SEROLOGICAL AND PCR DETECTION OF CYTOMEGALO VIRUS INFECTION AMONG PREGNANT WOMEN ATTENDING SOME HOSPITALS IN LOKOJA KOGI STATE, NIGERIA

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NOVEMBER, 2022

DECLARATION

I hereby declare that this worktitled **"Serological and PCR detection of Cytomegalovirus infection among pregnant women attending some hospitals in Lokoja, Kogi State Nigeria"** was undertaken by me under the supervision of Dr. A.B. Suleiman and Dr. M.B. Tijjani of the Department of Microbiology, Ahmadu Bello University, Zaria.

It has not been previously presented for the award of M.Sc. Microbiology in the Faculty of Science, Ahmadu Bello University, Zaria. The works of other researchers and authors are duly acknowledged and referenced accordingly.

_____ _____

Hannatu E.M. BALA Date

CERTIFICATION

This project dissertation entitled **"SEROLOGICAL AND PCR DETECTION OF CYTOMEGALOVIRUS INFECTION AMONG PREGNANT WOMEN ATTENDING SOME HOSPITALS IN LOKOJA, KOGI STATE, NIGERIA"** by HANNATU ELEOJO MARY BALA meets the regulations governing the award of Master of Science in Microbiology of Ahmadu Bello University, Zaria. It is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to my parents: Prof and Mrs A.E. Bala for their patience, constant support, encouragement and prayers.

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LIST OF ABBREVIATIONS

HCMV - Human Cytomegalovirus

CMV - Cytomegalovirus

ELISA - Enzyme Linked Immunosorbent Assay

IgM - Immunoglobulin M

KSSH - Kogi State Specialist Hospital

FMC - Federal Medical Centre

DNA - Deoxyribo nucleic acid

PCR - Polymerase Chain Reaction

TORCH - *Toxoplasma gondii*, Rubella, Cytomegalovirus and Herpes Simplex Virus

ABSTRACT

Cytomegalovirus (CMV) is one of the most common virusesthat causes congenital defects in babies from infected mothers. Most cases of congenital defects are asymptomatic at birth but can lead to sensoneural hearing loss (SNHL) and neurodevelopmental delay which can appear later in childhood. This study was conducted to determine the cytomegalovirus infection among pregnant women attending antenatal clinics at Kogi State Specialist Hospital (KSSH) and Federal Medical Centre (FMC) Lokoja, Kogi state, Nigeria. A total of 184 serum samples were collected and analyzed using CMV IgM ELISA kit Diagnostic Automation, INC (USA).The study revealed that 40 out of the 184 samples turned out positive for CMV IgM antibodies giving a prevalence of 21.7% while the negative sample result had a prevalence of 78.3%.From the forty (40) positive samples, seven (7) samples were subjected to Polymerase Chain Reaction but it turned out negative. Although PCR is a very sensitive and reliable method for detecting the organism (CMV) it was discovered that peripheral blood leukocytes is a better medium of choice to use as against plasma which was used for the research. Based on the research, there was no significant association between socio-demographic factors such as marital status, type of marriage, occupation and scarification and the infection. This could be due to the limited number in sample size. Other socio economic factors such as education had a significant p value of 0.035 with the infection. In addition, the pregnant women who had deformed babies, do not practice hand wash and were not transfused had a significant difference with the infection (p= 0.046, 0.027 and 0.003 respectively).It is advised that screening and sensitization of CMV for pregnant women when they come for ante-natal classes be done in order to reduce the risk of congenital birth defects among infants.

CHAPTER ONE

1.0 INTRODUCTION

Cytomegalovirus (CMV) is derived from the Greek word “cyto” meaning cell and “megalo” meaning large.Cytomegalovirus is also known as the Human Herpes virus 5(HHV-5) that belongs to Herpesviridae and subfamily Betaherpesvirinae which also includes the genera *Muromegalovirus* and *Roseolvirus* (HHV- 6 and HHV -7) (Staras *et al.,* 2006, Moore *et al.,* 2007,Al.Baiati*et al*., 2014). All herpesviruses have a shared characteristic ability of exhibiting latency within the body overtime, although they may be found throughout the human body (Moore *et al*., 2007, Ryan and Ray, 2004, Musa *et al.,* 2014). Human cytomegalovirus (HCMV) infects more than 80% of the general population and up to 100% within some populations and/or geographic areas (Astegiano *et al.,* 2010, Yeroh *et al.,* 2014, Fowotade *et al.,* 2015 and Winter *et al.,*2020). CMV infection is common over age 35 years and most people are exposed to CMV early in life and do not realize it because they areasymptomatic (Fowotade *et al.,* 2015).

Cytomegalovirus (CMV) is a ubiquitous herpes virus that generally causes asymptomatic or mildly symptomatic infections in immunocompetent hosts. However, it carries a high morbidity and mortality among immunocompromised hosts having CMV infection (Springer and Weinberg, 2004).

Pregnant women infected for the first time with cytomegalovirus are likely to transmit the virus to their fetus (Hameed and Azeez 2015, Ndako, 2016). Maternal Cytomegalovirus (CMV) is the most common viral infection in perinatal period and it is the leading cause of congenital cytomegalovirus infection with a permanent hearing loss, vision loss and neurological impairment (Tabatabaee and Tayyebi, 2009,Cannon *et al*., 2010 and Umelo *et al.,* 2017).

Cytomegalovirus (CMV) infection in pregnancy may cause mental retardation, congenital deafness, and damage to the fetus following vertical transmission (Anigilaje *et al.,* 2015). Congenital lesions are more serious when following primary infectionthan with re-infection or reactivation during pregnancy. Prenatal infections of CMV infection have more severe fetal consequences, and infection in the first trimester may affect the sequence of organogenesis leading to congenital malformation while infection in the second and third trimesters may cause neurological impairment or growth.

In developing countries, CMV infection is more common and seroprevalence varies greatly with a variety of epidemiological factors such as (Zenebe 2021). In Bida, Nigeria reported a seroprevalence of 84.2% among pregnant mothers attending the Federal Medical Centre Bida, Niger State (Okwori *et al*., 2009). In Abakaliki, a prevalenceof 96.08% was recorded among pregnant women and a seroprevalence of between 40% and 90% was recorded from other parts of the world including India. (Anuma and Umeora, 2016).

1.1 Statement of Research Problem

Cytomegalovirus infection is a leading cause of congenital viral infection as this infection is the most frequently transmitted infection to a developing fetus resulting into birth defects in later life therefore leading to an increase in mobidity and mortality (Bates and Brantsaeter 2016). In effect, the risk of congenital infection is much higher during primary or acute infection of the mother (Nasir *et al*., 2015 and Alghalibi *et al.,* 2016).

From some studies conducted in Africa, CMV prevalence rates for IgG among pregnant women in Egypt were found to be 96%, 85.7% for Tanzania, 97.2% for Benin and 86.4% for South Africa 88.4% for Kenya (Maingi and Nyamche, 2014; Edward *et al*., 2015).

It has been estimated that in United Kingdom, congenital CMV infection may account for 15% of sensori-neural hearing loss (SNHL) and 7% of cerebral palsy. The incidence of

congenital CMV infection in the United States was estimated to be around 1% of live birth and rates of 0.15% to 2.2% was reported (Edward*et al*., 2015. CMV infection is more widely spread in developing countries and in communities with lower socio-economic status and represents the most significant viral cause of birth defects in industrialized countries. Other studies have revealed that cytomegalovirus is found throughout all geographic locations and infect between 50% and 80% of adults in the United States as indicated by the presence of antibodies in much of the general populations (Edward*et al*., 2015).

In Nigeria, a study conducted in 2008, reported a prevalence of 45.0% IgG and 33% IgM antibodies among breastfeeding mothers and of the infants. Similarly, (Okwori*et al*., 2008) reported a prevalence of 86.1% IgG antibodies among multigravid women and 77.1% among primigravid women (Edward*et al*., 2015). In Abakaliki, a prevalence rate of 96.08% for IgG was reported among pregnant women (Anuma and Umeora, 2016).

Congenital defects is one of the greatest risk associated with Cytomegalovirus (Furui *et al.*, 2013). Congenital infection occurs in fetus if the mother has a primary infection or a viral reactivation during pregnancy. A typical clinical symptom could take the form of severe generalized or cytomegalic inclusion disease in which the infants usually have jaundice, hepatospleenomegaly, thrombocytopenia, haemolytic anaemia and pneumonia (Bates and Brantsaeter, 2011). The brain is almost always involved with microcephaly and motor disorder. Most infants with these symptoms do not survive. Infants are usually deaf and mentally retarded (Ndako *et al*., 2016).

