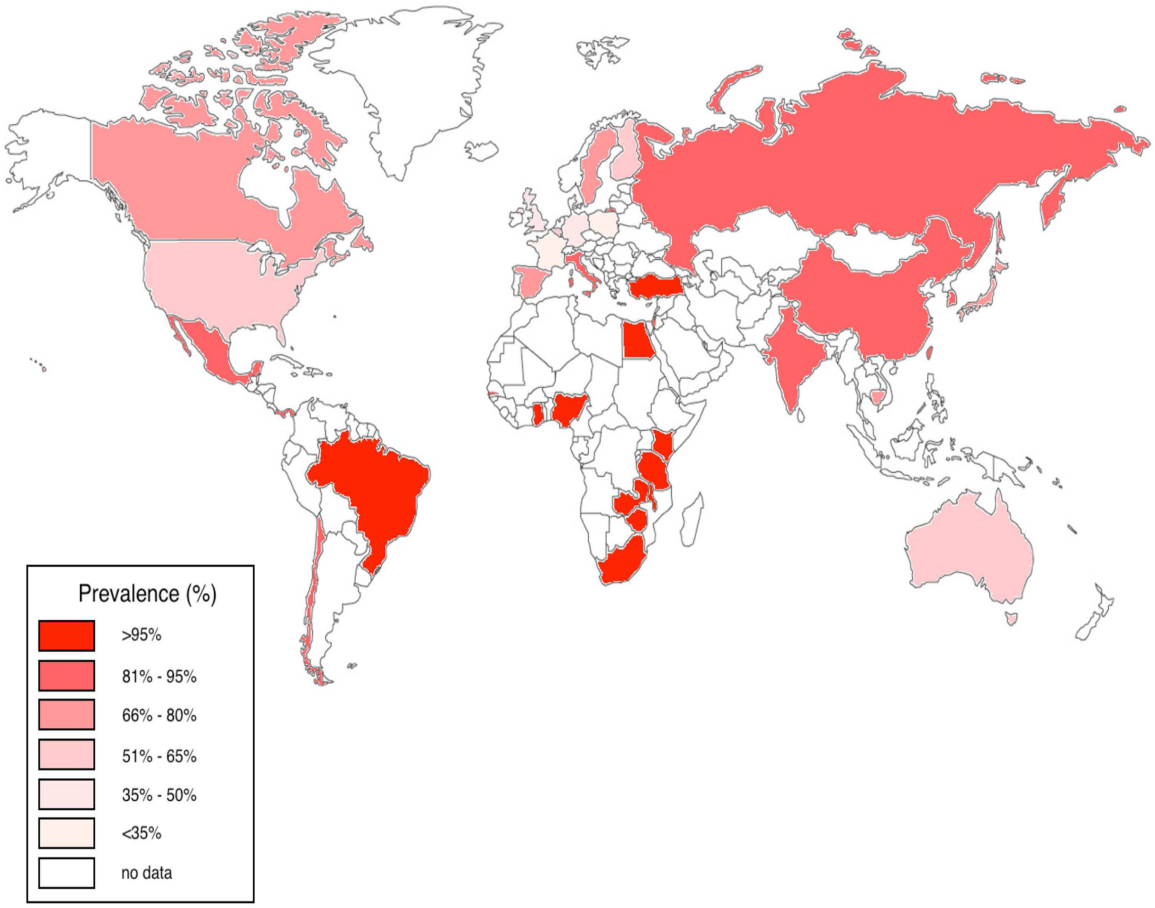


Figure 2.5: Worldwide hCMV seroprevalence rates in adults (Adland *et al.,* 2015)

2.8 Prevention and treatment of Human cytomegalovirus

The use of antiviral treatment for hCMV has proven to be of benefit in immunosuppressed individuals. However there has been scientific debate over the issues of treating hCMV infection in cases of immunocompetent individuals. The major therapeutic strategies used by clinicians are prophylactic or pre-emptive therapy. The purpose is to avoid the development of end stage organ disease. The first step is to start

the therapy universally (prophylactic therapy), and the next is to give antivirals to specific high-risk patients (pre-emptive therapy). The basic principle of pre-emptive therapy is to initiate antivirals for patients displaying viremia early in the clinical course to halt the progression to end organ disease (Meyer *et al.,* 2003). The drugs available for treating hCMV include Ganciclovir (GCV) and Valganciclovir (VGCV), Acyclovir (ACV) and valacylovir (VACV), Mirabivir, Foscarnet and Cidofovir. Ganciclovir is a deoxyguanosine analogue and in 1988 was the first drug to be approved for the treatment of hCMV. Since then, it has remained the first-line treatment for hCMV infections (Balfour 1999).

Most of the studies involving these treatments have been performed in immunocompromised patients and transplant recipients (Razonable *et al.,* 2008).Vaccine development has been tried with failure due to weak protection in human and in seronegative women of childbearing age it failed to provide protection (Plotkin *et al.,* 1989, Adler *et al.,* 1995).The most well documented attempt was a live attenuated hCMV vaccine, based on the Towne 125 strain. It was not successful due to the need of high attenuation to mount sterilizing immune response. It did however prevent or reduce disease in immunosuppressed individual (Plotkin & Huang, 1985). A randomised, double-blind, placebo-controlled clinical trial of a recombinant hCMV gB vaccine with MF59 adjuvant was later conducted. The vaccine protected only 50% of seronegative women against primary hCMV infection having the potential to decrease cases of maternal and congenital hCMV infection (Pass *et al.,* 2009).

The same vaccine was given to both seronegative and seropositive candidates of solid organ transplantation. The vaccine reduced both the level of viraemia and the need for pre-emptive treatment post transplant and the correlate of protective immunity was found to be the antibody titre against gB (Griffiths*et al.,* 2011). Another phase 2, randomised, placebo-controlled clinical trial used two DNA plasmids (gB and pp65) in seropositive patients undergoing stem cell transplants. The need for preventative treatment was reduced and the correlate of protection was the number of ELISPOT forming cells (Kharfan *et al.,* 2012). The western world has given the hCMV infection a priority and even carried out vaccine trials (Adland *et al.,* 2015).The trials have suggested that hCMV vaccine can both protect against primary hCMV infection and boost natural immunity in the seropositive host.

2.9 Human Cytomegalovirus diagnostic methods

The hCMV diagnostic tests can be grouped into 2 categories: direct detection and indirect examination (virus isolation) (Kohler & Milstein, 1995). In direct examination, the selected 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 & Milstein, 1995). The clinical specimens such as urine or blood are inoculated onto human fibroblast cells and incubated. The cells are observed for a period of time ranging from 2 to 21 days. In the standard tube cell culture technique, hCMV exhibits cytopathic effect (CPE) characterized by foci of flat, swollen

cells. The CPE is directly related to a virus's titer although this method is slow and requires 2–3 weeks before the results are declared positive (Ross *et al.,* 2011).

In serology, there are different methods namely: complement fixation, enzyme-linked immunosorbent assay (ELISA), anticomplement immunofluorescence, radioimmunoassay, and indirect hemagglutination (Pass, 1995). The enzyme-linked immunosorbent assays (ELISAs) are the most widely used. The detection of IgM antibodies has been used as an indicator of acute or recent infection. The IgM capture assays are widely employed and are based on selective binding of IgM antibody to the solid phase. Recombinant IgM assays using recombinant hCMV proteins and peptides have been developed in an attempt to standardize serological assays (Revello & Gerna, 2002). In addition, assays for IgM antibody lack specificity for primary infection because of false-positive results. This is because IgM can persist for months after primary infection, and also IgM can be positive in reactivated hCMV infections (Naumnik *et al.,* 2007).

Due to these limitations of the IgM assays, IgG avidity assays are utilized in some populations. This is to help distinguish primary from non-primary hCMV infection. These assays are based on observation for IgG antibodies of low avidity that could be present during the first few months. At the start of an infection the avidity increases over time reflecting maturation of the immune response. Therefore, high anti-CMV IgG avidity represents longstanding infection in an individual (Revello & Gerna, 2002).

Detection of hCMV DNA by polymerase chain reaction (PCR) is a highly specific and very sensitive method. This detects the onset of viremia 1 to 2 weeks prior to either culture or antigenemia test (Picone *et al.,* 2009). It is a widely available method for cytomegalovirus detection based on amplification of nucleic acids. The techniques usually target major immediate early and late antigen genes. This method can either be qualitative or quantitative, in which the amount of viral DNA in the respective sample is measured (Pignatelli *et al.,* 2004).

Immunohistochemistry is performed primarily on tissue or body fluid samples. The slides are made from frozen sections of biopsy tissue samples or centrifuged cells. Monoclonal or polyclonal antibodies against early hCMV antigens are applied and visualized by fluorescently labelled antibodies or enzyme labelled secondary antibodies which are visualized by the change of colour of the substrate. The stained slides are then examined by fluorescent or light microscopy. This technique is more sensitive and very specific although false negative results can also occur due to focal distribution of the virus (Ross *et al.,* 2011).

The prevalence of the hCMV infection in HIV-infected patients is an important problem that needs to be known. This is due to the effects it has on the HIV patients when their immunity is suppressed. Human cytomegalovirus causes mortality and even morbidity if not detected and treated early. This will provide information on the magnitude of the hCMV to the MOH and other health professionals on designing a policy that will help on control of hCMV in HIV patients.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at the comprehensive care clinic of Kenyatta National Hospital. The comprehensive care clinic provides comprehensive and coordinated HIV primary care services to HIV-positive adults, adolescents and children. The Kenyatta National Hospital is a referral hospital and has a bed capacity of 1,800.It is situated in Nairobi's South-western zone approximately 5 km from the Nairobi City Centre (Appendix I). The hospital also has a well-established comprehensive care clinic thus enhancing access of the target population.

3.2 Study design and population

This was a descriptive cross sectional study that targeted consenting HIV positive patients who were attending the KNH CCC.

3.2.1 Inclusion criteria

1. Above 18 years.
2. Gave consent.

3.2.2 Exclusion criteria

1. Those who were too sick to offer blood
2. Those who has other chronic opportunistic infections

3.3 Sample size determination

The sample size was obtained using the formula used to calculate the sample size of a cross sectional study (Charan & Biswas, 2013).

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

Where;

N - Minimum number of sample required

Z – Degree of confidence at 95% (1.96)

P - Proportion of the target population 3.6% (Njeru *et al*., 2009).

d - Precision required (95%, 0.05)

$$\frac{1.96^2 x 0.036 (1-0.036)}{(0.05)^2}$$

The minimum sample size = 54,

The sample size was increased to 400 in order to increase the chances of positivity especially for the active hCMV. In addition, between the two weeks of the sample collection this sample size would ensure that the 400 patients would benefit from the study.

3.4 Sampling procedure

Systematic random sampling was used.

The comprehensive care clinic at the Kenyatta national hospital runs daily from 8:00am to 3:00pm and serves an approximate of 200 HIV positive patients daily. Given that the questionnaire was to be administered for approximately 10 minutes. On average, 40 patients were interviewed every day. Therefore an approximate of 200 subjects patients were interviewed within a week.

Sampling interval I = $\frac{N \text{ (population size)}}{n \text{ (sample size)}}$ therefore $\frac{200}{40}$ = 5 sampling interval

The first patient to be recruited was determined by a random number between 1 and 5 (K). The 2nd patient was K+5, the 3rd patient was K+5+5.

3.5 Study procedures

The patients were informed of the study detailing its objectives, risks and benefits. Voluntary participation was sort through informed consent (Appendix II). A questionnaire (Appendix III) was administered to the study subjects in approximately 10 minutes and each filled questionnaires serialised. After informed consent,5ml of blood was aseptically collected from each participant. The samples were transported to the Kenya Medical Research Institute (KEMRI) Centre for Virus Research (CVR)for laboratory analysis. The samples were centrifuged at 1500 revolutions per minute at

room temperature for 10 minutes. The serum was separated and stored at -80^{o}C until use.

3.5.1 Enumeration of CD4 + T- cells

In CD4+ enumeration, FACSCalibur flow cytometer was used (Becton-Dickinson, NJ, and United Kingdom) according to the manufacturer's instructions. Briefly, solution of 20 µl monoclonal antibodies was pipetted in a labelled tube. Patient's whole blood (50 µl) was pipetted in the same labelled tube and after vortexing incubated for 15 minutes in the dark and at room temperature. Lysing solution (450 µl) was added in the mixture and incubated for another 15 minutes after vortexing. The flourochromes were analysed with anautomated acquisition and analysis software. The CD4+T cell counts results were reported as cells per cubic milimeter (Lihana *et al.,* 2009).

3.5.2 Quantification of HIV-1 viral load

The HIV-1 viral load was determined using the Abbott *m2000rt* System (Abbott Molecular Inc., Illinois, and U.S.A) with automated sample extraction, amplification and detection according to the manufacturers. Briefly, RNA was extracted from 0.2 mL of plasma. The RNA extraction and master mix addition protocol was done by the Abbott *m2000rt*sample preparation system form the 0.2 mL plasma. The viral RNA was transferred to Abbott *m2000rt*instrument for viral load detection using the program for 0.2mL RNA amplification with a set lower limit of quantitation at 150 copies/ml (2.18 $\log_{10}$) of plasma (Ochieng *et al.,* 2015).

3.5.3 Diagnosis of Human cytomegalovirus

Plasma samples were thawed at room temperature. The ELISA VIRO-IMMUN Labor-diagnostika GmbH kit was used. The IgM and IgG test were each carried out on separate kits depending on the plate coating. Eight controls and calibrator determinations containing one blank, one negative control for eight calibrators and one positive control per run were pipetted into the respective micro wells. A 100µL of the controls or diluted patients sample and Blank were added into the well. The solutions in the wells were incubated at room temperature (21-25^{0}c) for 30 minutes protected from intense light. This was followed by washing the wells with the washing buffer and adding ready to use peroxidase conjugate to each well. They were incubated at room temperature (21-25^{0}c) for 30 minutes protected from sunlight, followed by a washing step. A 100µLready to use TMB substrate was added into each well. The wells were again incubated at room temperature (21-25^{0}c) in the dark for 10 minutes. A100µL of stop solution was added to each well and a gentle tapping was to be done to ensure homogenous colour distribution and read within 10 minutes. The reading of plate was done when the bottom was free from moisture, with no air bubbles in the wells. Absorbance of the wells content was read at 450nm using an ELISA plate reader (Multiskan ex and Finland) (Alonso *et al.,* 2013).

3.6 Data management

3.6.1 Data collection

Data collection was done at the Comprehensive care clinic at Kenyatta National hospital. The qualitative data was collected by use of a questionnaire (Appendix III). A questionnaire was used to obtain demographical data and data pertaining to predisposing factors of hCMV such as history of blood transfusion, organ transplant among others. This data was collected by a means of an interviewer administered questionnaire, which took about 10 minutes to complete. The tools were pretested and refined before data collection. It was in two languages; English and Kiswahili. Quantitative was collected from analysis of blood samples.