1.2 Justification

Developing countries is been recorded as having the highest seroprevalence of Cytomegalovirus in adults and incidences of congenital Cytomegalovirus infection rates (1

to 5% of births) in which 90 % of infected infants are symptomatic at birth and are not recognized as at risk for CMV – associated infection. Consequently, 1% of pregnant women each year acquire acute CMV infection and out of this value, about 40% transmit the viral infection to their fetuses (Duff, 2007, Ndako *et al.*, 2016). There is dearth of information on the epidemiological data of Cytomegalovirus infection in Kogi State, Nigeria. This is a necessary tool for health planners and care providers in order to provide adequate health care for patients especially pregnant women who might have been infected with the virus. Consequently, this study will seek to provide information on the prevalence of Cytomegalovirus infection among pregnant women in the study area. Acute CMV infection is critical because it is a significant risk factor for vertical transmission of CMV infection to newborn babies with its entire associated clinical conditions (Umeh *et al*., 2009, Ndako *et al.*, 2016).

Nigeria is grappling with inadequate funding in the health sector and managing conditions such as congenital CMV disease is an issue, hence the need to investigate the IgM level with a view to guiding policy makers and educating pregnant mothers on ways of preventing infection to reduce birth of otherwise helpless children (Hamid *et al*., 2014). Therefore, in view of this, the information gotten will instigate relevant bodies in the State health sector to educate *en masse* on the need to take precautionary methods against contracting the virus and managing its treatment in the case of an infection and if necessary, include it in the routine test screening for pregnant women.

1.3 Aim

The aim of this research was to determine the seroprevalence and detect cytomegalovirus using ELISA and PCR respectively among pregnant women attending some hospitals in Lokoja, Kogi State, Nigeria.

1.4 Objectives

The objectives of this study were to:

1. determine the seroprevalence of Cytomegalovirus (CMV) infection among pregnant women in the study areas using ELISA;
2. confirm CMV among some ELISA positive samples using conventional Polymerase Chain Reaction (PCR) and
3. determine some socio demographic and possible risk factors associated with CMV infectionusing structured questionnaires.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Herpes Viruses

Since 1971, the International Committee on Taxonomy of Viruses(ICTV) addressed the taxonomy of herpesviruses (Wildy, 1971). A provisional approach to endowing herpesviruses with formal names was followed by classification into subfamilies largely on the basis of biological criteria (Roizman *et al.,* 1981). However, this effort was rather successful but was not free from what turned out in hindsight to be a few misclassifications and further division of the subfamilies into genera utilized molecular data to a greater extent than before primarily in relation to genome characteristics such as size and structure (Roizman *et al.,* 1992).

The ICTV herpesvirus study grouped the family *Herpesviridae* consist of three subfamilies: *Alphaherpesvirinae* which consist of *Simplexvirus, Varicellovirus, Mardivirus* and *Iitovirus* genera). *Betaherpesvirinae* which consist of the *Cytomegalovirus, Murimegalovirus* and *Roseolovirus* genera) and *Gammaherpesvirinae* which consist of *Lymphocryptovirus* and *Rhadinovirus* genera. In addition, genus *Ictalurivirus* is unattached to any subfamily and a large number of species not assigned to genera. All but one of the viruses assigned to taxa infect mammals or birds, although a substantial number of unassigned herpesviruses have lower vertebrate (reptilian, amphibian and fish) or invertebrate (bivalve) hosts.

The primary criterion for inclusion of an agent in the family Herpesviridae is that of virion morphology. The virion is spherical and comprises of four major components: the core, the capsid, the tegument and the envelope. The diameter of the virion depends on the viral species but is approximately 200nm (Edward *et al.,* 2015). The core consists of a single copy of a linear, double stranded DNA molecule packaged at high density into the capsid.

The capsid is an icosahedron and has an external diameter of 125-130nm. It consists of 162 capsomeres, 12 of which are pentons and 150 hexons each containing five and six copies respectively of the major capsid protein (Yeroh *et al.,* 2015). The capsomeres are joined via the triplexes, each of which contains two copies of one protein and one copy of another. The tegument which surrounds the capsid contains perhaps 30 or more viral protein species and is poorly defined structurally. In the tegument, structures positioned with symmetry corresponding to that of the capsid are detectable only in the region close to the capsid. The lipid envelope surrounds the exterior of the tegument and is studded with at least ten viral membrane glycoproteins, in addition to some cellular proteins. The protein composition of the tegument and envelope varies widely across the family (Chen*et al.,*1999).

2.2 Phenotypic Structures of Herpesviruses

The phenotypic architecture of the herpesviridae is characterized customarily by their possession of a central viral core that contains a linear double stranded DNA. This DNA is exemplified by a hole through the middle and the DNA is embedded in a proteinaceous spindle (Furlong *et al.,* 1972). The capsid is icosadeltahedral (16 surfaces) with 2-foldsymmetry and a diameter of 100-200nm that is partially dependent upon the thickness of the tegument. The capsid has 162 capsomeres. The three dimentional structure of the HHV-8 capsid was determined by cryo-electron microscopy (EM) and was found to be composed of 12 pentons, 150 hexons and 320 triplexes arranged as expected in the icosadeltahedral lattice with 20 faces: the capsids are 125nm in diameter (Wu *et al .,* 2000). Transmission EM showed a bulls-eye appearance in the virions with electron dense cores and amorphous teguments surrounding the viral core (Renne *et al.,* 1996). It is worthy to note that these structural characteristics were seen in endemic Kaposi Sarcoma lesions as early as 1984, but were not recognized at that time as the possible etiology of the disease (Walter *et al.,* 1984).

The herpesvirus tegument is an amorphous proteinaceous material which lacks distinctive features under the EM. This is found between the capsid and the envelope: it can be asymmetric in distribution. Thickness of the tegument is variable dependent upon its location in the cell and varies between different herpesviruses (McCombs *et al.*, 1971).

The herpesvirus envelop contains viral glycoprotein protrusions on the surface of the virus (Knipe and Howley, 2001). As shown by EM, there is a trilaminar appearance (Epstein, 1962) derived from the cellular membranes (Morgan, 1986) and contains some lipid. Glycoproteins protrude from the envelope and are more numerous and shorter than those found on other viruses. The presence of the envelope can influence the size measurement of the virusunder EM conditions (Knipe and Howley, 2001).

2.3 Genetic Properties of Herpesvirus

A virus species is defined as a polythetic class of viruses consisting a replicating lineage and occupying a particular ecological niche (Van Regenmortel, 1989, 1990). Members of a polythetic class share a subset of properties, with each property possessed by several members but no property possessed by all. Herpesviruses are defined as separate species if their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and if they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts (Roizman *et al.*, 1992, Roizman and Pelleet, 2001: Davison *et al.*, 2005) but no property possessed by all. However, genomic data have come to dominate biological properties with taxa corresponding to genetic lineages definedby sequences comparisms and identification of genes unique to certain lineages. An increasing number of herpesviruses inthe tissues of various animals was inferred from short PCR-derived sequences, usually from a single locus in the genome and often in the absence of any other information. These ‘virtual viruses’ cannot readily be classified under the current species definition. However, their

incorporation(perhaps in a special category) couldbe facilitated. Members of the family *Herpesvividae* replicate their tegument, capsid and core (Davison, 2005)

Electronmicroscopy of negatively stained capsids gives the impression that the core consists of the viral DNA molecule wrapped toroidally around a protein spindle (Furlong *et al.,* 1972). Images reconstructed from electron micrographs of virions frozen in ice in the absence of stain, a technique by which morphology is better preserved, show that the core consists of the DNA packed at high density in liquid crystalline form, probably as a spool lacking a spindle (Booy *et al.,* 1991; Zhou *et al.,* 1999). Herpesvirus genomes consist of linear, double-stranded DNA molecules that range in size from about 125-240kbp and in a nucleotide composition from 32-75% G+C depending on the virus species (Honess,1984). The genome termini are not covalently closed as in the*poxviridae* (Moss, 2001) or covalently linked to a protein as in the *Adenoviridae* (Shenk,2001). In those herpesvirus, genomes which have been thoroughly examined, have unpaired nucleotides present at the termini, for example, Herpessimplexvirus-1, Varicella zoster and HCMV have asingle 3 overhanging nucleotide at each terminus (Morcarski and Tan, 2001). Herpesvirus genomes are enclosed within larger capsids, but the relationship is not proportional, as the packing density of the DNA varies somewhat between species (Trus *et al.*, 1999; Bhella,*et al.,* 2000). The reasons for the striking range in nucleotide composition of herpesvirus genomes are not clear, but a similar phenomenon is found in other virus families and in cellular organisms.

In contrast to the Alpha and Beta*herpesvirinae*, the genomes of most Gamma*herpesvirinae* are generally deficient in the Cytosine-Guanine dinucleotide. In vertebrate genomes, this phenomenon is thought to be due to spontaneous deamination of 5-methylcytosine residues in DNA to thymidine residues, followed by fixation through DNA replication. CG depletion in herpesviruses and concomitant enrichment in TG and CA has been taken as

indicative of latency in dividing cell populations in which the latent genome is obliged to replicate as host cells divide (Honess *et al*., 1989). Thus, HSV-1 is resident in non-dividing neurons has a CG content consisting with its nucleotide composition, where EBV which latently infects dividing B cell populations is depleted. Local CG suppression of the major immediate early gene locus of HCMV has also been noted (Honess *et al.*, 1989).

The herpesvirus family consists of a group of viruses distinguished by the large size of their linear doublestranded DNA genomes (130-250kpb) and a common architecture of infectious particles (Gibson, 1996, Chiu and Rixon, 2002). Indeed, before the birth of molecular biology and the availability of genomic sequencing, the common hallmark structural features shared by these viruses were the most important criteria for the classification of a herpesvirus (Roizman and Pellett, 2001). Eight different kinds of herpesviruses were discovered to infect humans with more than 170 other viruses found in animals, fish and amphibians that show an identical structural design(Roizman and Pellet, 2001). These viruses have a highly ordered icosahedral- shaped nucleocapsid of about 125-130nm in diameter which encases the viral DNA genome. The nucleocapsid is surrounded by a partially ordered proteinaceous layer called the tegument, which in turn is enclosed within the envelope, a polymorphic lipid bilayer containing multiple copies of more than 10 different kinds of viral glycoproteins that are responsible for viral attachment and entry to host cells (El zein*et al.*, 2008).

2.4 Classification of Herpes Virus

Herpes virus have had more than a 100 of them discovered, of which are all double – stranded DNA viruses. These DNA viruses can establish latent infections in their respective vertebrate hosts. However, only 8 commonly establishes infection in humans (Knipe and Howley, 2001).

The *herpesviridea* family is subdivided into 3 families; alpha, beta or gamma *herpesvirinae*. The International Committee on Taxonomy of Viruses classified the herpesvirus study group using biological properties and it does not rely upon DNA sequence homology. However, researchers have been able to identify and characterize the viral subfamilies using DNA sequence analysis of the DNA polymerase gene, other investigators have been successful using the glycoprotein Bgene.

Upon research based on the biological properties such as growth characteristics and tissue tropism, herpes viruses can be further divided into 3 subfamilies among which the eight human herpes virus belong; the alpha subfamily which include neurotropic viruses and contains the herpes simplexvirus (HSV)1 and 2 andVaricella zoster virus (VZV). The members of the gamma subfamily are lymphotropic viruses which include Epstein- Barr virus(EBV) and Kaposi's sarcoma associated herpesvirus (KSHV). The viruses of the beta subfamily appear to be able to establish infections in many different types of cells and tissues; it also include human cytomegalovirus (HCMV) and human herpesvirus 6 and 7. This subfamily classification system is largely consistent with the extensive genomic information that is now available (McGeoch *et al.,* 2000).

While studies have been attempted to investigate the structure and architecture of each of the eight human herpesviruses, virion and virus related particles of herpes simples virus 1 (HSV-1), the prototype of all herpesviruses have been subjected to the most extensive structural studies (Booy *et al.,* 1991;Newcomb *et al.,* 1993; Zhou *et al.,* 1999, 2000). Significant progress have been made in understanding the structure of cytomegalovirus (Bhella *et al.,* 2000; Chen *et al.,* 1999; Trus *et al.,* 1999), the prototype of the beta herpesvirus family and KSHV, a representative of the gamma herpesvirus family (Wu *et al.,* 2000; Nealon *et al.,* 2001; Trus *et al.,* 2001 and Lo *et al.,* 2003).

2.4.1 The Alphaherpesviridae

The *Alphaherpesviridae* are characterized by variable cellular host range, shorter viral reproductive cycle, rapid growth in culture, high cytotoxic effects and the ability to establish latency in sensory ganglia. In humans, these are termed herpes simplex viruses 1 and 2(HSV 1 and HSV 2) and Varicella zoster virus (VZV) which represent human herpesviruses 1, 2 and 3 (Knipe and Howley, 2001).

Human herpesviruses 1, 2 and 3 have been classified as alphaherpesviruses based originally upon their biological properties and subsequently on the sequences of their respective genomes (Minson *et al.*, 2000). All of these viruses maintain latent infections in sensory ganglia and can productively infect a variety of human cells including the living cells of mucous membranes and skin. These epithelial sites also provide exit points for the virus to infect other individuals.

2.4.2 The Betaherpesviridea

The *Betaherpesviridea* have a more restricted host range with a longer reproductive viral cycle and slower growth in culture. Infected cells show an enlargement of infected cells (cytomegalia). Latency is established in secretory glands, lymphoreticular cells and in tissues such as the kidneys among others. In humans, these are termed human cytomegalovirus (HCMV or herpesvirus 5). Human herpesviruses 6A and 6B (HHV-6A and 6B) and human herpes virus 7 (HHV -7). HHV-7 has also been called the roseolavirus named after the disease roseola infant, which causes disease in children (Knipe and Howley, 2001).

The two major lineages in the *Betaherpesvirinae* are the Cytomegaloviruses (the *Cytomegalovirus* and *Muromegalovirus* genera, plus a number of other viruses whose taxonomy is defined partially) and the *Roseolovirus* genus. The best characterized members

of these lineages are HCMV (the prototype of the subfamily) and HHV-6. Cytomegaloviruses are present in a wide range of mammalian species and have been termed “salivary gland viruses” because of their ease of isolation from explained tissue. A divergence of the*Betaherpesvirinae* is represented by a herpes virus of elephants (Ehlers *et al.,* 2001).

2.4.3 The Gammaherpesviridea

The *Gammaherpesviridea* have a host range found within organisms that are part of the Family or Order of the natural host. *In - vivo* replication of the viruses occurs in lymphoblastoid cells, but some lytic infections occur in epithelial and fibroblasts for some viral species in this subfamily. They have evolved actively to protect their latent cells from being destroyed by the immune functions of their natural host.*Gammaherpesviruses* are specific for either Bor T cells with latent virus found in lymphoid tissues. Only 2 human gammaherpesviruses are known, human herpesvirus 4, referred to as Epstein –Barr virus (EBV) and human herpesvirus 8 referred to as HHV-8 or Kaposi’s sarcoma- associated herpesvirus (KSHV) (Knipe and Howley, 2001, Ackermann, 2006).

The Gammaherpesviruses subfamily contains two genera (a classification of closely related viruses) that include gamma-1 or *Lymphocryptovirus* (LCV) and the gamma-2 or *Rhadinovirus* (RDV) virus genera. EBV is the only LCV and HHV-8 is the only RDV discovered in humans. LCV is found only in primates but RDV can be found in both primates and subprimate mammals. RDV DNAs are more diverse across species and are found in a broader range of mammalian species. It is thought that RDVs evolved before LCVs (Knipe and Howley, 2001).

2.5 Human Cytomegalovirus

Human cytomegalovirus is a widely distributed human herpesvirus with a highly restricted host range and it commonly affects individuals from diverse geographical and socio-economic backgrounds. It is a prototypic betaherpesvirus having a life cycle with the ability to establish a life-long latent infection within the host. During latency, viral gene expression is highly restricted and infectious virions are not produced (Akinbami, 2009, Morcaski and Tan, 2001).

Cytomegalovirus (CMV) was first described in the year 1881 when large cytoplasmic inclusion bodies (protozoan-like cells) were seen in the kidney of a still-born infant. The term “cytomegalia” was introduced in the year 1921, but the viral aetiology of the disease was confirmed in 1926. CMV was reported as the leading cause of congenital viral infection in developed countries, occurring with an incidence of 83% (Winter*et al.*, 2020).