3.6.2 Data storage

All the data collected was coded, cleaned and entered into a password-protected computer using Microsoft Access. The filled questionnaires were kept under lock and key at the CCC for two weeks till all data was collected. The data was imported into a Microsoft Excel spread-sheet in a password protected computer. Each entry was assigned a unique subject identifier, which was not linked to subject's personal data. A back up was created and updated as data entry progressed using a compact disk, which was stored away from the computer containing the original data.

3.6.3 Data analysis

The data was exported to SPSS software version 22.0 for analysis. Descriptive statistic was done for general description of the study subjects, their CD4, viral load, predisposing factors, and prevalence of hCMV among participants. Bivariate analysis was carried out using chi-square and student t-test. Chi-square was used to test the association between the categorical variables while t-test was used for determining differences of quantitative variables between sub groups. Multivariate analysis (step-wise logistic regression) was performed to identify factors that were independently associated with occurrence of hCMV after adjusting for confounding factors. This was to assess the significance of difference among the groups at 95% confidence interval. Statistical significance was presumed where $p \leq 0.05$.

3.7 Ethical considerations

Informed consent

Informed consent was sought from all participating adults as indicated in the inclusion criteria (Appendix II). For this study, volunteers' signature consent was obtained. Samples from volunteers were identified only by the study code number and their names did not appear in the final data files. Volunteers were at liberty to refuse consent with or without explanation, and without penalty or prejudicial action towards them. Appropriate and valid consent was obtained and documented for all subjects.

Institutional Review Board Approval

The study was undertaken only after obtaining approval from Kenyatta national hospital and university of Nairobi ethics and research review committee (Appendix IV).

CHAPTER FOUR

RESULTS

4.1 Patient socio-demographic characteristics

A total of 400 patients consented to participate and were recruited in the study (Table 4.1). Their mean age (SD) was 42.73 (9.5) years. Of these, 246(61.5%) were females and 154(38.5%) were males. However, age difference between males and females was significant ($p<0.001$). Majority (159, 39.8%) of participants were aged between 39 and 48 years with most of them (253, 63.2%) being either divorced or separated. Half of the participants, 199 (50.4%) had multiple sexual partners. Majority 345 (93.5%) had been treated for more than 12 months. There were no participants who had a history of blood transfusion and intravenous drug use (Table 4.1).

Table 4.1: Socio-demographic characteristics of HIV-infected patients attending the Kenyatta National Hospital comprehensive care clinic

Variable	Frequency(N=400)	Percentages (%)
Age		
19-28 years	26	6.5
29-38 years	113	28.2
39-48 years	159	39.8
≥ 49 years	102	25.2
Gender		
Male	154	38.5
Female	246	61.5
Marital status		
Single	17	4.3
Married	130	32.5
Divorced/Widowed/Separated from spouse	253	63.2
Employment		
Not employed	85	21.2
Informal employment	169	42.3
Formal employment	146	36.5
Income* (ksh) 398		
≤9999	158	39.7
10000-19999	66	16.6
≥20000	174	43.7
Education		
Primary level	90	22.5
Secondary level	183	45.8
Tertiary level	127	31.8
Sex partners* 395**		
1	196	49.6
≥2	199	50.4
Children		
0	30	7.5
1-2	187	46.7
≥3	183	45.8
Ksh=Kenya shillings		

4.2 The Sero prevalence of human cytomegalovirus

The sero prevalence of human cytomegalovirus was 99 % (Figure 4.1)**.**

The sero positive patients with IgM were 8% while as the seronegative patients were 92%. Patients identified to be seropositive for IgG were 99% (Figure 4.1).

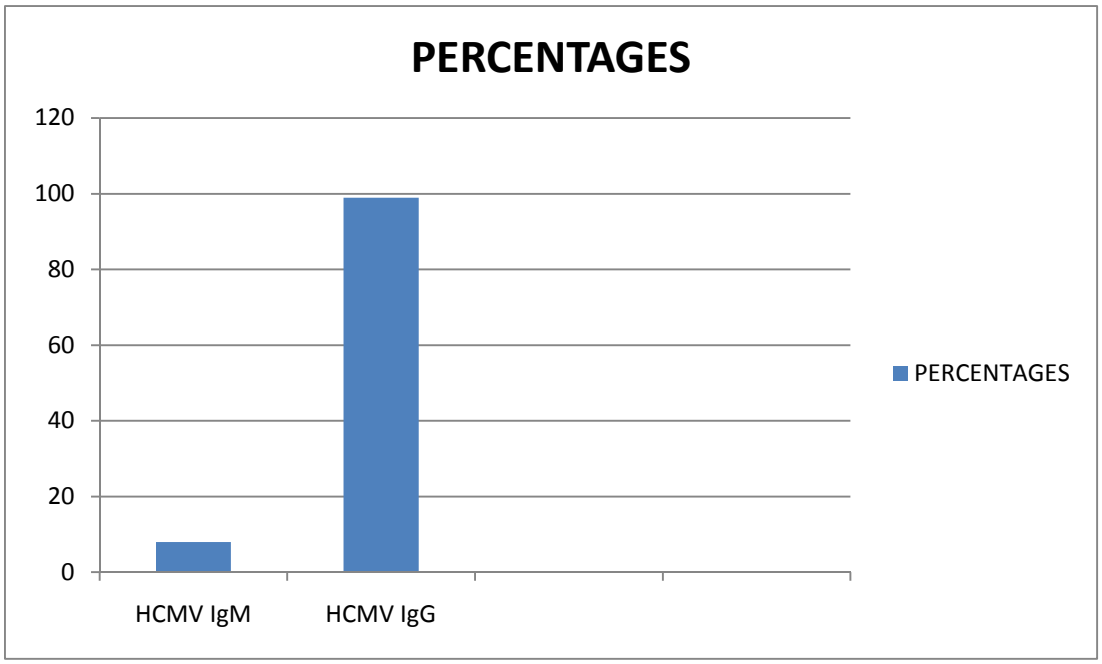


Figure 4.1: The seroprevalence of hCMV among HIV infected patients attending the comprehensive care clinic at Kenyatta National Hospital.

4.3 Factors associated with the occurrence of active human cytomegalovirus (IgM)

Human cytomegalovirus was significantly associated with the age group between 19 and 28 years with a prevalence of 23.1% as compared to 5.9% in the older age group of 49 years and above [OR = 4.8 (95% CI1, 4-16.4)] (Table 4.2). Also, those that had never been married had a higher prevalence of hCMV (23.5%) compared to the widowed/divorced/separated (6.7%) [OR = 4.3 (95% CI1.3-14.5)]. Similarly, patients who had never had children had a significantly higher prevalence (23.3%) as compared to those with more than 3 children (8.7%) [OR=3.2 (95% CI1.2-8.5)] (Table 4.2).Use of HAART treatment for 12 months and below was associated with higher prevalence of hCMV (20.8%) compared to those that were on HAART for more than 12 months [OR=3.5 95% CI (1.2-10.3)] (Table 4.2). Similarly, Bivariate analysis showed that CD4 and viral loads had a statistical significance of ($p < 0.001$) in the respondents who tested positive for hCMV. Therefore there was a positive correlation between CD4 and viral load in the occurrence of an active infection. Other characteristics of the respondents such as gender, employment, income, education and blood transfusion were not significantly associated with the risk of acute cytomegalovirus infection (Table 4.2).

Table 4.2: Factors associated with active HCMV (IgM) infection among HIV-infected patients attending the Kenyatta National Hospital comprehensive care clinic

	IgM			P value
Variables	**Positive (%)**	**Negative (%)**	**OR (95% CI)**	
Gender				
Male	15 (9.7)	139 (90.3)	1.5 (0.7-3.0)	0.310
Female	17 (6.9)	229 (93.1)	1.0	
Age				
19-28	6 (23.1%)	20 (76.9%)	4.8 (1.4-16.4)	0.012
29-38	6 (5.3%)	107 (94.7%)	0.9 (0.3-2.9)	0.855
39-48	14 (8.8%)	145 (91.2%)	1.5 (0.6-4.2)	0.389
≥49	6 (5.9%)	96 (94.1%)	1.0	
Marital status				
Single	4 (23.5)	13 (76.5)	4.3 (1.3-14.5)	0.020
Married	11 (8.5)	119 (91.5)	1.3 (0.6-2.8)	0.536
Divorced/Widowed/Separated	17 (6.7)	236 (93.3)	1.0	
Employment				
Not employed	9 (10.6%)	76 (89.4%)	1.3 (0.5-3.3)	0.547
Informal employment	11 (6.5%)	158 (93.5%)	0.8 (0.3-1.8)	0.562
Formal employment	12 (8.2%)	134 (91.8%)	1.0	
Income (Ksh)				
≤9999	17 (10.8%)	141 (89.2%)	2.0 (0.9-4.5)	0.100
10000-19999	5 (7.6%)	61 (92.4%)	1.3 (0.4-4.1)	0.602
≥20000	10 (5.7%)	164 (94.3%)	1.0	
Education				
Primary level	5 (5.6)	85 (94.4)	0.9 (0.3-2.8)	0.820
Secondary level	19 (10.4)	164 (89.6)	1.7 (0.7-4.1)	0.214
Tertiary level	8 (6.3)	119 (93.7)	1.0	

Variables	Positive (%)	Negative (%)	OR (95% CI)	
Sex partners				
1	15 (7.7)	181 (92.3)	0.9 (0.4-1.8)	0.746
≥2	17 (8.5)	182 (91.5)	1.0	
Number of children				
0	7 (23.3%)	23 (76.7%)	3.2 (1.2-8.5)	0.022
1-2	9 (4.8%)	178 (95.2%)	0.5 (0.2-1.2)	0.138
>3	16 (8.7%)	167 (91.3%)	1.0	
Blood transfusion				
Yes	3 (7.9)	35 (92.1)	1.0 (0.3-3.4)	1.000
No	29 (8.1)	331 (91.9)	1.0	
HAART				
Yes	29 (7.9)	340 (92.1)	1.0	
No	3 (9.7)	28 (90.3)	1.3 (0.4-4.4)	0.727
Duration of HAART				
Up to twelve months	5 (20.8)	19 (79.2)	3.5 (1.2-10.3)	0.031
More than twelve months ago	24 (7.0)	321 (93.0)	1.0	
	Median (95% CI)			P value
	Positive	Negative		
Median CD4 (IQR) cell/ml^3	81.5 (45.0-98.0)	480.5(333.0-658.0)	–	<0.001
Median viral load (IQR) copy/ml	4633(150-177770)	150 (150-150)	–	<0.001

OR = Odds Ratio, CI= Confidence Interval;, IQR= interquartile ratio; HAART= Highly active antiretroviral therapy; IgM= Immunoglobulin M; Ksh=Kenya shillings

4.4 Factors associated with the occurrence of chromic human cytomegalovirus (IgG)

Gender, age, marital status, employment, income, sexual partners, number of children, blood transfusion, HAART and duration of HAART taken did not show any statistical significance. This shows that the above variables did not show any association with the occurrence of chronic hCMV infection (Table 4.3).

Table 4.3: Factors associated with chromic HCMV (IgG) infection among HIV-infected patients attending the Kenyatta National Hospital comprehensive care clinic

	IgG		**P value**
Variables	**Positive (%)**	**Negative (%)**	
Gender			
Male	152(98.7)	2(1.3)	0.148
Female	246(100.0)	0(0.0)	
Age			
19-28	26 (100.0)	0 (0.0)	0.414
29-38	112 (99.1)	1 (0.9)	
39-48	159 (100.0)	0 (0.0)	
≥49	101 (99.0)	1(1.0)	
Marital status			
Single	17 (100.0)	0 (0.0)	1.000
Married	129 (99.2)	1 (0.8)	
Divorced/Widowed/Separated	252 (99.6)	1 (0.4)	
Employment			
Not employed	85 (100.0)	0 (0.0)	0.511
Informal employment	167 (98.8)	2 (1.2)	
Formal employment	146 (100.0)	0 (0.0)	
Income (Ksh)			
≤9999	158 (100.0)	0 (0.0)	0.305
10000-19999	65 (98.5)	1 (1.5)	
≥20000	173 (99.4)	1 (0.6)	
Education			
Primary level	90 (100.0)	0 (0.0)	1.000

Variables	Positive (%)	Negative (%)	
Secondary level	182 (99.5)	1 (0.5)	
Tertiary level	126 (99.2)	1 (0.8)	
Sex partners			
1	195 (99.5)	1 (0.5)	1.000
≥2	198 (99.5)	1 (0.5)	
Number of children			
0	30 (100.0)	0 (0.0)	1.000
1-2	186 (99.5)	1 (0.5)	
>3	182 (99.5)	1 (0.5)	
Blood transfusion			
Yes	38 (100.0)	0 (0.0)	1. 000
No	358 (99.4)	2 (0.6)	
HAART			
Yes	31 (100.0)	0 (0.0)	1.000
No	367 (99.5)	2 (0.5)	
Duration of HAART			
Up to twelve months	24 (100.0)	0 (0.0)	1.000
More than twelve months ago	343 (99.4)	2 (0.6)	

Continuation of table 4.3

Log10 CD4 cells+ 1 count, mean (SD) cell/ml^3	3.6 (0.4)	3.7 (0.0)	0.752
Log10 viral load + 1, mean (SD) copy/ml	3.6 (0.9)	3.2 (0.0)	0.532
OR = Odds Ratio, CI= Confidence Interval;, IQR= interquartile ratio; HAART= Highly active antiretroviral therapy; IgM= Immunoglobulin M; Ksh Kenya shillings			

4.5 Multivariable analysis

After the analysis of the factors associated with IgM active infection of hCMV, those that showed a statistically significance proceeded to multivariable (Step-wise logistic regression) analysis. This was done in order to remove any confounding factors giving the exact factors that show an association to active hCMV infection.The CD4 (p=<0.001) and viral loads (p <0.001) were the only variables independently associated with the occurrence of active hCMV(Table4.4).