Periodically, HCMV is able to reactivate from latency, resulting in the production of new infectious virus, a process which often leads to life-threatening disease in immunosuppressed individuals. While HCMV infection causes mild or clinically non-apparent disease in the immunocompetent host, it is a major cause of morbidity and mortality in immunocompromised individuals such as patients with acquired immunodeficiency syndrome, allograft recipients (bone marrow and solid organ) and neonates (Pass, 2001). Many aspects of HCMV biology and pathogenesis still remain poorly understood despite the major clinical impact of this virus on selected populations. In particular, little is known about the molecular mechanisms that underlie the ability of the virus to persist in a latent form (Boeckh *et al.,* 2001).

Human cytomegalovirus is the main cause of congenital viral infection (Ljungman *et al.*, 2002; Sissons and Carmichael, 2002). The viral genome is one of the largest and most

complex genomes known and many regions of variation have been identified, more so in clinical isolates than in laboratory-adapted strains (Cha *et al.,* 1996; Prichard *et al.,* 2001). Human cytomegalovirus can replicate in several cell types including epithelial cells which is important in intrauterine infections as well as in associations with cardiovascular disease (Jarvis and Nelson 2002, Maidji *et al.,* 2002). Cytomegalovirus has the ability to establish a life-long relationship with its host known with the term "latency". Patients with compromised immunity which include those with human immunodeficiency virus infection, malignancies and organ transplants or individuals receiving immunosuppressive therapy are at high risk for reactivation of latent CMV. Most knowledge regarding CMV reactivation has come from studies of transplant recipients and AIDS patients (Limaye *et al.,* 2001; Alberola *et al.,* 2001 and Chemaly *et al.,* 2004).

2.5.1 Structure of Human Cytomegalovirus

Human cytomegalovirus is a ubiquitous beta human herpesvirus type 5. Compared to other human herpesviruses, HCMV is the largest with a genome of 235kb encoding 165 genes (Davison *et al.,* 2003). The virion consists of a double-stranded linear DNA core in an icosahedral nucleocapsid enveloped by a proteinaceous matrix (the tegument) (Chen *et al.,* 1999). These components are enclosed in a lipid bilayer envelope that contains a number of viral glycoproteins (Morcaski *et al.,* 2007). Mature virions range in diameter from 200 to 300 nanometres (Morcaski *et al.,* 2007, Prescott *et al.,* 2011, Edward*et al.,* 2015).

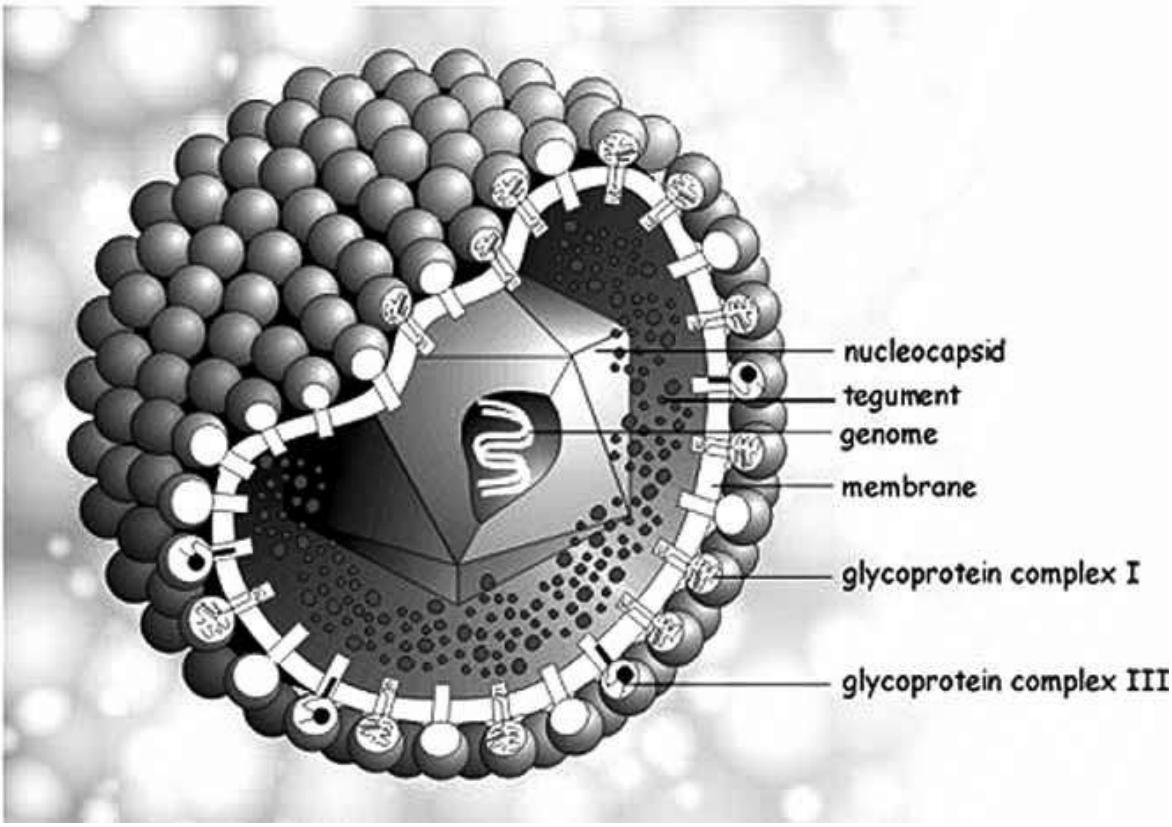


Figure 2.1: Structure of Cytomegalovirus

The tegument compartment contains the majority of the virion proteins with the most abundant tegument protein being the lower matrix phosphoprotein 65 (pp65) also termed as unique long 83 (UL 83) (Varnum *et al.,* 2004). Other major tegument proteins include the virion transactivator pp71 (upper matrix protein, UL82 gene product), the herpesvirus core virion maturation protein pp150 (large matrix phosphoprotein, UL 32 gene product), the largest tegument protein (UL48 gene product) and the UL 99 encoded pp28 (Varnum *et al.,* 2004). In addition, the tegument also contains additional proteins that are present in small amounts and some cellular and viral RNA (Morcaski*et al.,* 2007).

The functions of the tegument proteins are sub-divided into two classes:

Class 1- proteins that play a structural role and are important in the assembly of virions and the disassembly of the particle during entry

Class 2- proteins which modulate the host cell response to infection (Morcaski *et al.*, 2007). The host cell endoplasmic reticulum-golgi intermediate compartment-derived lipid bilayerenvelope surrounding the tegument contains at least 20 virus-encoded glycoproteins that are involved incell attachment and penetration (Morcarski *et al.*, 2007). These include glycoprotein B (gB), gH, gL, gM, gN and gO (Varnum *et al.*, 2004). Productive infection leads to the coordinated synthesis of proteins in three overlapping phases based on the time of synthesis after infectionnamely, immediate early (IE) (0-2hrs), delayed early (24hrs)and late (24hrs)viral proteins.

Surface projections of envelope are distinct and spikes are dispersedevenly over the entire surface The nucleocapsid is isometric and surrounded by the tegument that consists of globular material which is frequentlyasymmetrically distributed and may be variable in amount (Tsaparas *et al.*, 2003). The nucleocapsid is sometimes penetrated by stain (although an intact envelope is impermeable to stain) viral nucleocapsid is angular and has an icosahedral symmetry. There are 162 capsomeres per nucleocapsid and capsomeres are hexagonal in cross section with a hole running half way down the long axis. The viral core consist of a fibrillary spool on which DNA is wrapped while the ends of the fibres are anchored to the underside of the CMV.

CMV is inactivated by a number of physical and chemical treatments including; heat (56^{o}C for 30 mins), low pH, ether,ultraviolet light and cycles of freezing and thawing (Richardson *et al.*, 2004). CMV virions contain one molecule of linear double stranded DNA. Total genome length is 120,000-220,000bp. Molecular virology techniques have been used to study variations among CMV strains. DNA strains have been shown to have similar but distinctive fragment migration patterns. Antigenic heterogeneity among CMV strains has been detected in cross neutralization andother serologic assays; however evidence for distinct serotypes is limited (Patel *et al.*, 2009).

The HCMV envelope is exceedingly complex and currently incompletely defined. Its genome encodes Open Reading Frames to at least 57 putative glycoproteins far more than other herpesviruses. However, the extent of transcription, translation and function of the majority ofthese glycoproteins remains unknown. Biochemical studies of the virions have revealed 14 structural glycoproteins remain unknown, eight of which have been experimentally shown toreside in the envelope (Britt*et al.,* 2004).