Table 4.4: Factors independently associated with occurrence of active human cytomegalovirus among HIV-infected patients attending the Kenyatta National Hospital comprehensive care clinic

Variables	Adjusted OR (95% CI)	P value
Age		
19-28	2.3 (0.1-42.6)	0.565
29-38	1.8 (0.3-12.1)	0.540
39-48	2.1 (0.5-9.5)	0.333
≥49	1.0	
Marital status		
Single	2.7 (0.2-38.6)	0.467
Married	0.7 (0.2-2.8)	0.630
Divorced/Widowed/Separated	1.0	
Income (Ksh)		
≤9999	1.1 (0.2-4.8)	0.923
10000-19999	1.2 (0.2-6.6)	0.805
≥20000	1.0	
Number of children		
0	2.9 (0.3-27.1)	0.360
1-2	0.8 (0.2-3.1)	0.732
>3	1.0	
Duration of HAART		
Up to twelve months	2.5 (0.5-13.8)	0.289
More than twelve months ago	1.0	
Log10 CD4 cells+ 1 count, mean (SD) cell/ml^3	0.984 (0.978-0.990)	<0.001
Viral load copy/ml		
>=1000	11.9 (4.5-31.4)	<0.001
<1000	1.0	
OR = Odds Ratio, CI= Confidence Interval, IQR= interquartile range; HAART= Highly active antiretroviral therapy; Ksh =Kenya shillings		

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 The sero-prevalence of human cytomegalovirus

The study aimed to establish the seroprevalence of hCMV infections and its associated factors among HIV infected patients attending the Kenyatta National Hospital comprehensive care center. There is a high prevalence of HIV/AIDS in Sub Saharan Africa (Akinbami *et al.,* 2010). This study showed that majority 99% of the HIV participants were sero positive hCMV IgG. This does comply with what has been previously reported in Nigeria, where the prevalence was up to 100% among HIV-1 seropositive patients (Fowotade *et al*., 2015, Akinbami *et al.,* 2010).This is evidence that the virus remains an important co-factor in HIV disease progression. The observed high prevalence suggest that participants in the study had previously been exposed to hCMV.

The findings were different from many developed countries where the hCMV seroprevalence has been found to range from 50-60% (Adeiza *et al.,* 2016, Lübeck *et al.,* 2010).The participants who developed symptomatic hCMV infection may have the infection for a long time. During episodes of immunosuppression by HIV, hCMV becomes pathogenic (Akinbami *et al.,* 2010). The high prevalence could be due to the fact that in Africa the diagnosis of HIV is usually administered at advanced stages.

There has also been a record of poor adherence to treatment providing a chance for hCMV infection (Dyrehave *et al*., 2015).

The anti hCMV IgM sero positive was 8.0 % and 99.0 % for anti-hCMV IgG. The hCMV-specific IgM is a marker of active or recent primary infection while as hCMV-specific IgG is a marker of chronic infection (Adeola *et al.,* 2015).The IgM could be as a result from reactivation of latent hCMV infection or a new exposure to hCMV (Adeiza *et al.,* 2016).The ability to reactivate from latency is a common feature of hCMV. It is likely to occur in healthy individuals but is normally controlled by the host immune response. However, in the immunocompromised individual this can lead to disease and later cause morbidity (Griffiths *et al.,* 2009).

The study result are in compliance with a study reported in Nigeria that 11.1% of HIV-1 infected persons were seropositive for anti-CMV IgM and 93.9% anti-CMV IgG. It is also in agreement with a study in India that reported 89.4% IgG and 10.6% IgM in HIV positive patients (Basawaraju *et al.,* 2011). The high occurrence (99%) of anti hCMV IgG in the study indicates that these patients have been previously infected. The study is also in agreement with a recent study conducted in Nigeria that reported seroprevalence of 86% hCMV IgG and13.2% hCMV IgM among HIV-positive patients (Adeiza *et al.,* 2016).

The study is also in compliance with a study carried out in Lagos where the prevalence was 25% hCMV IgM and 75% hCMV IgG (Akintunde *et al.,* 2014).This was also similar in India where the seroprevalence was between 90 –100% amongst

immunocompetent subjects (Akinbami *et al.,* 2010). Most patients with AIDS who develop clinical signs and symptoms of hCMV infection are usually from reactivation rather than from a primary infection (Akinbami *et al.,* 2010). The high prevalence of hCMV in HIV patients is also similar to blood donors. This is confirmed by a study in Kenya where the sero positivity of hCMV IgG and IgM positivity was 97.0%, (95% CI 96.45-97.53%), and 3.6% (95% CI 1.7-5.2%) respectively (Njeru *et al.,* 2009). This is similar to a study in India where the seroprevalence amongst blood donors was 95.0% hCMV IgG (Kothari *et al.,* 2002).

This high prevalence of hCMV among the HIV patients may pose a danger to HIV progression in various ways. The hCMV may alter the tropism of HIV by helping it form pseudotypes that are no longer restricted to CD4 positive cells. This makes it capable for HIV to infect other hCMV susceptible cells. The hCMV can induce cytokine release to the neighbouring cell activating latent HIV DNA. The hCMV may also activate CD4 expression or other receptors permitting HIV cell entry in cells usually non-susceptible to HIV (Griffiths *et al.,* 2006). The US28 gene of hCMV may encode a chemokine receptor that can substitute for CCR5. This results in entry of the HIV into a CD4 positive cells and fibroblasts (Pleskoff *et al*., 1997). With this state present, hCMV may trigger all these effects leading to progression of HIV infection and replication.

5.1.2 The demographic associated factors of human cytomegalovirus (hCMV) infection

Human cytomegalovirus IgG seropositivity was generally high in the age-group 39-48 [159 (100.0%)] years. This is in agreement with previous findings where hCMV infections occurs worldwide (Bernard *et al*., 2007). It is documented that about four out of five people over age 35 have been infected with cytomegalovirus, usually during childhood or adulthood (Bernard *et al*., 2007). Childhood infection could also be a major form of hCMV acquisition. This is agreement to a study done in Kenya that showed 90% hCMV was detected in HIV-exposed uninfected infants (Slyker *et al.,* 2009) and also high detection of maternal hCMV DNA in the blood near the time of delivery (Slyker *et al*., 2009). There are several mechanisms that explain the increased prevalence of hCMV in HIV-infected neonates. A HIV-infected mother can transmit HIV *in utero* and is expected to be immunosuppressed. This does result in increased risk of reactivating and transmitting hCMV to the infant (Slyker *et al.,* 2009).

Having a single or multiple sexual partners did not show any statistical significance in the study. This is with the knowledge that HIV and hCMV share several modes of transmission (Compston *et al*., 2009). The most common mode of transmission for the two viruses is through sexual contact (pass, 2001).

There was a higher sero positivity of hCMV IgG and hCMV IgM in females than in male. This was in compliance with other studies done in the United States of America and Brazil that identified the female's had a higher seropositivity (Cannon *et al*., 2010;

Adisa *et al.,* 2008).This is in agreement that women are put at a greater risk of acquiring hCMV through sexual intercourse with a Sero positive male partner (Fowler & Pass, 2006).Having such a high seropositivity in female introduction of preventative programs has been implemented. This is due to congenital hCMV infection. The Center for Disease Control and Prevention (CDC) and the American College of Obstetricians and Gynaecologists in the United States have educated pregnant women. This is to reduce their exposure to hCMV to their young children through Saliva and urine (Staras *et al.,* 2008). The educating of pregnant women in good personal hygiene practices such as hand washing, not sharing food utensils and not kissing young children on the mouth has reduced the risk of hCMV infection when compared to non-pregnant mothers attempting conception (Adler *et al.,* 2004).

High parity was not observed as a factor for increased susceptibility to acquisition of hCMV infection. However in other studies high parity has showed that direct contact with secretions from their children's or possibly due to poor hygiene could expose them to hCMV infection(Hamdan *et al.,* 2011).Therefore in a recent randomised controlled trial good hygiene practise showed that hygiene information given to hCMV seronegative pregnant women significantly prevented maternal infection (Revello *et al.,* 2015).This could also be related to mode of transmission (sexual) therefore repeated sexual exposure associated with both HIV and hCMV could lead to this observation (Wester *et al*., 2006).

Distribution of anti-CMV IgM in relation to marital status showed statistical association in the anti-CMV seropositivity among the various marital groups. This is in agreement to other cross sectional studies done in Kenya and Nigeria (Maingi & Nyamache, 2014, Fowotade *et al*., 2015).

5.1.3 The biological associated factors of human cytomegalovirus (hCMV) infection

The CD4 and the occurrence of active hCMV infection showed a statistical significance. This is in concordance with the knowledge that the risk of hCMV disease increases as CD4+ T cell counts drop in the HIV patient. A decrease in CD4+ counts (<50 cells per millilitre) does lead to reactivation or easy reinfection of the hCMV. Studies from Africa have shown that patients with hCMV viremia (hCMV DNA > 200 copies/mL) had a significantly lower CD4 cell count than patients with undetectable hCMV levels (Brantsæter *et al.,* 2012).This is also in concordance with a study in Kenya that showed a trend for lower CD4 cell count in hCMV viremic HIV-infected pregnant women (Slyker *et al.*, 2009). Since advanced HIV disease lowers CD4 cell count, it allows reactivation and replication of latent CMV infections.

Further there was a correlation in the occurrence of hCMV and HIV viral loads in participants who tested positive for active hCMV. This confirms indirect effect on replication of both viruses. Diverse mechanisms used by hCMV can possibly activate latent HIV proviral DNA (Griffiths, 2006).The hCMV may impact on HIV disease progression and death (Estibaliz *et al*., 2008).The progression of AIDS has not only been related to CD4 and HIV viral load but also to hCMV DNA (Fielding *et al.,* 2011). This

has been reported among HIV-infected haemophiliacs, coinfected with hCMV. It has been reported that individuals who develop AIDS have a 2.5 times likelihood to develop the disease as compared to those who were hCMV seronegative (Webster *et al.,* 1989). It has been confirmed that there is a correlation in CD4, viral load and hCMV by a study in Kenya. This is where maternal HIV-1 infected patients CD4 measurements, HIV-1 RNA viral load, hCMV viral load and death was reported to correlate. It was confirmed by subsequent infant disease progression and mortality with no precise mechanisms on how the three factors interacted (Slyker *et al.*, 2009).

The participants who were on HAART showed a statistical significance, this could be due to the fact the HAART reduce the replication of the HIV virus. This results in decreased opportunistic infections (Connick, 2001). When the HAART is not adhered to well or in a drug resistance scenario hCMV Infection occurs (Ying *et al.,* 2011).

Human cytomegalovirus infection and disease are difficult to diagnose and treat in resource-limited settings. There is a lack of sufficient research on effects of preemptive therapy in African settings, and there is no licensed vaccine. The WHO guideline for treatment of HIV infection does not mention hCMV coinfection or hCMV EOD treatment. This shows that the consequences of hCMV coinfection to HIV infected patients is a neglected area warranting future studies to enhance more knowledge on the consequences of hCMV and HIV coinfection. Despite these findings, this study had some limitations; the study was not able to confirm the active disease by PCR. In addition, there was also re-call bias from the patients during data collection.

5.2Conclusion

1. In this study, the prevalence of hCMV IgG was 99.0%, an indication that hCMV is hyper endemic. The hCMV IgM sero positivity was at 8%. The HIV-infected CMV IgM antibody positive patients were also CMV IgG antibody positive.
2. The CD4 and HIV viral load were found to be predisposing factors for active hCMV infections. CD4 and HIV viral load were the major predisposing factors to active hCMV infections after confounding factors were considered.
3. Age, marital status, parity, did not show any statistical significance in the occurrence of HCMV infection among HIV patients. This is with the knowledge that the HCMV is a herpes virus is a virus that is mostly obtained during birth.

5.3 Recommendations

1. There is need for a continuous screening for active hCMV infections in HIV patients. This will be advisable in order to avoid active human cytomegalovirus.
2. It will be important to ensure that the CD4 and viral load levels of the HI V patients are well managed by ensuring that patients are educated in importance of adhering to drugs and clinic to avoid HCMV infection
3. Preventive measures such as education on the modes of transmission and importance of adhering to the HAART will be important. This will be a measure taken to decrease the mortality and morbidity related to hCMV infections.

REFERENCES

Adeiza, M. A., Dalhat, M. M.,Musa, B., Muktar, H. M., Garko, S. B., & Habib, A. G. (2016). Sero epidemiology of cytomegalovirus antibodies in HIV positive and HIV negative adults in Nigeria Sub Saharan. *African journal medical*. *3,* 142-7.

Adeola, F., Iheanyi, O., Olajide, O., & Suleiman, S. (2015) High seropositivity of IgG and IgM antibodies against cytomegalovirus (CMV) among HIV-1 seropositive patients in Ilorin, Nigeria. *African Health Sciences, 15*(1), 1–9.

Adler, S. P., Starr, S. E., Plotkin, S. A.,Hempfling, S. H., Buis, J., Manning, M. L., & Best, A. M.(1995). Immunity induced by primary human cytomegalovirus infection protects against secondary infection among women of childbearing age. *Journal Infectious Disease*, *171*, 26–32.

Adler, S.P., Finney, J.W., Manganello, A.M., & Best, A.M. (2004). Prevention of child-to-mother transmission of cytomegalovirus among pregnant women. *Journal of Paediatric*, *145*, 485-491.

Adland, E., Klenerman, P., Goulder, P., & Matthews, P. C. (2015). Ongoing burden of disease and mortality from HIV/CMV coinfection in Africa in the antiretroviral therapy era.*Frontiers in Microbiology, 6,* 1016.

Adisa, T. M., Bukbuk, D. N., & Harry, T. O. (2008). Maternofoetal transfer of Cytomegalovirus IgG antibodies in Maiduguri, North Eastern Nigeria. *The Internet Journal of Microbiology*; *4*(2).

Ahmad, R. M., Kawo, A. H., Udeani, T. K. C., Manga, S. B., & Ibrahim, M. L., (2010). Seroprevalence of cytomegalovirus antibodies in pregnant women attending two selected hospitals in Sokoto State, Northwestern Nigeria. *Journal of Pure and Applied Science, 3*, 298-303.

Akintunde, O. O., Ayolabi, C ., Opara, M., Akanmu, S. A.,Bamiro, B. S., Adeleye, I. A., Adeoye, G. O., & Seriki, A. T. (2014).Seroprevalence of Human Cytomegalovirus and Rubella Virus Antibodies among Anti-retroviral Naive HIV Patients in Lagos.*International Journal of Tropical Disease and Health,4*(9), 984-992.

Akinbami, A. A., Akanmu, A. S., Adeyemo, T. A., Wright K.O., Dada, M.O., & Dosunmu, A.O., (2010). Cytomegalovirus Antibodies amongst Immunocompromised (HIV) Patients at Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos. *Journal of Medicine*, *11,* 151-154.

Alonso, A. R, Moro-García, M. A, Echeverría, A., Solano-Jaurrieta, J. J., Suárez-García, F. M, & López-Larrea, C. (2013). Intensity of the Humoral Response to Cytomegalovirus Is Associated with the Phenotypic and Functional Status of the Immune System. *Journal of Virology*, *87*(8), 4486–4495.

Anne, B. S., Patricia, G. S.,Sian, F., Rosemary, E. W., Maeve, K. L., Hazzie, M., ... & Hazel, M. D. (2008). Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for Cytomegalovirus. *BMC Infection Disease*, *8*, 139.

Arabzadeh, A.M., Mosavat, S. A., & Eftekhari, N. (2007). Seroepidemiology of Human Cytomegalovirus in Pregnant Women and their Neonates in Kerman City During 2005. *Journal of Kerman University of Medical Sciences*, *4*(14), 279-288.

Arne, B. B., Asgeir, J., Mona, H., Leiv, S., Ezra, N., Sokoine, L. K.,Halvor, R., Johan, N. B., & Dag, K. (2012).Cytomegalovirus viremia in dried blood spots is associated with an increased risk of death in HIV-infected patients: a cohort study from rural Tanzania. *International journal of infectious desease*, *10*, 1016.

Atabani, S. F., Smith, C., Atkinson, C., Aldridge, R. W., Rodriguez-Peralvarez, M., Rolando, N., ... & Griffiths, P. D.(2012). Cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy. *Am. J .Transplant,12*, 2457-2464.

Barbi, M., Binda, S., Caroppo, S., Primache, V. Dido, P., & Guidotti, P. (2001). CMV gB genotypes and outcome of vertical transmission: Study on dried blood spots of congenitally infected babies. *Journal of Clinical virology, 21*(1), 75-9.

Balfour, H. H. Jr., (1999). Antiviral drugs. *The New English journal of Medicine, 340*, 1255-1268.

Basawaraju, A., Mane, P. M., & Vijayadurga, S. (2011). Reactivation of cytomegalovirus in HIV *Journal of Clinical and Diagnostic Research*, *5*(4), 749-751.

Bernard J. T. (2007). Human Cytomegalovirus Species.*Classification of Viral Family (Herpesviridae)*, *2,* 127-128.

Boehme, K.W., Guerrero, M., & Compton, T. (2006). Human cytomegalovirus envelope glycoproteins B and H are necessary for TLR2 activation in permissive cells. *Journal of Immunology, 177*(10), 7094-7102.

Boppana, S. B., Rivera, L. B., Fowler, K. B., Mach, M., & Britt, W. J. (2001).Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. *New England Journal of Medicine, 344*, 1366–71.

Brantsæter, A. B., Johannessen, A., Holberg-Petersen, M., Sandvik, L., Naman, E., Kivuyo, S.L., Rollag, H., Bruun, J. N, Kvale, D. (2012).Cytomegalovirus viremia in dried blood spots is associated with an increased risk of death in HIV-infected patients: a cohort study from rural Tanzania. *International journal of infectious disease*, *16*(12), e879-85.

Chakravarti, A., Tewari, S., & Bhalla, P. (2009).Human Cytomegalovirus Infection among Patients Living with AIDS in a Tertiary Level Hospital in India. *Journal of the International Association of Physicians in AIDS Care, 9*, 94 – 7.

Crough, T. & Khanna, R. (2009). Immunobiology of human cytomegalovirus: from bench to bedside. *Clinical Microbiology Revision, 22*, 76-98.

Cannon, M. J., Scott, S. D., & Terri, B. H. (2010). Review of Cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Revision Medical Virology, 20*, 202–213.

Compston, L. I., Li, C., Sarkodie, F., Owusu, O. S., Opare-Sem, O., & Allain, J. P. (2009). Prevalence of persistent and latent viruses in untreated patients infected with HIV-1 from Ghana, West Africa. *Journal Medical Virology, 81,* 1860-8.

Compton, T. (2004). Receptors and immune sensors: the complex entry path of human cytomegalovirus." *Trends in Cell Biology, 14*(1), 5-8.

Compton, T. & Feire, A., (2007). Early events in human cytomegalovirus infection. *McArdle Laboratory for Cancer Research*, Madison: University of Wisconsin

Connick, E. (2001). Immune reconstitution in HIV-1-infected individuals treated with potent antiretroviral therapy. *Journal Investigation Dermatology Symptoms proceedings*, *6*, 212–218.

Charan, J. & Biswas, T. (2013). How to Calculate Sample Size for Different Study Designs in Medical Research? *Indian Journal of Psychological Medicine*, *35*(2), 121-126.

Deayton, J.R., ProfSabin, C.A., Johnson, M.A., Emery, V.C., Wilson, P., & Griffiths, P.D. (2004). Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV infected patients receiving highly active antiretroviral therapy. *Lancet, 363*, 2116 2121.

Docke, W. D., Prosch, S., & Fietze, E. (1994).Cytomegalovirus reactivation and tumor necrosis factor. *Lancet*, *3436*, 268-9.

Dyrehave, C., Rasmussen, D. N., Honge, B. L., Jespersen, S., Correia, F. G., Medina, C., Wejse, C., & Rodkjaer, L.(2015).Nonadherence is associated with lack of HIV-related knowledge: a cross-sectional study among HIV-infected individuals in Guinea-Bissau. *Journal of the International Association of Providers of AIDS Care*, 1-9.

Emery V. C. (2001).Investigation of CMV disease in immunocompromised patients. *Journal Clinical Pathology, 54*, 84-88.

Estibaliz, L., Gaelle, C., Jérémie, G., Patrick, B.,Isabelle, P., Daniel, C., François, D., ... & Rodolphe, T. (2008). Change in T-lymphocyte count after initiation of highly active antiretroviral therapy in HIV-infected patients with history of Mycobacterium avium complex infection.*Antiviral Therapy*, *11*(3), 343–350.

Enders, G., Bäder, U., & Lindemann, L. (2001). Prenatal diagnosis of congenital cytomegalovirus infection in 189 pregnancies with known outcome. *Prenatal Diagnosis*, *21,* 362.

Elkington, R., Walker, S., Crough, T., Menzies, M., Tellam, J., Bharadwaj, M., & Khanna, R. (2003). Ex vivo profiling of CD8+-T-cell responses to human cytomegalovirus reveals broad and multispecific reactivities in healthy virus carriers. *Journal of Virology, 77*, 5226-5240.

Erlandson, K.M., Allshouse, A.A., Rapaport, E., Palmer, B.E., Wilson, C.C., Weinberg, A., ...& Campbell T. B. (2015). Physical function impairment of older, HIV-

infected adults is associated with cytomegalovirus immunoglobulin response. *AIDS Research and Human Retroviruses, 31*, 905–912.

Fielding, K., Koba, A., Grant, A. D., Charalambous, S., Day, J., Spak, C., Wald, A., ...& Churchyard, G. J.(2011). Cytomegalovirus viremia as a risk factor for mortality prior to antiretroviral therapy among HIV-infected gold miners in South Africa. *PLoS ONE, 6*(10)

Fowotade, A., Okonko, I.O., Agbede, O.O., & Suleiman, S.T. (2015). High seropositivity of IgG and IgM antibodies against cytomegalovirus (CMV) among HIV-1 seropositive patients in Ilorin, Nigeria, *African Health Sciences*, *15*(1).

Fowler, K. B. & Pass, R.F. (2006). Risk factors for congenital cytomegalovirus infection in the offspring of young women: exposure to young children and recent onset of sexual activity. *Pediatrics, 118*, e286-e292.

Fortunato, E. A. & Spector, D. H., (1999). Regulation of human cytomegalovirus gene expression. *Advances in Virus Research*, *54*, 61-128.

Gandhi, M.K., & Khanna, R. (2004). Human cytomegalovirus: Clinical aspects, immune regulation, and emerging treatments. *Lancet Infectious Diseases*, (12), 725-38.

Gregory, H.T. (2003). Cytomegalovirus. *American Family Physician*, *67*(3), 519-524, 526.

Gibson, L., Piccinini, G., Lilleri, D., Revello, M. G., Wang, Z., Markel, S., Diamond, D. J., & Luzuriaga, K. (2004). Human cytomegalovirus proteins pp65 and immediate

early protein 1 are common targets for CD8+ T cell responses in children with congenital or postnatal human cytomegalovirus infection. *Journal of immunology, 172,* 2256-2264.

Griffiths, P. D. (2006). CMV as a cofactor enhancing progression of AIDS.*Journal of Clinical Virology, 35*, 489–492.

Griffiths, P. D., Stanton, A., McCarrell, E., Smith, C., Osman, M., Harber, M., ... & Burroughs, A. K. (2011). Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial. *Lancet,377*, 1256-1263.

Griffiths, P. D., Emery, V. C., & Milne, R. C. (2009). Cytomegalovirus. In: D. D. Richman, R. Whitley, F. Hayden (Eds.), *Clinical virology*., (pp. 475-506). Washington DC: ASM Press.

Heiden, D., Ford, N., Wilson, D., Rodriguez, W.,Margolis, T., Janssens, B., ... & Drew, L. (2007). Cytomegalovirus retinitis: the neglected disease of the AIDS pandemic. *PLoS Medicine, 4*, e334.

Hamdan, Z.H., Ismail, E.A., Nasser, M. N., & Ishag, A. (2011).Seroprevalence of cytomegalovirus and rubella among pregnant women in western Sudan. *Virology Journal*, *8,* 21.

Http://www.mapsofworld.com/kenya/cities/nairobi.html read on 22/1/2017 2:00pm

Isaacson, M. K., Juckem, L. K., & Compton, T. (2008).Virus entry and innate immune activation. Current Top. *Microbiology Immunology*, *325*, 85-100.

Jarvis, M. A. & Nelson, J.A. (2002).Human cytomegalovirus persistence and latency in endothelial cells and macrophages. *Current Opinion in Microbiology*, (4), 403-7.

Juckem, L. K., Boehme, K. W., Feire, A. L., & Compton, T. (2008). Differential initiation of innate immune responses induced by human cytomegalovirus entry into fibroblast cells. *Journal of Immunology*, *180*(7), 4965-4977.

Kothari, A., Ramachandrum, V. G., Gupta, P., Singh, B., & Talwar, V. (2002). Seroprevalence of Cytomegalovirus among voluntary blood donors in Dehli, India. *Journal of Health Population and Nutrition, 20*(4), 348–351.

Kohler, C. & Milstein, C. (1995). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature Journal*, 256, 495-497.

Kenneson, A. & Cannon, M. J., (2007). Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Revision of Medical Virology*, *17*, 253-276.

Knipe, D. M., & Howley, P. M. (eds.) (2007).*Fields virology.* philadelphia: Lippincott williams & wilkins.

Kharfan-Dabaja, M. A., Boeckh, M., Wilck, M. B., Langston, A. A., Chu, A. H., Wloch, M. K., ... & Kenney, R. T.(2012). A novel therapeutic cytomegalovirus DNA vaccine in allogeneic haemopoietic stem-cell transplantation: a randomised,

double-blind, placebo-controlled, phase 2 trial. *Lancet Infectious Disease.12*, 290-299.

King, C.C., Ellington, S. R., & Kourtis, A.P. (2013).The role of co-infections in mother-to-child transmission of HIV. *Current HIV Resources. 11,* 10–23.

Landolfo, S., Gribaudo, G., & Lembo, D. (2003). The human cytomegalovirus. *Pharmacology & therapeutics*, *98*(3), 269-297.

Lübeck, P. R., Doerr, H. W., & Rabenau, H. F. (2010). Epidemiology of human cytomegalovirus (HCMV) in an urban region of Germany: What has changed? *Medical Microbiology Immunology, 199*, 53-60.

Leila, B., Hossein, M., Narges, S., & Mohammad, G. (2012). Seroprevalence of cytomegalovirus infection among pregnant women in Eastern Iran. *The brazilian journal of infectious diseases*, *16*(4), 402–403.

Legendre, C. & Pascual, M. (2008). Improving outcomes for solid-organ transplant recipients at risk from cytomegalovirus infection: late-onset disease and indirect consequences. *Clinical Infectious Disease, 46*, 732-40.

Lihana, R.W., Khamadi, S.A., Lubano, K., Lwembe, R., Kiptoo, M.K., Lagat, N., ... &, Ichimura, H., (2009). HIV Type 1 Subtype Diversity and Drug Resistance among HIV Type 1-Infected Kenyan Patients Initiating *Antiretroviral Therapy. AIDS Research and Human Retroviruses*, *25*(12), 1211-1217.

Leach, C. T., Detels, R., Hennessey, K., Liu, Z., Visscher, B. R., Dudley, J. P., & Cherry, J. D (1994). A longitudinal study of cytomegalovirus infection in human immunodeficiency virus type 1-seropositive homosexual men: molecular epidemiology and association with disease progression. *Journal of Infectious Disease*, *170*(2), 293-298.

Lilleri, D., Zelini, P., Fornara, C., Comolli, G., Revello, M.G., & Gerna, G. (2009). Human cytomegalovirus-specific CD4+ and CD8+ T cell responses in primary infection of the immunocompetent and the immunocompromised host. *Clinical Immunology,131*(3): 395-403.

Lichtner, M., Cicconi, P., Vita, S., Cozzi-Lepri, A., Galli, M., LoCaputo, S., ...& Monforte, A.D. .(2015). Cytomegalovirus coinfection is associated with an increased risk of severe non-AIDS-defining events in a large cohort of HIV-infected patients. *Journal of Infectious Disease,211*, 178–186.

Lopo, S., Vinagre, E., Palminha, P., Paixão, M. T., Nogueira, P., & Freitas, M. G. (2011).Seroprevalence to cytomegalovirus in the Portuguese population, 2002-2003. *Eurosurveillance, 16*(25).

Matthew, B., Kunda, M., & Alimuddin, Z. (2013). Human Cytomegalovirus (HCMV) Infection in Sub-Saharan Africa, Manifestations of Cytomegalovirus Infection, Prof. Patricia Price (Ed.), 978-953-51-1116-0.

Maingi, Z., & Nyamache, A. K. (2014). Seroprevalence of Cytomegalovirus (CMV) among pregnant women in Thika, Kenya. *BMC Research Notes*, *7*, 794.

Maartens, G. (2002) Opportunistic infections associated with HIV infection in Africa. *Oral Disease*, *8(*Supply 2), 76-9.

MacGregor, R. R.,Pakola, S. J., Graziani, A. L., Montzka, D. P., Hodinka, R. L., Nichols, C.W., & Friedman, H. M. (1995). Evidence of active cytomegalovirus infection in clinically stable HIV-infected individuals with CD4+ lymphocyte counts below 100/microliters of blood: features and relation to risk of subsequent CMV retinitis. *Journal of Acquired Immune Deficiency Syndome in Human Retrovirology, 10*, 324-30.

Murphy, E., Rigoutsos, I., Shibuya, T., Shenk, T. E.(2003).Reevaluation of human cytomegalovirus coding potential. *Proceedings of the National Academy of Science of the United States of America, 100*(23), 13585-90.

Meyer, E., Boland, G. J., & Verdonck, L. F. (2003). Prevention of cytomegalovirus disease in recipients of allogeneic stem cell transplants. *Clinical Microbiology Revision, 16*, 647-657.

Mettenleiter, T.C., Klupp, B. G., & Granzow, H. (2009). Herpesvirus assembly: an update. *Virus Research, 143*, 222-234.

Mettenleiter, T.C., Muller, F., Granzow, H., & Klupp, B. G. (2013). The way out: what we know and do not know about herpesvirus nuclear egress. *Cell Microbiology, 15*, 170-178.