2.5.2 Immunity against Cytomegalovirus Infection

The cell- mediated immune response plays an important role in the control of HCMV infection. In particular, clinical observations indicate the severity of HCMV disease parallels the degree of impairment of T cell responses (Pass, 2001). Both major histocompatibility complex class 1 (MHC-I) and MHC-II restricted HCMV specific cytotoxic T lymphocyte responses are generated and are important for virus clearance (Lindsley *et al.,* 1986). MHC-II- restricted CD4 cells act as critical antiviral effectors as well as providing helper functions for maintaining HCMV specific CD8 T-cell responses (Komanduri *et al.,* 2001) and impairment of the HCMV- specific CD4 T -cell response has been associated with prolonged secretion of virus in young children (Tu*et al.,* 2004).

In a study of CD4 T-cell responses following primary HCMV infection in renal transplant recipients, the results provided evidence for the emergence of HCMV –specific CD4 T cells that were represented during the latent phase of infection and that had the capacity to lyse HCMV antigen expressing cells in an MHC-II dependent manner (Vanleeuwen *et al.,* 2006). Thus MHC-II-restricted CD4 T cells likely play an important role in the control of productive infection and latency/reactivation. These findings have led to the hypothesis that HCMV encoded modulation of MHC II would enhance the capacity of the virus to limit the host immune response during both productive and latent phases of infection. During latency, interference with MHC-II expression leading to impairment of CD4T cell

surveillance of latently infected cells which may enhance chances for a long term maintenance of the virus. In this respect, experimental latent infection of cultured granulocyte macrophage progenitor (GM-P) cells has been reported to result in a decrease in the expression of cell surface MHC-II molecules (Slobedman *et al.,* 2004). Human cytomegalovirus clinical strains display genetic polymorphisms, possibly related to strain – specific tissue tropism and HCMV induced immunopathogenesis. A substantial portion of the genome encodes proteins with the potential to affect virulence through cell tropism, immune evasion, molecular mimicry or interference with host chemokines (Hahn *et al.,* 2004).

2.6 Antibody and Complement against Human Cytomegalovirus Infection

Following acute or primary infection, antibodies specific to numerous HCMV encoded proteins are readily detectable in serum (Britt, 1991; Landini and Michelson, 1988). These include structural tegument proteins (such as pp65 and pp150), envelope glycoproteins (predominantly gBand gH) as well as non-structural proteins such as the Immediate Early 1 protein(IE 1, UL 123) and a DNA binding protein (UL44). Viral neutralizing activity *in vivo* is predominantly mediated by antibodies specific for gB and gH (Britt *et al.,* 1988 and Urban *et al.,* 1996). Both mouse and guinea pig animal models suggest that antibody is important in protection from a lethal infective dose and in reducing fetal infection (Rapp *et al.,* 1992; Harrison *et al.,* 1995). In humans, pre-existing humoral immunity to cytomegalovirus also plays an important role in preventing congenital infection of the fetus during pregnancy (Fowler *et al.,* 1992) and in preventing transfusion –associated infection in premature infants (Yeager *et al.,*1981). The role of humoral immunity in the pathogenesis of HCMV disease in immunosuppressed patients is uncertain(Guglielmo *et al.,* 1994 and Munoz *et al.,* 2001).

2.7 Pathogenesis of CMV

Following infection or contact with cytomegalovirus, the DNA enters the nucleus of the host cells and begins the process of replication and shedding leading to the release of new virions (viral particles) into the blood and other body fluids. The replication cycle takes approximately 24 hours and consist of 3 regulated phases; an immediate early phase (4hours)during which regulator proteins are made followed by an early phase (8hours)during which viral DNA polymers are made and a late phase (12hours) during which structural proteins and new progeny viral particles are assembled (Schleiss, 2011).

In an immunocompetent host, most of the virus are destroyed by CMV- specific cytotoxic cells and the infectious process becomes asymptomatic. The presence of asymptomatic CMV infection is based on the detection of CMV in body fluids or on seroconversion from a negative to a positive status. The latter is defined as the appearance of IgM or a fourfold rise in the level of CMV specific IgG antibodies. In immunocompromised patients who are not receiving prophylactic treatment against CMV, symptomatic CMV infection, CMV disease or organ involvement may result (Wingard *et al.,* 1999).

There are various mechanisms by which CMV disease may occur and these include direct cellular loss through viral infection, both of individual cells and of larger foci because of propensity to spread leading to coalescing cells. This close cell-cell interaction protects virus from antibody inactivation. Systemic hypoxic- Ischemic insult due to systemic hypotension and immunologic injury, involving antigen-antibody complex deposition may also be involved in organs such as kidney. Eventually, CMV attains a latent state and persistent infection within T cells, endothelial cells and monocyte-derived macrophages ensues (Wingard *et al.,* 1999).

Cell-mediated immunity is required for resolution of symptoms and contributes to symptoms while the role of antibodies are limited. Suppression of cell mediated immunity as seen in HIV infection allows recurrence of symptoms and can result in exacerbation of disease. The virus also has the ability to induce immunosuppression in the body (Cicognac, 1993). The degree of morbidity induced by CMV is influenced by the degree of immunosuppression. In transplant recipients, two factors influence the degree of morbidity due to CMV infection, the type and extent of immunosuppression and the type of transplant. Morbidity is highest among bone marrow transplant (BMT) patients and lowest among kidney transplant patients. Multiple mechanisms of immune evasion by CMV could relate to the pathogenic role of the virus. The expression of immune evasion genes US3, US6 and US11 of CMV in the blood of solid organ transplant recipients has been investigated (Patel *et al.*, 2009).

2.8 Pathology of CMV

CMV produces characteristic cytopathic changes on infected cells and they appear large and rounded containing grounded glass which appear like inclusion bodies in the cytoplasm. These infected cells are the hallmark of CMV infection and indicates the presence of CMV in a sample. The nuclear inclusion has the appearance of an owl's eyes because it is typically surrounded by a clear halo that extends to the nuclear membrane (Euright *et al.*, 2001).

2.9 Clinical Manifestation of CMV

Following a first exposure to CMV, the virus lies dormant in the body for life and can be reactivated. Usually a reactivated infection causes few or no symptoms in an individual. CMV disease may manifest in any of the following underlisted ways;

2.9.1 Congenital Infection

Congenital infection is defined as the isolation of CMV from the saliva or urine of the neonate within 3 weeks of birth (Meyers and Flournoy, 1996). The virus may also be transmitted from the mother to the infant in breast milk. CMV was reported to be the commonest cause of congenital infection and affects around 0.3-1% of all livebirths(Boeckh *et al.,* 2001).. A total of 5-10% of congenitally infected infants have symptoms at birth while fatal disease occurs in 20% of these infants (Boeckh *et al.,* 2001).

Ninety percent (90%) of the symptomatic survivors have long term sequelae while 15% of the asymptomatic survivors also have long term sequelae. CMV is the second commonest cause of mental retardation after Down's syndrome and causes more cases of congenital damage than Rubella (Meyers and Dandliker, 1998). Cytomegalic inclusion disease refers to any group of diseases caused by cytomegalovirus infection and marked by characteristic inclusion bodies in enlarged infected cells. An infected pregnant woman may pass the virus to her developing baby or during childbirth as a newborn and through breastfeeding (Edward*et al.,* 2015). The classic disease is congenital, been acquired in utero from the mother and typically presents with intrauterine growth retardation, jaundice, hepatosplenomegaly, thrombocytopenia and encephalitis with or without microcephaly. It is often difficult to differentiate on clinical grounds between the several agents that may cause intrauterine infection. The severe thrombocytopenia, hepatitis, pneumonia and myocarditis attributed to the virus may be life threatening. The involvement of the central nervous system may lead to seizures, focal neurological signs and mental retardation. Unlike rubella, there is no evidence that CMV is teratogenic (Meyers and Danndliker 1998).

Most of the damage is caused by destruction of target cells once they have been formedand unlike rubella, the fetus can bedamaged by infection during any stage of pregnancy. In 20%

of cases (1% of those who are congenitally infected), the infection is so severe that mortality occurs during infancy, while the rest are likely to sustain serious abnormality on follow up, and thus may manifest as microcephaly, mental retardation and seizures. The spectrum of brain damage varies from mild to severe. Optic atropy, deafness and blindness may also be present (Atkinson and Downs, 1995). The effects of congenital CMV infection are as follows: CNS abnormalities, microcephaly, mental retardation, spasticity, epilepsy, periventricular calcification, eyechoriodoretinitis and optic atropy, ear-sensorineural deafness, liver hepatosplenomegaly and jaundice which is due to hepatitis, hematological thrombocytopenic purpura, haemolytic anaemia, neutropenia and lymphocytosis. Late sequelaeinclude damage to the enamel - forming organ of the teeth, resulting inyellow discolouration of the teeth and brittleness. This occurs in 40% of infants (Atkinson and Downs, 1995). Ndako *et al.,* 2016 also reported symptoms from intrauterine infection which include any combination of microcephaly, intracranial calcification, chorioretinits, jaundice, low birth weight, hepatospleenomegaly and purpura.