Mustakangas, P., Sarna, S., & Ammälä, P. (2000). Human cytomegalovirus seroprevalence in three socioeconomically different urban areas during the first trimester: a population-based cohort study. *International Journal of Epidemiology*, *29*, 587.

Mocarski, E. S., Jr., & Courcelle, C. T. (2001). *Cytomegaloviruses and Their Replication*, (4th ed.)., vol. 2. Philadelphia, PA: Lippincott, Williams, & Wilkins.

Mocarski, E.S., Shenk, T., & Pass, R.F. (2007). Cytomegaloviruses. in *Fields Virology*.(5th ed.). Edited by: Knipe DM, Howley PM. Philadelphia, PA: Lippincott Williams & Wilkins.

McSharry, B. P., Avdic, S., & Slobedman, B. (2012). Human Cytomegalovirus Encoded Homologs of Cytokines, Chemokines and their Receptors: Roles in Immunomodulation. *Viruses*, *4*(11), 2448–2470.

Miles, D. J., Sande, M., Kaye, S., Crozier, S., Ojuola, O., Palmero, M. S., ... & Marchant, A. (2008). CD4 (+) T cell responses to cytomegalovirus in early life: a prospective birth cohort study. *Journal of Infectious Disease, 197*, 658–662.

Marshall, E.E., & Geballe, A.P., (2009). Multifaceted evasion of the interferon response by cytomegalovirus.*Journal of Interferon Cytokine Research,* 29, 609-619.

Njeru, D.G., Mwanda, W.O., Kitonyi, G.W., & Njagi, E.C. (2009). Prevalence of cytomegalovirus antibodies in blood donors at the National Blood Transfusion Centre, Nairobi: *East Africa Medical Journal*, *86*(12 Supply), S58-61.

Nissapatorn, V. (2008). Lessons learned about opportunistic infections in south east Asia. Southeast Asian *Journal Tropical Medicine Public Health*, *39*, 625-41.

Novak, Z., Chowdhury, N., Ross, S. A., Pati, S. K., Fowler, K., & Boppana, S. B. (2011). Diagnostic consequences of cytomegalovirus glycoprotein B polymorphisms. *Journal Clinical Microbiology, 49*(8), 3033-3035.

Naumnik, B., Malyszko, J., Chyczewski, L., Kovalchuk, O., & Mysliwiec, M. (2007) Comparison of serology assays and polymerase chain reaction for the monitoring of active cytomegalovirus infection in renal transplant recipients. *Transplantation Proceedings, 39*(9), 2748–2750.

Ornoy, A & Diav-Citrin, O. (2006). Fetal effects of primary and secondary cytomegalovirus infection in pregnancy. *Reproductive Toxicology, 21*, 399.

Ochieng, W., Kitawi, R.C., Nzomo, T.J., Mwatelah, R.S., Kimulwo., M.J., Ochieng, D.J., ... &, Aman, R. (2015). Implementation and Operational Research: Correlates of Adherence and Treatment Failure Among Kenyan Patients on Long-term Highly Active Antiretroviral Therapy. *Journal Acquired Immune Deficiency Syndrome*, *69*(2), e49-56.

Pass, R. F (2001). Cytomegalovirus, p. 2675-2706. In D. M. K. a. P. M. H. (ed.) (ed.), *Fields Virology*, (4th ed.). vol. 2. Philadelphia, PA: Lippincott, Williams, & Williams,

Pass, R. F., Zhang, C., Evans, A., Simpson, T., Andrews, W., Huang, M. L., ... & Cloud, G. (2009). Vaccine prevention of maternal cytomegalovirus infection. *The New English Journal of Medicine, 360,* 1191-1199.

Pass, R. F., Britt, W. J., & Stagno, S. (1995). Cytomegalovirus. In: Lennette EH, Lennette DA, Lennette ET, editors. *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections.* (5th ed.) Washington D.C.: American Public Health Association.

Patrica, P. (2013). The Footprint of CMV Infection May Last a Lifetime. 2013 Price; licensee *InTech*

Picone, O., Vauloup-Fellous, C., & Cordier, A. G. (2009). A 2-year study on cytomegalovirus infection during pregnancy in a French hospital. *Journal of Gynaecology*, *116*, 818.

Pignatelli, V., Cepko, C. L., & Strettoi, E. (2004).gN variants in cytomegalovirus infection. *Journal of Congenital Neurology, 469*, 351–359.

Pleskoff, O., Treboute, C., Brelot,A., Heveker, N., Seman, M., Alizon, M. (1997) Identification of a chemokine receptor encoded by human cytomegalovirus as a cofactor for HIV-1 entry. *Science*, *276*, 1874–1878.

Plotkin, S.A., & Huang, E.S. (1985). Cytomegalovirus vaccine virus (Towne strain) does not induce latency. *Journal of Infectious Disease, 152*, 395-397.

Plotkin, S.A., Starr, S.E., Friedman, M.H., Gonczol, E., & Weibel, R.E. (1989) Protective effects of Towne cytomegalovirus vaccine against low-passage cytomegalovirus administered as a challenge. *Journal of Infectious Disease, 159*, 860-865.

Razonable, R.R. (2008). Cytomegalovirus infection after liver transplantation: Current concepts and challenges. *World Journal of Gastroenterology : WJG*, *14*(31), 4849–4860.

Rasmussen, L., Geissler, A., Cowan, C., Chase, A., & Winters, M. (2002). The genes encoding the gCIII complex of human cytomegalovirus exist in highly diverse combinations in clinical isolates. *Journal of Virology*, *76*(21), 10841-10848.

Rasmussen, L., Geissler, A., & Winters, M. (2003). Inter- and intragenic variations complicate the molecular epidemiology of human cytomegalovirus. *Journal of Infectious Disease*, *187*(5), 809-819.

Rasmussen, L., Hong, C., Zipeto, D., Morris, S., Sherman, D., Chou, S., ... & Merigan, T. C. (1997). Cytomegalovirus gB genotype distribution differs in human immunodeficiency virus-infected patients and immunocompromised allograft recipients. *Journal of Infectious Disease*, *175*(1), 179-184

Rafailidis, P.I., Mourtzoukou, E.G., Varbobitis, I.C., & Falagas, M.E. (2008). Severe cytomegalovirus infection in apparently immunocompetent patients: a systematic review. *Virology journal,* 5, 47.

Revello, M. G., Tibaldi, C., Masuelli, G., Frisina, V., Sacchi, A., Furione, M.,... & Todros, T. (2015). Prevention of Primary Cytomegalovirus Infection in Pregnancy. *EBio Medicine*, *2*(9), 1205-10

Ross, S. A., Novak, Z., Pati, S., & Boppana, S. B. (2011).Diagnosis of Cytomegalovirus Infections. *Infectious Disorders Drug Targets*, *11*(5), 466–474.

Reus, S., Portilla, J., Gimeno, A., Sánchez-Payá, J., García-Henarejos, J.A., Martínez Madrid, O., ... & López-Aldeguer J (2004). Predictors of progression and death in patients with advanced HIV infection in the era of highly active antiretroviral therapy. *EnfermedadesInfecciosasy MicrobiologíaClínica, 22*, 142–149.

Revello, M.G., & Gerna, G. (2002). Diagnosis and Management of Human Cytomegalovirus Infection in the Mother, Fetus, and Newborn Infant. *Clinical Microbiology Reviews*, *15*(4), 680–715.

Ryckman, B.J., Jarvis, M.A., Drummond, D.D., Nelson, J.A., & Johnson, D.C., (2006). Human cytomegalovirus entry into epithelial and endothelial cells depends on genes UL128 to UL150 and occurs by endocytosis and low-pH fusion. *Journal of Virology*, *80*, 710-722.

Scrivano, L., Sinzger, C., Nitschko, H., Koszinowski, U. H., & Adler, B. (2011). HCMV Spread and Cell Tropism are Determined by Distinct Virus Populations. *PLoS Pathogens*, *7*(1).

Steininger, C., Puchhammer-Stockl, E., & Popow-Kraupp, T. (2006).Cytomegalovirus disease in the era of highly active antiretroviral therapy (HAART). *Journal Clinical Virology*; *37*, 1-9.

Slyker, J.A., Lohman-Payne, B.L., John-Stewart, G.C., Maleche-Obimbo, E., Emery, S., Richardson, B., ... & Rowland-Jones, S.L. (2009). Acute cytomegalovirus infection in Kenyan HIV-infected infants: *AIDS, 23(*16), 2173-81.

Slyker, J.A., Lohman-Payne, B.L., Rowland-Jones, S.L., Otieno, P., Maleche-Obimbo, E., Richardson, B., ... & John-Stewart, G.C. (2009). The detection of cytomegalovirus DNA in maternal plasma is associated with mortality in HIV-1-infected women and their infants. *AIDS*, *23*, 117–124.

Staras, S. A., Flanders, W. D., Dollard, S. C., Pass, R. F., McGowan, J. E., Jr., & Cannon, M. J. (2008). Cytomegalovirus seroprevalence and childhood sources of infection: A population-based study among pre-adolescents in the United States. *Journal of Clinical Virology, 43*, 266-271.

Staras, S. A., Dollard, S. C., Radford, K. W., Flanders, W. D., Pass, R. F., & Cannon, M. J. (2006). Seroprevalence of cytomegalovirus infection in the United States, 1988-1994.*Clinical Infectious Disease, 43*, 1143-1151.

Syggelou, A., Iacovidou, N., Kloudas, S., Christoni, Z., & Papaevangelou, V. (2010). Congenital cytomegalovirus infection. *Annals of the New York Academy of Sciences, 1205*, 144-147.

Tembo, J., Kabwe, M., Chilukutu, L., Chilufya, M., Mwaanza, N., Chabala, C., Zumla, A., & Bates, M. (2015) Prevalence and risk factors for betaherpesvirus DNA emia in children >3 weeks and <2 years of age admitted to a large referral hospital in sub-Saharan *Africa Clinical Infectious Disease, 60*(3), 423-31.

Tookey, P. A., Ades, A. E., & Peckham, C. S., (1992). Cytomegalovirus prevalence in pregnant women: the influence of parity. *Archives of Disease in Childhood, 67*, 779-783.

Vanarsdall, A. L & Johnson, D. C. (2012). Human cytomegalovirus entry into cells.*Current Opinion in Virology*, *2,* 37-42.

Wester, C.W., Bussman, H., Moyo, S, Avalos A, Gaolathe, T., Ndwapi, N., Essex M... & Marlink, R.G. (2006). Serological Evidence of HIV-Associated Infections among HIV-1-infected Adults in Botswana.*Clinical Infectious Diseases*, *43*, 1612-1615.

Webster, A., Grundy, J. E., Lee, C.A., Emery, V. C., Cook, D. G., Kernoff, P. B., & Griffiths, P. D. (1989). Cytomegalovirus infection and progression to AIDS. *Lancet, 2*, 681.

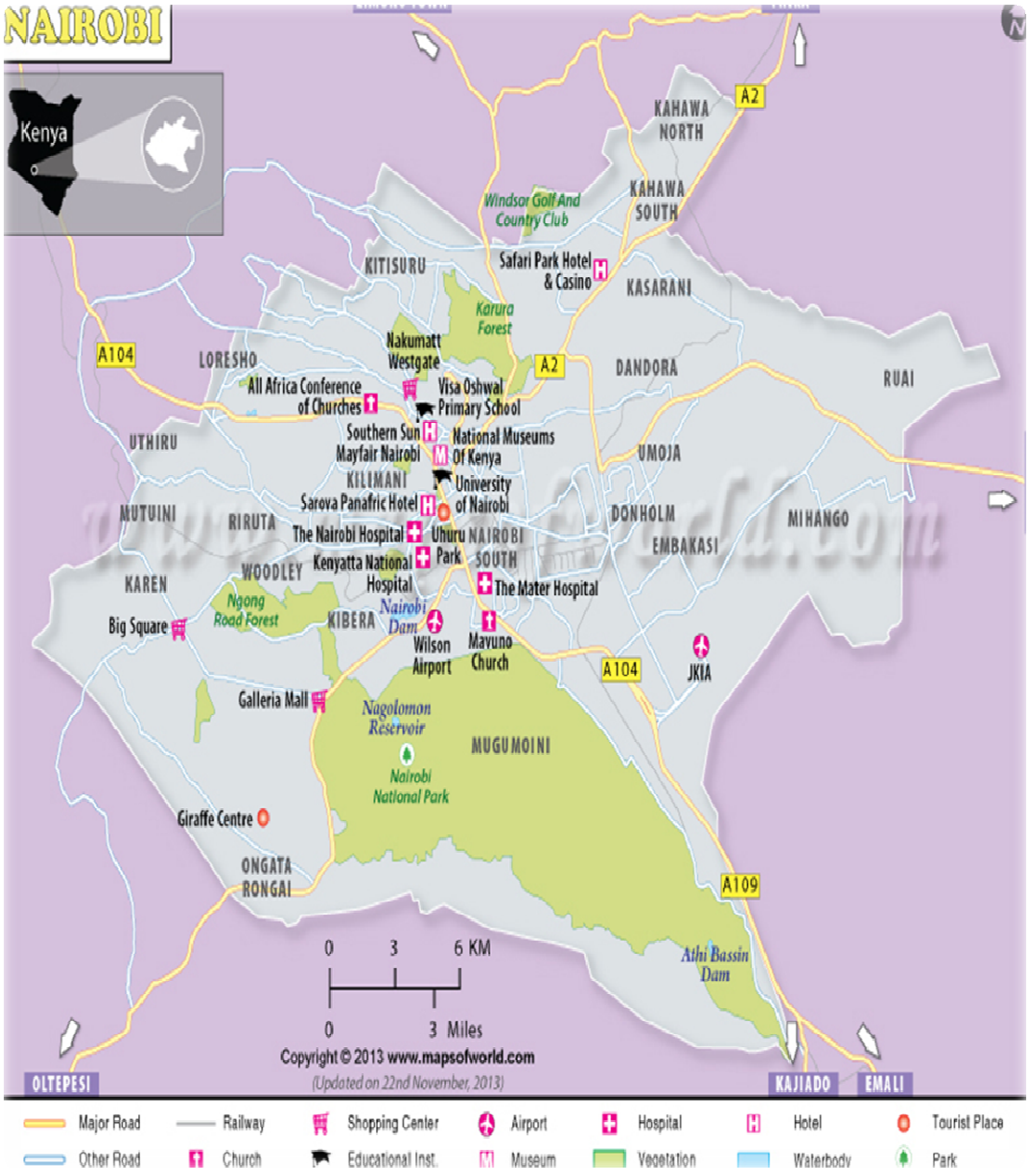
Wohl, D.A., Zeng, D., Stewart, P., Glomb, N., Alcorn, T., Jones, S., ... & van der H, C. (2005). Cytomegalovirus viremia, mortality, and end-organ disease among patients with AIDS receiving potent antiretroviral therapies. *Journal of Acquired Immune Deficiency Syndromes, 38*, 538–544.

Ying, S., Hongzhou, L., Taiwen, H., Yalin, Y., Li, L., Renfang, Z., ... & Zhiyong, Z. (2011) Prevalence and clinical management of cytomegalovirus retinitis in AIDS patients in shanghai, china.*BMC Infectious Diseases,11***,** 326.

APPENDICES

Appendix i: Study area map

Kenyatta National Hospital (www.mapsofworld.com).



Appendix ii: Informed consent document

Part A: Informed consent document

Title of the study:

SEROPREVALENCE OF HUMAN CYTOMEGALOVIRUS INFECTION AND ITS ASSOCIATED FACTORS AMONG HIV INFECTED PATIENTS ATTENDING THE COMPREHENSIVE CARECLINIC AT KENYATTA NATIONAL HOSPITAL.

Consent forms for adults above 18

My name is……and I am a Medical virology student at the JKUAT ITROMID. I am conducting a study on the seroprevalence of Human cytomegalovirus infection and their associated factors among HIV infected patients attending the comprehensive careclinic at Kenyatta national hospital. The information that I will gather will be useful to the government and other policy makers. I will summarize the findings from this study and distribute it to various stakeholders including the Ministry of Health, KEMRI, and JKUAT. TheKenyatta national hospital and University of Nairobi ethics and research review committee, who will be responsible for conducting approval of this study. We are seeking permission from you to take part in this study.

Research Procedures: This study will take place at Kenyatta National Hospital, comprehensive care clinic where HIV positive patients receive care. Blood will be drawn (5ml) aseptically to determine if HCMV are in your blood, the blood will also be used to measure the level of CD4 T-cell counts as well as amount of HIV in your blood. You will be interviewed on Age, gender, marital status, occupation, level of education, and history of blood transfusion. Your blood sample will be transported to KEMRI for analysis on HCMV.

This study is cross-sectional, meaning that you will meet the researcher only once. However, you are free to contact the person mentioned below at any time. You will not be required to come to any additional appointments for this research study. In order to ensure complete confidentiality of the test results, no names will be attached to the blood samples, but an identification number assigned to you will be used to label the sample.

Potential Risks: During this procedure there will be no long-lasting effect. However, you may feel a brief moment of pain or fear during the blood draw.

Potential benefits: This study may benefit the HIV infected patients and help the government to understand the risk the community is facing as a result of unavailability of data on the sero prevalence of the virus. The study may be able to advice on appropriate interventions to minimize the impact of the virus. This study may benefit you because the results of the tests may be communicated to your doctor and to you enhancing to the management of the HIV.

Participant's Rights: Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Confidentiality: We will make every effort to protect your identity. You will not be identified in any report or publication of this study or its results.

Contact Information: If you have any question now or in the future regarding your rights or participation in this study, **you may contact the secretary, Kenyatta national hospital and university of Nairobi ethics and research review committee.** P. O. Box 19676 Code 00202

Nairobi Tel. (254-020) 2726300-9 Ext 44355 E-mail:uonknh_erc@uonbi.ac.ke

Part B: Participant consent form

May I now ask if you would like to participate in the study? ……..The above details about the study and the basis of participation have been explained to me and I agree to take part in the study. I understand that I am free to choose to be part of the study. I also understand that if I do not want to go on with the study, I can withdraw at any time. I give my consent for my blood to be used for this study. Any questions I have concerning the study have been adequately answered and I have been given the persons to contact in case of any questions regarding this study as follows.

Investigator: Ruth Wambui Gicho, P. O Box 45028 – 00100 Nairobi, Mobile No. 0710359025, or E-mail address: ruthgicho24@gmail.com

Lead supervisor: Dr Raphael Lihana, P.O Box 54840-00200 NAIROBI, Mobile No. 0733735562 or E-mail address: Lihana@gmail.com

Secretariat, Kenyatta National Hospital/University of Nairobi - Ethics & Research Committee, P.O Box 19676- 00202 Nairobi, **Telephone:** +254-20-726300-9, **Cell phone:** (+254)-735274288 / 0721665077 or E-mail: uonknh_erc@uonbi.ac.ke

Please sign here or put your right hand thumb mark if you agree:

Signature/ Thumb mark----------

Date ----------

Witness Signature/ Thumb mark----------Date ----------

Appendix iii: Questionnaire

Title: SERO PREVALENCE OF HUMAN CYTOMEGALOVIRUS INFECTION AND ITS ASSOCIATED FACTORS AMONG HIV INFECTED PATIENTS ATTENDING THECOMPREHENSIVE CARECLINIC AT KENYATTA NATIONAL HOSPITAL.

Investigator: Mrs Ruth Gicho

Administered by: ……

Date of interview: ……

Subject code…..…

Part A: Personal & Social Demographic Characteristics

1) **Age …….years**
2) **Sex**
    1. Male
    2. Female
3) **Marital status?**
    1. Single
    2. Married
    3. Divorced/Widowed/Separated from spouse
4) **What is your employment status?**
    1. Not employed
    2. Informal employment

3. Formal

employmentRetired

5) How much is your monthly income?

1. ≤9999
2. 10000-19999
3. ≥20000

6) Highest education status?

1. Primary level
2. Secondary level
3. Tertiary level

7) Number of sexual partners?

1. 1
2. >2

8) How many children have you had?

1. 0
2. <2
3. >3

9) Do you have a history of organ transplant?

1. Yes
2. No

10) Do you have any history of blood transfusion?

1. Yes
2. No
3. Cannot recall

11) When was the last blood transfusion?

1. Last three months
2. Last six months
3. Last twelve months
4. More than twelve months

12) Have you ever been an intravenous drug user?

1. Yes
2. No

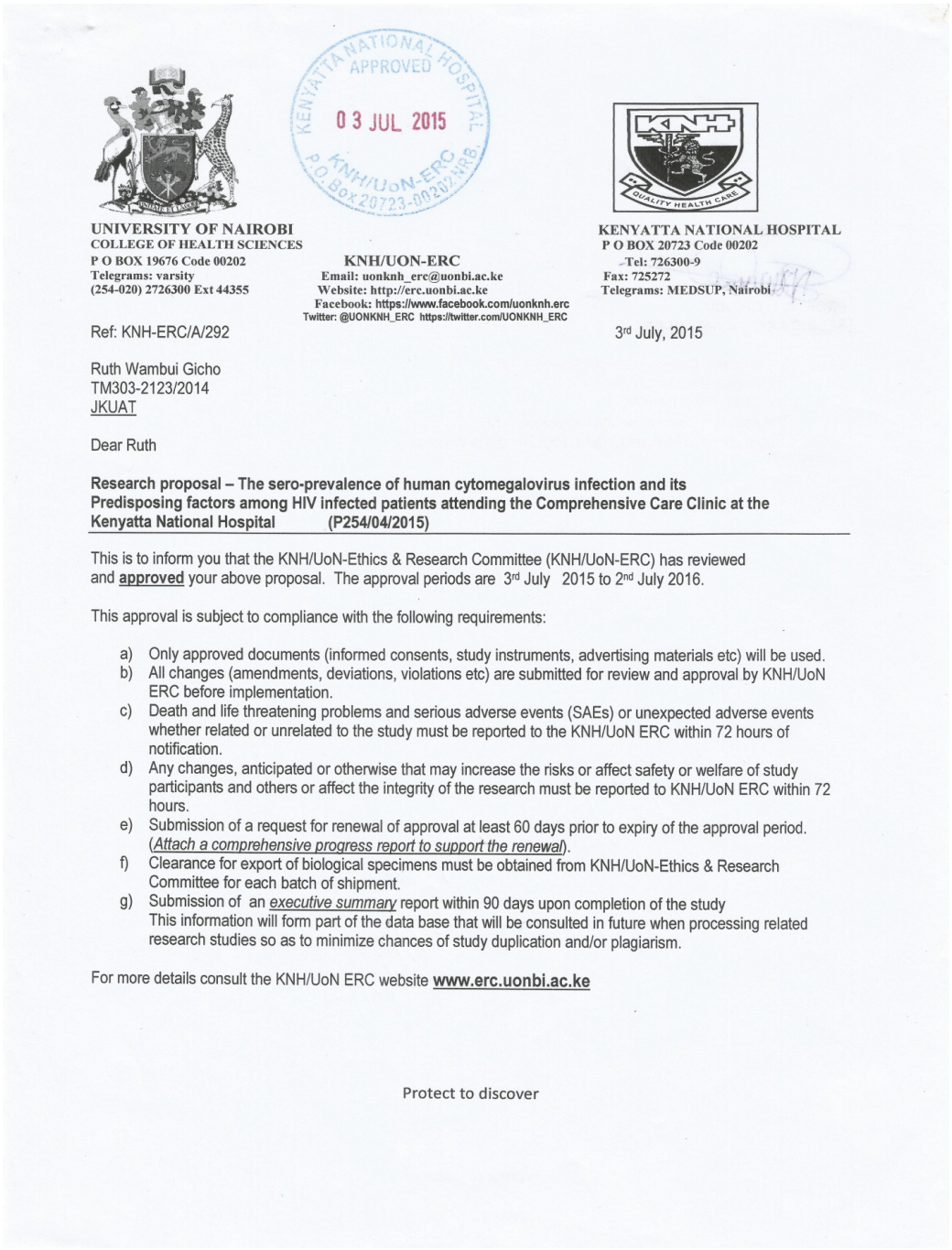
13) Are you currently on antiretroviral treatment?

1. Yes
2. No

14) If your answer to above is yes, for how long?

1. Up to twelve months
2. More than 12 month

Appendix iv: Ethical and SSC approval



KENYATTA NATIONAL HOSPITAL APPROVED
0 3 JUL 2015
KNH/UoN-ERC P.O. Box 20723-00202 NAIROBI

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Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/292

3rd July, 2015

Ruth Wambui Gicho
TM303-2123/2014
JKUAT

Dear Ruth

Research proposal – The sero-prevalence of human cytomegalovirus infection and its Predisposing factors among HIV infected patients attending the Comprehensive Care Clinic at the Kenyatta National Hospital (P254/04/2015)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 3rd July 2015 to 2nd July 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

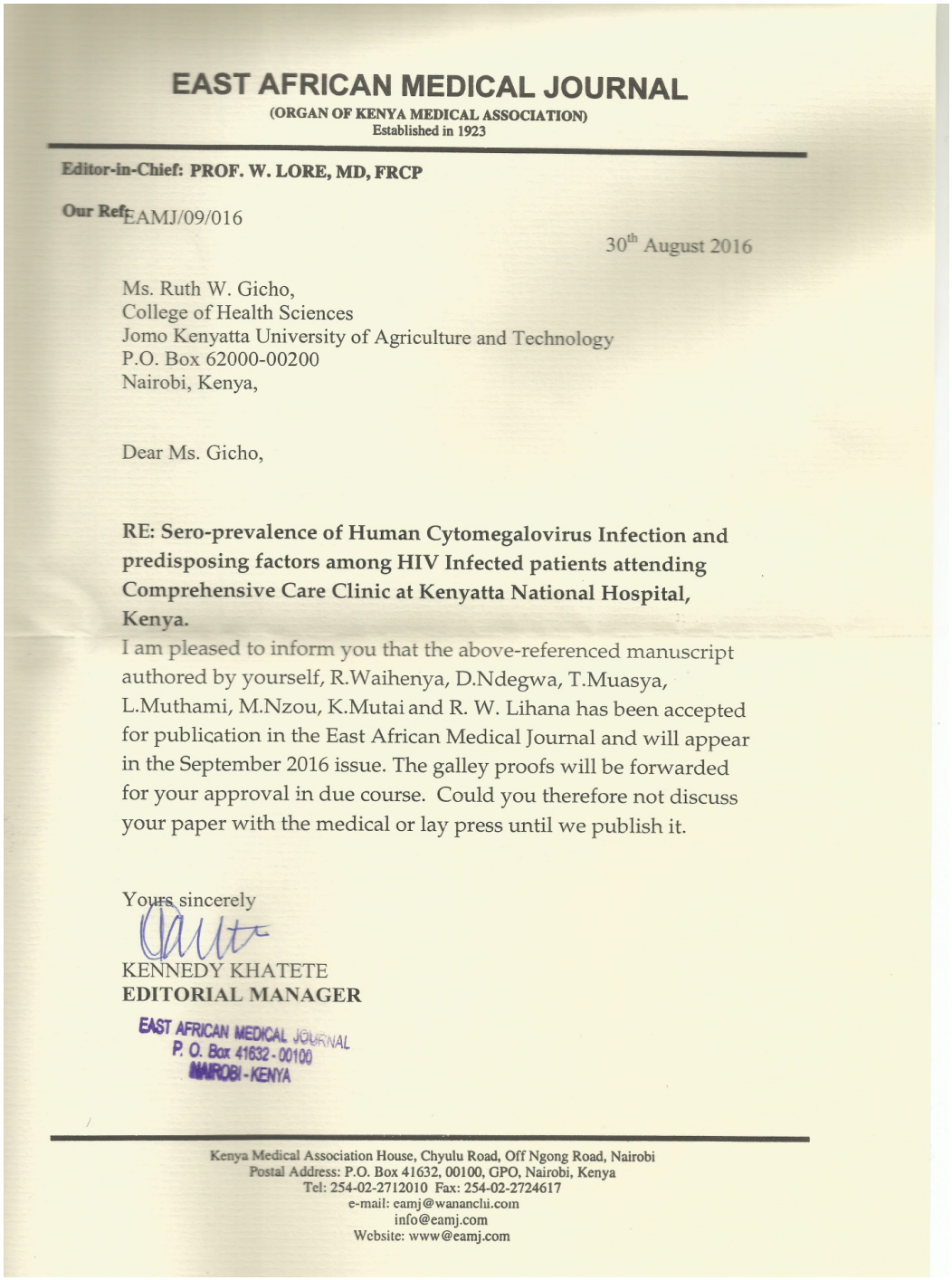
Protect to discover

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
The Assistant Director, Health Information, KNH
Supervisors: Raphael Lihana, Prof.Rebecca Waihenya

Appendix v: Publication letter



EAST AFRICAN MEDICAL JOURNAL

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30th August 2016

Ms. Ruth W. Gicho,
College of Health Sciences
Jomo Kenyatta University of Agriculture and Technology
P.O. Box 62000-00200
Nairobi, Kenya,

Dear Ms. Gicho,

RE: Sero-prevalence of Human Cytomegalovirus Infection and predisposing factors among HIV Infected patients attending Comprehensive Care Clinic at Kenyatta National Hospital, Kenya.

I am pleased to inform you that the above-referenced manuscript authored by yourself, R.Waihenya, D.Ndegwa, T.Muasya, L.Muthami, M.Nzou, K.Mutai and R. W. Lihana has been accepted for publication in the East African Medical Journal and will appear in the September 2016 issue. The galley proofs will be forwarded for your approval in due course. Could you therefore not discuss your paper with the medical or lay press until we publish it.