2.9.2 Perinatal Infection

Despite the continued excretion of high titres of virus in theurine for many months, the vast majority of perinatally infectedinfants do not develop acute symptoms, although few cases of infantile pneumonitis have been reported.This appears to be an exceedingly rare event. (Meyers and Dandliker, 1998; You and Dimopoulous, 2001).

2.9.3 Postnatal Infection

The incubation period for CMV ranges from 4-8 weeks. Acute infection in the postnatal period is usually mild or asymptomatic. Occasionally, the infectionmay be accompanied by the syndrome of infectious mononucleosis with atypical lymphocytosis. This is similar to the syndrome produced by Epstein Barr virus (EBV) except that lymphadenopathy is uncommon and Paul-Bunnell test is negative. CMV-induced mononucleosis (Goldrich and

Mori, 2004). Malaise, fever of up to 39.4^{o}C, chills, sore throat, headache and fatigue can be the predominant features of both viruses. Many of the same clinical manifestations that are typical of EBV induced mononucleosis (e.g. lymphadenopathy, spleenomegaly, pharyngeal erythema) also can occur with CMV, although less frequently. Presentation of acute cytomegalovirus infection in an immunocompetent host may be in form of mononucleosis. Patients with mononucleosis may present with non-specific skin rashes such as; generalized maculopapular, urticarial and scarlatiniform rashes. These rashes are not a direct cause of CMV proliferation within the skin but occur due to an immunologic response to the virus. The classic hypersensitivity drug rash is associated with ampicillin therapy given to patients with EBV induced mononucleosis is essentially CMV mononucleosis acquired by blood transfusion (Canpolat and Culbert, 1997).

2.10 CMV Infection in the Immunocompromised Patients

Acute CMV infection in immunocompromised individuals is far less likely to beasymptomatic. These patients develop spiking pyrexia which resolves within a few days (Canpolat and Culbert, 1997). Some may develop a viremia with septicaemia- like syndrome in the presence or absence of hepatitis. Pneumonitis may develop which is associated with grave prognosis. The virus may disseminate to involve the retina, causing CMV retinitis which is the commonest ocular opportunistic infection and the most common cause of visual loss in people with AIDS (You and Dimopoulous, 2001). CMV may disseminate to the guts where it may cause an asymptomatic infection or ulceration or haemorrhage by the erosion of nearby blood vessels. In patients with CMV-induced immune suppressive syndrome the patient becomes unable to deal with opportunistic infections such as Pseudomonas. AIDS patients may develop lowgrade encephalopathy and CMV adenitis.

Kaposi sarcoma is another disease that has been associated with past CMV infection which is the most common infection in bone marrow transplant recipients. Infection by this virus occurs in over one half of the patients. The virus is an immunomodulatory and CMV disease exacerbates an ongoing immunosuppression in transplant recipient, thereby the risk for bacterial and fungal infection such as *Norcardia asteriodes, Mycoplasma* and *Pneumocystis carini* (Sisson *et al.,* 2002).

2.11 Immunosuppression by CMV

Often, it is stated that HCMV is "immunosuppressive" froma clinical perspective, the association is somewhat anecdotal; consequently, CMV disease frequently arises in the context of immunosuppression, iatrogenic or otherwise and it is difficult conclusively to implicate HCMVin the causation of immunosuppression. The disease associated with primary HCMV infection in the normal immunocompetent host is characterized by marked T- cell proliferation, but not obviously associated with immunosuppression. The possession of a large number of gene functions which modulate the expression of MHC molecules, cytokines and NK cell interactions, is the mostspecific evidence that HCMV exerts "immunosuppressive" effects, although it is obvious these effects do not prevent the normal host from mounting a sustained and effective immune response against the virus. In thewhole organism, the effect of HCMV- induced downregulation of MHC molecules may be counteractedby other influences for instance, it has been shown that IFN and TNF can upregulate MHC Class I molecules and counteract the downregulating effect of the HCMV genes (Hengel *et al.,* 1995).

Much of the *in - vivo* evidence for "immunosuppression" comes from experiments in which investigators have added HCMV to cultures of peripheral blood mononuclear cells and then used a readout such as Tcell proliferation in response to exogeneous mitogens or a similar assay (Raftery *et al.,* 2001).

2.12 Epidemiology of Cytomegalovirus

Human cytomegalovirus is common in the general population which in effect infects both males, females, young and the old.Consequently, it is one of the most common viral infections during perinatal period which leads to congenital CMV infections. Its clinical manifestations range from asymptomatic forms (90% of cases) to severe fetal damage that may include permanent hearing, vision loss, neurological impairment and, in rare cases, death due to abortion (Maingi and Nyamache 2014).

Most infections are mild or without symptom. However,two major group of patients who may manifest serious CMV infections. One group includes congenitally infected newborn infants and the other group is patients receiving immunosuppressive therapy. Between 0.3% and 2% of newborn infants excrete CMV in their urine (Tan, *et al.,* 2014). The need for a rapid CMV diagnosis is increasing accordingly.

Cytomegalovirus is ubiquitous virus found universally throughout all geographic locations and socioeconomic groups and infects between 50% and 85% of adults in the United States by 40 years of age (Leinikki *et al.,* 1996). CMV infection is more widespread in developing countries and in areas of low socio economic condition (Cabau *et al.,* 2000). The early acquisition and high prevalence of CMV antibody has been ascribed to low socio economic conditions, poor hygienic and overcrowding conditions (Umelo, *et al.,* 2017). Studies carried out among people the of the New Guinea highland villages, people with primitive standard of hygiene and who often lived apartfrom childhood until marriage as well as the studies carried out on people of the New Hebrides and Solomon Island who lived in crowded population but are scrupulously clean, showed that the prevalence of CMV infection was higher in the latter than the former group. It was concluded that close personal contact, rather than poor hygiene was more important for the acquisition and spread of CMV (Jemal, 2011).

Human cytomegalovirus infections have been recognized in every human population that has been studied (Krech *et al.,* 1971; Gold and Nankervis, 1976). It is endemic without seasonal variation (Gold and Nankervis, 1976) In the developing world, acquisition of HCMV is nearly universal in early childhood. Studies have shown that most preschool children (>90%) in South America, sub-saharan Africa, East Asia and India are HCMV antibody positive (Gold and Nankervis, 1982; Stagno, 2001). In Chengdu, China a population survey observed 60% of children between 4-7 years of age were HCMV seropositive (Liu *et al.,* 1990). Similarly, 58% of children between 4-12 years of age in Taipei, Taiwan, 61% of hospitalized pediatric patients from a low income population in Rio de Janeiro, Brazil and 56% of children aged 1-4 years in Jamaica were HCMV antibody positive (Prabhakar *et al.,* 1992; Shen *et al.,* 1992; Suassuna *et al.,* 1995). In Finland, the HCMV seroprevalence rates increased from 27% in children of 7 months of age to 41% in children 8 years of age in a cohort study of children followed for 8 years (Aarnisalo *et al.,* 2003). In a population survey in Parma, Italy, age specific HCMV seroprevalence increased from 28% in two year olds to 96% in 45-54year old resident(Natali *et al.,* 1997).

Similarly, in Spain, the CMV seroprevalence rate in children 2-5 years of age was 42% increasing to 79% in adults between 31-40 years of age (de Ory *et al.,* 2004). Recent studies in blood donors have demonstrated that populations in Asia and Africa continue to have CMV seropositivity rates of 95-100% Serological surveys have shown CMV infections invirtually every population that have been tested with seropositivity ranging from 40 – 100% in different parts of the world. For example, a serological survey of over 20,000 women in London found 54.4% of these women were seropositive for CMV. The African Continent is now known to have the highest prevalence of CMV IgG antibodies. Seroprevalence of 72.2% and 96.0% have been reported in Western Sudan and Egypt. In

Asian countries such as Malaysia, the seroprevalence of CMV IgG antibodies among pregnant women was reported to be 84.0%. In Nigeria, serological survey conducted in Bida, Lagos and Sokoto among pregnant women found a seroprevalence of 84.2%, 97.2% and 98.7%respectively (Okwori *et al.,* 2008, Akinbami *et al.,* 2011, Ahmad *et al.,* 2011)These results shows that CMV is on the rise in Nigeria.