Yours sincerely

KENNEDY KHATETE
EDITORIAL MANAGER

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Appendices VI: Manuscript

East African Medical Journal Vol. 93 No. 10 October 2016
SERO-PREVALENCE OF HUMAN CYTOMEGALOVIRUS INFECTION AND PREDISPOSING FACTORS AMONG HIV INFECTED PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC AT KENYATTA NATIONAL HOSPITAL, KENYA R. W. Gicho, BSc, R. Waihenya, PhD, D. Ndegwa, BSc, Jomo Kenyatta University of Agriculture and Technology, M. Timothy, BSc, MSc, PhD, Centre for Virus Research-Kenya Medical Research Institute, L. Muthami, BSc, MSc, Centre for Public Health-Kenya Medical Research Institute, M. Nzou, PhD, Nagasaki University Institute of Tropical Medicine (NUITM), Kenya Medical Research Institute (KEMRI), K. Mutai, BSc, Kenyatta National Hospital and R. W. Lihana, PhD, Jomo Kenyatta University of Agriculture and Technology, Centre for Virus Research-Kenya Medical Research Institute

Request for reprints to: R. W. Gicho, Jomo Kenyatta University of Agriculture and Technology, P.O BOX 62000-00200 Nairobi, Kenya.

SERO-PREVALENCE OF HUMAN CYTOMEGALOVIRUS INFECTION AND PREDISPOSING FACTORS AMONG HIV INFECTED PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC AT KENYATTA NATIONAL HOSPITAL, KENYA

R. W. GICHO, R. WAIHENYA, D. NDEGWA, M. TIMOTHY, L. MUTHAMI, M. NZOU, K. MUTAI and R.W. LIHANA

ABSTRACT

***Background*: Human Cytomegalovirus (hCMV) is one of the opportunistic infections in HIV patients. During an active infection it's a common cause of Pneumonia, retinitis, gastro-intestinal disease, and hepatitis. It is significantly associated as a HIV disease co-factor. It does quicken HIV acquisition, disease progression and high mortality and morbidity in HIV patients. Currently there is scanty data on this disease in Kenya leading to lack of recognition on the magnitude especially in HIV patients.**

***Objective*:To determine the sero-prevalence and predisposing factors associated with hCMV infection among HIV infected individuals attending comprehensive care clinic (CCC) at Kenyatta National Hospital, Nairobi County, Kenya.**

***Design*: A cross sectional study.**

***Setting*: Kenyatta National Hospital.**

***Subjects*: A total of 400 consenting patients were systematically sampled from HIV comprehensive clinic of Kenyatta National Hospital between July and August 2015**

***Results*: A total of 400 HIV-infected individuals who were 18 years and above with an average age of 42.73 (SD, 9.5) years were screened for CMV infections. Of these, 246(61.5%) were female and 154(38.5%) were male. Of 400, 398 (99.0%) were hCMV IgG sero-positive, 32 (8.0%) were hCMV IgM sero-positive. Age group between 19 and 28 years [OR = 4.8 95% CI: (1.4-16.4); P=0.012], never been married [OR = 4.3 95% CI: (1.3-14.5); p=0.020], never had children [OR=3.2 95% CI: (1.2-8.5); p=0.022] and use of highly active anti-retroviral therapy (HAART) [OR=3.5 95% CI: (1.2-10.3); p=0.031]were found to be significantly associated with CMV sero-positivity in bivariate analysis. In multivariate analysis, bothCD4 (p <0.001) and viral loads (p <0.001) were found to be significantly associated with CMV sero-positivity.**

***Conclusion*: The 99.0% sero-prevalence of hCMV in the HIV patient's calls for routine screening for hCMV infections in order to prevent neurological clinical manifestations associated with CMV in HIV patients. Human cytomegalovirus preventive measures may be necessary to decrease mortality and morbidity associated with hCMV infections.**

INTRODUCTION

Human Cytomegalovirus (hCMV) is a widespread virus that presents asymptomatically and is a persistent infection in immunocompetent people (1). After primary infection, the virus remains latent until when it reactivates. Transmission from person to person is through body fluids such as saliva, blood, urine, semen and transplanted organ fluids (2). Upon infection the hCMV establishes latency in various types of cells but mostly in the primary fibroblast (3).

Reactivation occurs in human immunodeficiency Virus (HIV) infected patients and due to immune system suppression, it potentially translates into a

more rapid disease progression (4). During advanced AIDS, hCMV produces debilitating end-organ disease (EOD) including retinitis, colitis and pneumonitis (5). People who have experienced primary infection can be re-infected with another or the same strain of hCMV. This re-infection does not differ clinically from reactivation though it may be important epidemiologically to distinguish between reactivation and re-infection (6). Immunologically, it has been shown that the risk of hCMV disease increases as CD4+ T cell counts drop. It has also been reported that after T-cell has been infected by hCMV it undergoes apoptosis. The most common manifestation of hCMV disease in patient with AIDS is retinitis (7, 8).

Developing countries and lower socio-economic sections of society seems to have more hCMV infection. The sero-prevalence varies greatly with a variety of epidemiological factors such as age, geography, socio-economic status, marital status and parity (5). Human cytomegalovirus sero-positive individuals with sexual exposure as the risk factor for acquisition of HIV are reported to have higher risk of hCMV disease (7). In resource-poor settings, examining the occurrence of hCMV disease in HIV-infected individuals has been neglected (9). Preventive measures of vaccine development have failed due to weak protection in human and in sero-negative women of child bearing age it failed to provide protection (10, 11). In the western world hCMV infection has been a priority and even they have carried out vaccine trials resulting to no success (12).

In Western Europe and United States its prevalence is 80%. In South America, Africa and Asia the prevalence is at 78% -100% (13, 14). Currently there is scanty data on this disease in Kenya. In previous studies, the hCMV prevalence among pregnant women attending a hospital in Thika was 88.4% (15) while it was shown that anti- CMV IgG and IgM positivity in blood donors was 97.0% and 3.6%, respectively at the Kenyatta National Hospital (16).

Human cytomegalovirus is one of the opportunist pathogen that does result in end organ disease in HIV patients. It is associated significantly as co-factor in HIV disease, prompting HIV acquisition, disease progression and high mortality and morbidity in immunocompromised individuals. Currently hCMV data is limited on HIV patients in Kenya, resulting to under-estimation of the magnitude of the disease. This leads to inadequate information on disease burden hence less attention in guiding treatment or intervention. Since hCMV presentation mimics signs and symptoms of other pathogens it is easily misdiagnosed. Establishing the burden of the disease is important in prevention of classical cytomegalovirus syndrome. We set out to establish sero-prevalence of hCMV and predisposing factors among HIV infected adults attending the comprehensive care clinic (CCC) in Kenyatta National Hospital, Nairobi County, Kenya.

MATERIALS AND METHODS

Study Design, Population and Setting: A descriptive cross sectional study was conducted among 400 HIV positive adults attending the CCC of Kenyatta National Hospital between July and August 2015. Systematic sampling was used to select the study participants.

Sample collection and processing: After ethical approval, informed consent was sought from patient before recruitment into the study. Five milliliters of blood was drawn from each patient using aseptic procedures. The sample was centrifuged, sera separated and stored at -80°C for hCMV serology and HIV viral load. The qualitative and quantitative data were double entered in MS excel. In order to identify errors the two image data sets were compared to identify discrepancies. Discrepant values were compared against the original data set and the correct values included.

Laboratory Methods: Enumeration of CD4 T–cell counts A total of 50µl of whole blood, was used to determine CD4+ T-cell counts using a FACSCalibur flow cytometer (Becton-Dickinson, NJ) equipped with fluorochrome-tagged monoclonal anti-bodies to detect; anti-CD3, anti-CD45, and anti-CD4. This was done within six hours of sample draw and according to the manufacturer's instructions (17).

Quantification of HIV-1 viral load: The HIV-1 viral load was determined using the Abbott m2000rt System (Abbott Molecular Inc., Illinois, U.S.A) with automated sample extraction, amplification and detection according to the manufacturer's instructions (18).

Human cytomegalovirus Serology: Enzyme linked immunosorbent assay (ELISA) was done using the Vir-ELISA assay (Viro-Immun Labor-Diagnostika GmbH, Oberursel, Germany) to determine hCMV IgG and IgM antibodies as per the manufacturer's instruction. Briefly, patients' sera were diluted and added to wells already coated with purified hCMV-Ag. The antibodies, if present, would bind to the antigen. All unbound materials were washed away and an enzyme conjugate added. The plates were incubated to allow hydrolysis of the substrate by the enzyme. The intensity of the color generated was proportional to the amount of antibodies against hCMV in the sample. Absorbance of the well contents was read at 450nm using an ELISA plate reader (19).

Questionnaire: A pre-tested structured questionnaire was administered and socio-demographic data werecollected. Data captured in questionnaires was double entered into a computer database designed using MS- excel application. Data cleaning and validated was analysed using SPSS version 22.0.

Statistical Analysis: Univariate analysis was used to measure frequencies and bivariate analysis was used to test the association. Multivariate analysis (step-wise logistic regression) was performed to identify factors that were independently associated with occurrence of hCMV after adjusting for confounding factors. Statistical significance was presumed where $p < 0.05$.

RESULTS

A total of 400 patients consented to participate and were recruited in the study (table 1). Their mean age was 42.73 (SD, 9.5) years. Of these, 246(61.5%) were female and 154(38.5%) were male. However, age difference between males and females was significant ($p<0.001$). Majority (159, 39.8%) of participants were aged between 39 and 48 years with most of them (253, 63.2%) being either divorced or separated. In number of sexual partners, half 199 (50.4%) of them having multiple sexual partners. Majority 369 (92.3%) had undergone HIV treatment with 345 (93.5%) treated for more than 12 months (Table 1).

The sero-prevalence of hCMV IgM was 8.0 % (95% CI of 5.5% - 10.8 %) (32/400) while that of hCMV IgG was 99.0 % (95% CI 98.8% - 100.0%) (398/400) (Table 1).

Factors associated with the occurrence of active human cytomegalovirus (IgM) Human cytomegalovirus was significantly associated with the age group between 19 and 28 years with a prevalence of 23.1% as compared to 5.9% in the older age group of 49 years and above [OR = 4.8 (95% CI1, 4-16.4)] (Table 2). Also, those that had never been married had a higher prevalence of hCMV (23.5%) compared to the widowed / divorced / separated (6.7%) [OR = 4.3 (95% CI1.3-14.5)]. Similarly, patients who had never had children had a significantly higher prevalence (23.3%) as compared to those with more than 3 children (8.7%) [OR=3.2 (95% CI1.2-8.5)](Table 2).

Use of HAART treatment for 12 months and below was associated with higher prevalence of hCMV (20.8%) compared to those that were on HAART for more than 12 months [OR=3.5 95% CI (1.2-10.3)] (Table 2). Similarly, Bivariate analysis showed that CD4 and viral loads had a statistical significance of($p <0.001$) in the respondents who tested positive for hCMV. Therefore there was a positive correlation between CD4 and viral load in the occurrence of an active infection. Other characteristics of the respondents such as gender, employment, income, education and blood transfusion were not significantly associated with the risk of acute cytomegalovirus infection (Table 2).

Patients' characteristics did not show any statistical significancewith the occurrence of chronichCMV infection (Table 3).

Multivariate (Step-wise logistic regression)

Table 1
Socio-demographic characteristics of HIV-infected patients attending the Kenyatta National Hospital comprehensive care center

Variable	Frequency (N=400)	Percentages (%)
Age		
19-28 years	26	6.5
29-38 years	113	28.2
39-48 years	159	39.8
≥ 49 years	102	25.5
Gender		
Male	154	38.5
Female	246	61.5
Marital status		
Never married	17	4.3
Married	130	32.5
Divorced / Widowed / Separated from spouse	253	63.2
Employment		
Not employed	85	21.2
Informal employment	169	42.3
Formal employment	146	36.5

Income*		
≤9999	158	39.7
10000-19999	66	16.6
≥20000	174	43.7
Education		
Primary level	90	22.5
Secondary level	183	45.8
Tertiary level	127	31.8
Sex partners***		
1	196	49.6
≥2	199	50.4
Children		
0	30	7.5
1-2	187	46.7
≥3	183	45.8
Blood transfusion*		
Yes	38	9.5
No	360	90.5
Last blood transfusion**		
Last three months	0	0
Last six months	1	2.6
Last twelve months	0	0
More than twelve months ago	37	97.4
Intravenous drug user		
No	400	100.0
Yes	0	0.
HAART		
No	31	7.8
Yes	369	92.3
Duration of HAART****		
Up to twelve months	24	6.5
More than 12 months	345	93.5

Variable	Frequency (%) (N=400)	95% CI
IgM		
Positive	32 (8.0)	5.5, 10.8
Negative	368 (92.0)	89.3, 94.5
IgG	398 (99.0)	98.8,
Positive	2 (1.0)	100.0
Negative		0.0, 1.3

** n=398; ** n=38; *** n=395; **** n=369; HAART= highly active antiretroviral therapy; CI= Confidence Interval; IgM= Immunoglobulin M ; IgG= Immunoglobulin G*

Table 2
Factors associated with active HCMV (IgM) infection among HIV-infected patients attending the Kenyatta National Hospital comprehensive care center

	IgM		OR (95% CI)	P value
	Positive (%)	Negative (%)		
Gender				
Male	15 (9.7)	139 (90.3)	1.5 (0.7-3.0)	0.310
Female	17 (6.9)	229 (93.1)	1.0	
Age				
19-28	6 (23.1%)	20 (76.9%)	4.8 (1.4-16.4)	0.012
29-38	6 (5.3%)	107 (94.7%)	0.9 (0.3-2.9)	0.855
39-48	14 (8.8%)	145 (91.2%)	1.5 (0.6-4.2)	0.389
≥49	6 (5.9%)	96 (94.1%)	1.0	
Marital status				
Never married	4 (23.5)	13 (76.5)	4.3 (1.3-14.5)	0.020
Married	11 (8.5)	119 (91.5)	1.3 (0.6-2.8)	0.536
Divorced/Widowed/Separated	17 (6.7)	236 (93.3)	1.0	
Employment				
Not employed	9 (10.6%)	76 (89.4%)	1.3 (0.5-3.3)	0.547
Informal employment	11 (6.5%)	158 (93.5%)	0.8 (0.3-1.8)	0.562
Formal employment	12 (8.2%)	134 (91.8%)	1.0	
Income				
≤9999	17 (10.8%)	141 (89.2%)	2.0 (0.9-4.5)	0.100
10000-19999	5 (7.6%)	61 (92.4%)	1.3 (0.4-4.1)	0.602
≥20000	10 (5.7%)	164 (94.3%)	1.0	
Education				
Primary level	5 (5.6)	85 (94.4)	0.9 (0.3 2.8)	0.820
Secondary level	19 (10.4)	164 (89.6)	1.7 (0.7-4.1)	0.214
Tertiary level	8 (6.3)	119 (93.7)	1.0	
Sex partners				
1	15 (7.7)	181 (92.3)	0.9 (0.4-1.8)	0.746
≥2	17 (8.5)	182 (91.5)	1.0	
Number of children				
0	7 (23.3%)	23 (76.7%)	3.2 (1.2-8.5)	0.022
1-2	9 (4.8%)	178 (95.2%)	0.5 (0.2-1.2)	0.138
>3	16 (8.7%)	167 (91.3%)	1.0	
Blood transfusion				
Yes	3 (7.9)	35 (92.1)	1.0 (0.3-3.4)	1.000
No	29 (8.1)	331 (91.9)	1.0	
ART				
Yes	29 (7.9)	340 (92.1)	1.0	
No	3 (9.7)	28 (90.3)	1.3 (0.4-4.4)	0.727
Duration of ART	5 (20.8)	19 (79.2)	3.5 (1.2-10.3)	0.031
Up to twelve months	24 (7.0)	321 (93.0)	1.0	
More than twelve months ago				
			Median (95% CI)	
Median CD4 (IQR)	81.5 (45.0-98.0)	480.5 (333.0-658.0)	461 (431-485)	<0.001
Median viral load (IQR)	4633 (150 177770)	150 (150 150)	150 (150 150)	<0.001

OR = Odds Ratio, CI= Confidence Interval;, IQR= interquartile ratio; HAART= Highly active antiretroviral therapy; IgM= Immunoglobulin M

Table 3
Factors associated with chromic HCMV (IgG) infection among HIV-infected patients attending the Kenyatta National Hospital comprehensive care center

	IgG		P value
	Positive (%)	Negative (%)	
Gender			
Male	152 (98.7)	2 (1.3)	0.148
Female	246 (100.0)	0 (0.0)	
Age			
19-28	26 (100.0%)	0 (0.0%)	0.414
29-38	112 (99.1%)	1 (0.9%)	
39-48	159 (100.0%)	0 (0.0%)	
≥49	101 (99.0%)	1 (1.0%)	
Marital status			
Never married	17 (100.0)	0 (0.0)	1.000
Married	129 (99.2)	1 (0.8)	
Divorced/Widowed/Separated	252 (99.6)	1 (0.4)	
Employment			
Not working	85 (100.0)	0 (0.0)	0.511
Informal employment	167 (98.8)	2 (1.2)	
Formal employment	146 (100.0)	0 (0.0)	
Income			
≤9999	158 (100.0)	0 (0.0)	0.305
10000-19999	65 (98.5)	1 (1.5)	
≥20000	173 (99.4)	1 (0.6)	
Education			
Primary level	90 (100.0)	0 (0.0)	1.000
Secondary level	182 (99.5)	1 (0.5)	
Tertiary level	126 (99.2)	1 (0.8)	
Sex partners			
1	195 (99.5)	1 (0.5)	1.000
≥2	198 (99.5)	1 (0.5)	
Number of children			
0	30 (100.0)	0 (0.0)	1.000
1-2	186 (99.5)	1 (0.5)	
>3	182 (99.5)	1 (0.5)	
Blood transfusion			
Yes	38 (100.0)	0 (0.0)	1. 000
No	358 (99.4)	2 (0.6)	
ART			
Yes	31 (100.0)	0 (0.0)	1.000
No	367 (99.5)	2 (0.5)	
Duration of ART			
Up to twelve months	24 (100.0)	0 (0.0)	1.000
More than twelve months ago	343 (99.4)	2 (0.6)	
Log10 CD4 cells+ 1 count, mean (SD)	3.6 (0.4)	3.7 (0.0)	0.752
Log10 viral load + 1, mean (SD)	3.6 (0.9)	3.2 (0.0)	0.332

OR = Odds Ratio, CI= Confidence Interval, SD =standard deviation; HAART= Highly active antiretroviral therapy; IgG= Immunoglobulin G

Table 4
Factors independently associated with occurrence of active human cytomegalovirus among HIV-infected patients attending the Kenyatta National Hospital comprehensive care center

Variable	Adjusted OR (95% CI)	P value
Age		
19-28	2.3 (0.1-42.6)	0.565
29-38	1.8 (0.3-12.1)	0.540
39-48	2.1 (0.5-9.5)	0.333
≥ 49	1.0	
Marital status		
Never married	2.7 (0.2-38.6)	0.467
Married	0.7 (0.2-2.8)	0.630
Divorced / Widowed / Separated	1.0	
Income		
≤9999	1.1 (0.2-4.8)	0.923
10000-19999	1.2 (0.2-6.6)	0.805
≥20000	1.0	
Number of children		
0	2.9 (0.3-27.1)	0.360
1-2	0.8 (0.2-3.1)	0.732
≥3	1.0	
Duration of ART		0.289
Up to twelve months	2.5 (0.5-13.8)	
More than twelve months ago	1.0	
Median CD4 (IQR)	0.984 (0.978-0.990)	<0.001
Median viral load (IQR)	1.0 (1.0-1.0)	<0.001

OR = Odds Ratio, CI= Confidence Interval, IQR= interquartile range; HAART= Highly active antiretroviral therapy

analysis showed that CD4 (p=<0.001) and viral loads (p <0.001) were the only variables independently associated with the occurrence of active hCMV (Table 4).

DISCUSSION

In this study, we report the prevalence of hCMV infections and its predisposing factors among HIV infected patients attending the Kenyatta National Hospital comprehensive care center.

It has been shown that 8.0 % of HIV sero-positive patients attending the CCC tested positive for anti-hCMV IgM and 99.0 % for anti-hCMV IgG. This is in agreement with what has been previously reported in Nigeria where the prevalence was at 93.9% and 100% for IgG among HIV-1 sero-positive patients. The recorded high prevalence's, confirm constant exposure to the population to CMV infections (20, 21).

There was no statistical association between the positivity of anti-CMVIgG and anti-CMV IgM ingender. Though, we identified higher sero-positivity of CMV IgG and CMV IgM in females. This was in agreement with other studies done in the United States of America and Brazil that identified the females having a higher sero-prevalence (22, 23).

Human cytomegalovirus IgG sero-positivity was generally high in the age-group 39-48 [159 (100.0%)] years. This is in agreement with previous findings where hCMV infections occur worldwide. This could be due to recurrent exposure to hCMV during sexual intercourse. About four out of five people over age 35 have been infected with cytomegalovirus, usually during childhood or adulthood (24). Childhood infection could also be a major form of hCMV acquisition. This is agreement to a study done in Kenya that showed 90% hCMV was detected in HIV-exposed uninfected infants (25) and also high detection of maternal hCMV DNA in the blood near the time of delivery(26).

High parity was observed as a factor for increased susceptibility to acquisition of hCMV infection. This could be as a result of direct contact with secretions from their children's or possibly due to poor hygiene (27). It could also be related to mode of transmission (sexual) therefore repeated sexual exposure associated with both HIV and hCMV could lead to this observation (28).

Having a single or multiple sexual partners did not show any statistical significance in our study although a large percentage (99.5%) was sero-positive for hCMV IgG. This is in agreement with the knowledge that HIV and hCMV share several modes of transmission (14).

Distributio of anti-CMV IgM in relation to marital status showed statistical association in the anti-CMV sero-positivity among the various marital groups. This is in agreement to other cross sectional studies done in Kenya and Nigeria (15, 20).

The CD4 and the occurrence of active hCMV infection showed a statistical significance. This is in agreement to the knowledge that the risk of hCMV disease increases as CD4+ T cell counts drop in the HIV patient (7).Further there was a correlation in the occurrence of hCMV and viral loads in the patients who tested positive for active hCMV. This is in agreement to the knowledge that there is an indirect effect on replication of both viruses.Diverse mechanisms used by hCMV can possibly activate latent HIV proviral DNA(29).

Despite these findings, this study had some limitations; the study was not able to confirm the active disease by PCR. In addition, there was also re-call bias from the patients during data collection.

In conclusion, in this study, the prevalence of hCMV IgG was 99.0%. It was shown that 8% of the study participants were hCMV IgM positive. Age, marital status, parity, CD4 and HIV viral load were predisposing factors to active hCMV infections. There is need for a continuous screening for active hCMV infections in HIV patients in order to prevent any transmission and risk to CMV infections.

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REFERENCES

1. Landolfo, Santo, Gariglio, M., Landolfo, S., *et al*. The human cytomegalovirus. *Pharmacology and therapeutics*, 2003; 98, 269-297.
2. Taylor, G. H. Cytomegalovirus. *American Family Physician*. 2003; 67, 519-524+526.
3. Emery, V. C. Investigation of CMV disease in immunocompromised patients.*Journal Clinical Pathology*,2001; 54: 84-88.
4. Chakravarti, A., Tewari, S. and Bhalla, P. Human Cytomegalovirus Infection among Patients Living with AIDS in a Tertiary Level Hospital in India. Journal of the International Association of Physicians in AIDS Care, 2009; 9: 94 – 7.
5. BasawarajuAnuradha, Mane Pratibha M., Vijayadurga S. Reactivation of cytomegalovirus in HIV. *Journal of Clinical and Diagnostic Research*. 2011August, Vol-5(4): 749-751.
6. Bagheri, L., Mokhtarian, H., Sarshar, N. *et al*. Sero-prevalence of cytomegalovirus infection among pregnant women in Eastern Iran. *The brazilian journal of infectious diseases*, 2012; 16:402–403.
7. Brantsæter, A. B., Johannessen, A., Holberg-Petersen, M., *et al*. Cytomegalovirus viremia in dried blood spots is associated with an increased risk of death in HIV-infected patients: a cohort study from rural Tanzania. *International journal of infectious disease*, 2012; 16:e879-85.
8. Böhler, T., Wintergerst, U., Linde, R., *et al*.CD95 (APO-1/Fas) expression on naive CD4 (+) T cells increases with disease progression in HIV-infected children and adolescents: effect of highly active antiretroviral therapy (HAART). (2001). PediatricResearch. 49, 101–110. 10.1203/00006450-200101000-00021 [PubMed] [Cross Ref]
9. Heiden, D., Ford, N., Wilson, D., *et al*. Cytomegalovirus retinitis: the neglected disease of the AIDS pandemic. PLoS Medicine, 2007; 4: e334.
10. Plotkin, S. A., Starr, S. E., Friedman, H. M., *et al*(1989). Protective effects of Towne cytomegalovirus vaccine against low-passage cytomegalovirus administered as a challenge. *Journal Infectious Disease*. 159, 860–865. 10.1093/infdis/159.5.860 [PubMed] [Cross Ref]
11. Adler, S. P., Starr, S. E., Plotkin, S. A., *et al*. (1995). Immunity induced by primary human cytomegalovirus infection protects against secondary infection among women of childbearing age. *J. Infect. Dis*. 171, 26–32. 10.1093/infdis/171.1.26 [PubMed][Cross Ref]
12. Adland, Emily, Klenerman, Paul, Goulder, Philip, *et al*. Ongoing burden of disease and mortality from HIV/CMV coinfection in Africa in the antiretroviral therapy era. Frontiers in Microbiology 6 1016.
13. Ben-Smith, A., Gorak-Stolinska, P., Floyd, S., *et al*. Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for Cytomegalovirus. BMC Infection Disease; 2008; 8: 139.
14. Compston, L. I., Li, C. and Sarkodie, F. Prevalence of persistent and latent viruses in untreated patients infected with HIV-1 from Ghana, *West Africa. Journal Medical Virology*, 2009; 81: 1860-8.
15. Maingi, Z. and Nyamache, A. K. Seroprevalence of Cytomegalovirus (CMV) among pregnant women in Thika, Kenya: BMC Research Notes.2014; 7:794.
16. Njeru, D. G., Mwanda, W. O., Kitonyi, G. W., *et al*. Prevalence of cytomegalovirus antibodies in blood donors at the National Blood Transfusion Centre, Nairobi: 2009; 86:358-61.
17. Lihana, R. W., Khamadi, S. A., Lubano, K., *et al*. HIV Type 1 Subtype Diversity and Drug Resistance among HIV Type 1-Infected Kenyan Patients Initiating Antiretroviral Therapy. AIDS Research and Human Retroviruses, 2009;December, 25(12): 1211-1217.
18. Ochieng, W., Kitawi, R. C., Nzomo, T. J., *et al*. Implementation and Operational Research: Correlates of Adherence and Treatment Failure Among Kenyan Patients on Long-term Highly Active Antiretroviral Therapy. *Journal Acquired Immune Deficiency Syndrome*. 2015; 69(2):e49-56.
19. Alonso, Arias, R., Moro-García, M. A., Echeverría, A., *et al*.Intensity of the Humoral Response to Cytomegalovirus Is Associated with the Phenotypic

and Functional Status of the *Immune System.Journal of Virology*, 2013; 87(8): 4486–4495.

20. Fowotade, A., Okonko, I. O., Agbede, O. O., *et al.* High seropositivity of IgG and IgM antibodies against cytomegalovirus (CMV) among HIV-1 seropositive patients in Ilorin, Nigeria, African Health Sciences,2015;Vol 15 Issue 1.
21. Akinbami, A. A., Akanmu, A. S., Adeyemo, T. A., *et al.* Cytomegalovirus Antibodies Amongst Immunocompromised (HIV) Patients At Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos. *Journal of Medicine*, 2010; 11: 151-154.
22. Cannon, M. J., Scott Schmid, D., Terri, B. Hyde review of Cytomegalovirus seroprevalence and demographic characteristics associated with infection. Revision Medical Virology,2010; 20:202–213.
23. Adisa, T. M., Bukbuk, D. N. and Harry, T. O. Maternofoetal transfer of Cytomegalovirus IgG antibodies in Maiduguri, North Eastern Nigeria. *The Internet Journal of Microbiology*, 2008; Volume 4 Number 2.
24. Bernard, J. T. Human. Cytomegalovirus Species. Classification of Viral Family (Herpesviridae), 2007; 2:127-128.
25. Slyker, J. A., Lohman-Payne, B. I., *et al.* The detection of cytomegalovirus DNA in maternal plasma is associated with mortality in HIV-1-infected women and their infants. AIDS. 2009; 23:117–124/
26. Hamdan Z Hamdan, Ismail E Abdelbagi,Nasser, M., Nasser, *et al.*Sero-prevalence of cytomegalovirus and rubella among pregnant women in western Sudan. *Virology Journal*,2011;8:21.
27. Wester, C. W., Bussman, H., Moyo, S, *et al.*Serological Evidence of HIV-Associated Infections among HIV-1-infected Adults in Botswana. *Clin ical Infectious Diseases* ,2006; 43, 1612-1615.
28. Griffiths, P. D. CMV as a cofactor enhancing progression of AIDS. Journal Clinical. *Virology*, 2006; 35, 489–492.