The prevalence of CMV has been studied in relation to other causes of congenital infections such as *Toxoplasma gondii* and rubella (TORCH agents) among pregnant women. In one of these comparative studies conducted in Turkey, CMV was found to be leading in prevalence with a seroprevalence of 96.4% for IgG, 0.7% for IgM and 1.9% for IgG + IgM. Similarly, a study in Turkey found higher CMV seroprevalence of 94.4%for IgG and 0.4% for IgM (Yeroh *et.al.,* 2015).

2.13 Modes of Transmission of Cytomegalovirus

Cytomegalovirus is the largest of the human herpesvirus family: its transmission occurs by close contact, through contamination from urine, saliva, blood, semen, and cervical secretions. However, the exact mode of HCMV transmission is unknown but it is assumed that to be through direct contact with body fluids from an infected person. The differences in age-related prevalence probably reflect differences in child rearing practices, sexual behaviours and possibly, living conditions. Breastfeeding, children day care, crowded living conditions and sexual activity have all been associated with high rates of HCMV infections. Sources of virus include oropharyngeal secretions, urine, cervical and vaginal secretions, semen, breast milk, blood products and allografts (Wang and Zhao, 2020).The primary mode of spread of the virus is presumed to be exposure to saliva and other body fluids containing the infectious virus and infected infants shed significant amount of HCMV for months to years following infection. Even older children and adults shed virus for prolonged periods (>6 months) following a primary HCMV infection. In addition, a

significant proportion of seropositive individuals continue to shed virus intermittently. An important determinant of the frequency of congenital and perinatal HCMV infections is the seroprevalence rate in women of child bearing age.

2.16 Treatment of CMV Infection

In the management of CMV disease, four different strategies can be utilized: antiviral therapy, prophylaxis, pre-emptive and suppressive treatment. Ganciclovir, a nucleoside analogue of guanine is a potent inhibitor of CMV replication in vivo and it is effective in preventing hearing deterioration (Kimberlin*et al.,* 2003).

Valganciclovir, a prodrug of ganciclovirhas also been associated with myelo-suppression which is often dose dependent. Oral valganciclovir has an improved hearing and neurodevelopmental effect (Kimberlin *et al.,* 2015).

Foscarnet, a pyrophosphate analogue with in vivo activity against all human herpes as well as HIV has also been useful in treatment of CMV retinitis in AIDS patients. Nephrotoxicity and electrolyte imbalance are the most common toxicity associated with foscarnet (Tan, 2014). In addition, foscarnet can be used as a second line therapy in CMV patients with ganciclovir resistance. Oral ganciclovir has been licensed for CMV prophylaxis in patients with advanced AIDS. Routine prophylaxis however has not become standard in most HIV care settings, mainly due to the high cost of prophylaxis, potential toxicity and the inconvenience of taking 12 capsules per day (Hilt and Buchhotz, 2001). However, there is no validated treatment for CMV during pregnancy but other studies have shown encouraging results for the effect use of intravenous hyperimmune globulin to prevent or treat congenital disease (Willame *et al.,* 2015).

2.15 Prevention and Control

The optimal prevention of CMV Disease is by vaccination. However, there is no effective vaccine available. The Towne strain reduced the severity of the disease without affecting the infection rate. Studies are currently going on to determine an alternative approach which is the use of a subunit recombinant or DNA vaccines (Einsele *et al.,* 2005).

Interventions, in effect should be targeted at women who wish to become pregnant, women who care for children and immunocompromised individuals. These individuals in whom exposure to CMV can be most detrimental will be the target groups for possible administration of a future vaccine. A possible approach to preventing congenital CMV infections includes improved hygiene behavior of seronegative pregnant women, administration of CMV hyperimmune globulin (HIG) to pregnant women with acute infection and vaccines once available is administered to girls or women before pregnancy (Adler, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area was Lokoja, the State capital of Kogi state, Nigeria. The samples were collected from both the Federal Medical Center and the Kogi State Specialist Hospitals located in Lokoja, Kogi State, Nigeria.Lokoja, which is the city capital of Kogi State, Nigeria lies at the confluence of the Niger and Benue rivers. Its coordinates are located between latitude 7^0 49'N and longitude 6^0 45'E. It has a landmass area of 3,180km^2 and a population of 195,261 at the 2006 census (Onuigbo *et al.*, 2017). (Figure 3.1)

3.2 Study Population

The study population were pregnant women attending antenatal clinics of the selected hospitals.

3.3 Inclusion Criteria

The inclusion criteria were pregnant women attending antenatal clinics of the selected hospitals who gave their consent to participate in the study.

3.4 Exclusion Criteria

The exclusion criteria were women who were not pregnant and not attending antenatal clinics, and those who refused consent to partake in the study.

3.5 Study Design

The study design is a laboratory based descriptive cross sectional study conducted at the study area to investigate the seroprevalence and associated possible risk factors among pregnant women attending antenatal care of the hospitals who voluntarily chose to participate in the research.

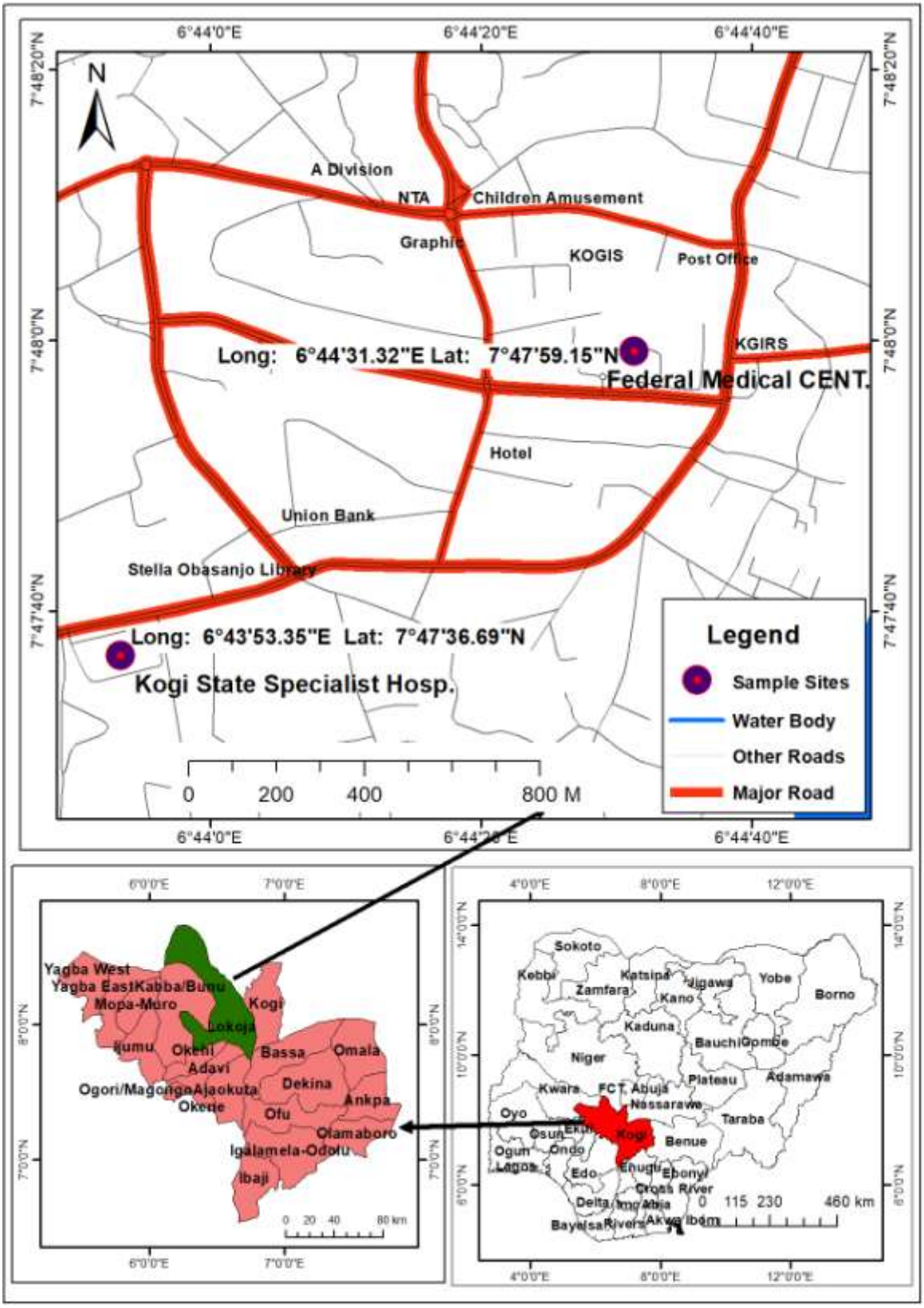


Figure 3.1: Map of the Study Area

Source: Administrative Map of Kogi State

3.6 Sample Size Determination

The sample size was determined using the formula below:

$$N= z^2Pq/d^2$$ (Naing*et al.*,2008)

Where,

n= the sample size

z=the standard normal deviate (1.96 for a 95% confidence level)

d=the level of the accuracy desired, or sampling error

p= the proportion of the population having the characteristic being measured

q= the proportion of the people that do not have the characteristic (that is 1-p)

using a prevalence of 10.5% (Edward *et al.,* 2015).

$$n= \frac{1.96^2 x\ 0.105(1-0.105)}{0.05^2}$$

$$=144.4047$$

A total of one hundred and eighty-four (184)blood samples was collected from willing participants in order to increase the chances of positivity.

3.7 Ethical Consent

The Ethical consent was obtained from Kogi State Ministry of Health as well as from both selected hospitals' Ethical approval committees.

3.8 Collection of Samples

A total of five-milliliter (5ml) of whole blood was collected from each consenting pregnant woman by venipuncture in which three milliliter (3ml) was collected in sterile anticoagulant-free plain bottles and two milliliter (2ml) in EDTA bottles appropriately labelled to avoid mix-up from both hospitals. Firstly, each respondent's arm was swabbed with 70% alcohol and blood collected was transferred into sterile anticoagulant-free sterile bottle or plain bottle and allowed to clot. The clotted blood sample was centrifuged at 3,000 rpm for five (5) minutes, and the serum (the supernatant) was transferred using micropipettes into microtubes and stored at -20°C until required for use. A Sample in the EDTA container was also centrifuged at 3,000 rpm for five (5) minutes and the plasma separated out into microtube using a micropipette. This was also stored at -20^{o}C until required for further use.

The cut-off optical density (O.D) obtained was in accordance with the manufacturer's instructions. The CMV index of the samples was calculated by dividing the O.D value of each sample(S) with the obtained Cut-off O.D value (C.OD).

S/C.OD Interpretation

CMV index<0.90 = Negative or is

CMV index 1.0 and above = Positive

Structured questionnaires were administered to the participants in order toobtain the Socio-demographic characteristics as well as risk factors associated with CMV infection.

3.9 Serological Test

The serum sample was tested for CMV-specific IgM antibodies using IgM ELISA kit Diagnostic Automation, INC (USA). All the samples, reagents and calibrators were

brought to room temperature at 25^{O}Can hour before the test for thawing to take place. The ELISA test was run as specified by the manufacturer's instructions using the manual.

The specimen samples and Durham tubes were arranged accordingly to avoid mix-up during dilution. The micro-titre wells also were labelled accordingly with blank and the control wells (positive and negative controls) as well as the sample wells. A wash buffer of 50ml concentration was prepared to 1000ml.

A 1:40 dilution of sample and diluent was done (5µl:200l) into the Durham tubes using micropipettes. A 100µl of the diluted samples was pipetted into each microtitre well accordingly. Incubation was done in the dark for thirty (30) minutes. After the elapsed 30minutes, the wells were rinsed with wash buffer for three (3) consecutive times to remove excess or unbound materials and blotted out onto paper serviettes to remove all air bubbles. A 100µl of enzyme conjugate was pipetted into the wells and incubated for 30 minutes after which the excess was washed off three (3) consecutive times. A 100µl of Tetra Methyl Benzidine (TMB) chromogenic substrate was pipetted into the wells and incubated at room temperature for 15 minutes. A 100µl of stop solution was added to the microwells to stop the reaction. The microtitre wells was loaded into the microwell reader at 450nm.

All samples with optical densities less than 10 U/ml were considered negative and those greater than or equal to 10 U/ml were considered positive.

3.10 PolymeraseChainReaction

The Polymerase Chain Reaction (PCR) was essentially to detect the presence of the virus by the glycogen B protein at 257 base pair. The first step was DNA extraction which was done by reconstituting 1,060 µl of storage buffer to the 20ml tube of proteinase k and stored at -20^{0}C prior to DNA extraction. The DNA extraction was carried out as follows:

for each of the blood samples, 200µl was transferred into a microcentrifuge tube; this was followed by the addition of 200µl of biofield and cell buffer and 20µl of proteinase K. The selected samples were allowed to thaw at room temperature after which 200µl of the sample was pipetted into a microcentrifuge tube and 200µl of biofluid and cell buffer (red) and 20µl of proteinase k was added. The sample thoroughly mixed and incubated at 55^{o}C for 10 minutes. One volume of genomic binding buffer was added and mixed thoroughly. The mixture was transferred to a zymo spin 11c-xl column in a collection tube and centrifuged for at greater or equal to 12,000µg for a minute and the collection tube was discarded with the flow through. A 400µl of DNA prewash buffer was added to the column in a new collection tube and centrifuged for a minute then the collection tube was emptied. A 700µl/g of DNA wash buffer was centrifuged for a minute and the collection tube emptied. A 200µl/g of DNA wash buffer was centrifuged for a minute after which the collection tube was discarded with the flow through.The DNA was eluted and transferred into a clean microcentrifuge tube and greater or equal of 50µl of DNA elution buffer was added. Incubation was done for 5 minutes and centrifuged for a minute and then while inside the centrifuge, another 50µl was added for 2minutes. After incubation, the tubes werelabelled accordingly and 420µl of genomic buffer was added. Centrifugation followed at 10,000 rpm for 2 minutes.

3.11 The PCR process

A 50 µl of reaction mixture was prepared comprising of 25 µl of master mix, 1µl of forward primer, 1µl of reverse primer and 3 µl of DNA extract18 µl of nuclease free waterwhich summed up to the 50µl. All these additions were done using micropipette into eppendorf tubes. These tubes were placed in the PCR machine in which the conditions was set at; 95^{o} C at 5 minutes for the initial denaturation phase, 95^{o} C at 30 seconds for the

denaturation phase, 56.5^{o} C at 30 seconds the annealing temperature phase and 72^{o} C at 30 seconds, 72 ^{o} C at 5 minutes for extention phase.

3.12 Gel electrophoresis

A 1.5g of agar rose gel was dissolved in 100ml of buffer, this was heated and stained with 2µl of ethidium bromide. This was cooled, casted on the gel trays with the gel combs already affixed in it and then allowed to gel. The amplified PCR product was loaded with a blank for 30 minutes in the electrophoresis tank set at 80 volts.After the time had elapsed, the gel was loaded onto the gel documentation chamber, viewed and snapped.

3.13 Data Analysis

The data was analysed using SPSS version 20 (2010). Pearson chi- square test was used to test level of significance in the occurrence of cytomegalovirus and other variables. The level of significance was set at 0.05 level.

CHAPTER FOUR

4.0 RESULTS

Of the one hundred and eighty four (184) pregnant women screened for Cytomegalovirus infection using IgM ELISA, ninety two (92) samples each was collected from Kogi State Specialist Hospital (KSSH) and Federal Medical Centre (FMC), Lokoja.

A total of thirty (30) samples were screened positive using CMV ELISA IgM giving 32.6% as positive percentage prevalence from Kogi State Specialist Hospital (KSSH) while ten (10) samples were screened positive of CMV ELISA IgM giving 10.86% aspositive percentage prevalence from Federal Medical Centre (FMC) Lokoja. However, the overall seroprevalence of IgM obtained from the study location was 21.7% (Fig 4.1).

Table 4.1 presents the seroprevalence of Cytomegalovirus with respect to age.The prevalence in relation to age in years was categorized into four (4) groups; 15-24 years, 25-34 years, 35-44 years and 45-54 years. The result showed that age group 35-44 years had the highest prevalence value of 23.53% closely followed by age group 25-34 years which had a prevalence of 23.08% respectively. However, age group 15-24 years had a prevalence value of 15.63%, while age group 44-54 years had a zero prevalence. The *p* value was not statistically significant ($p = 0.7607$).

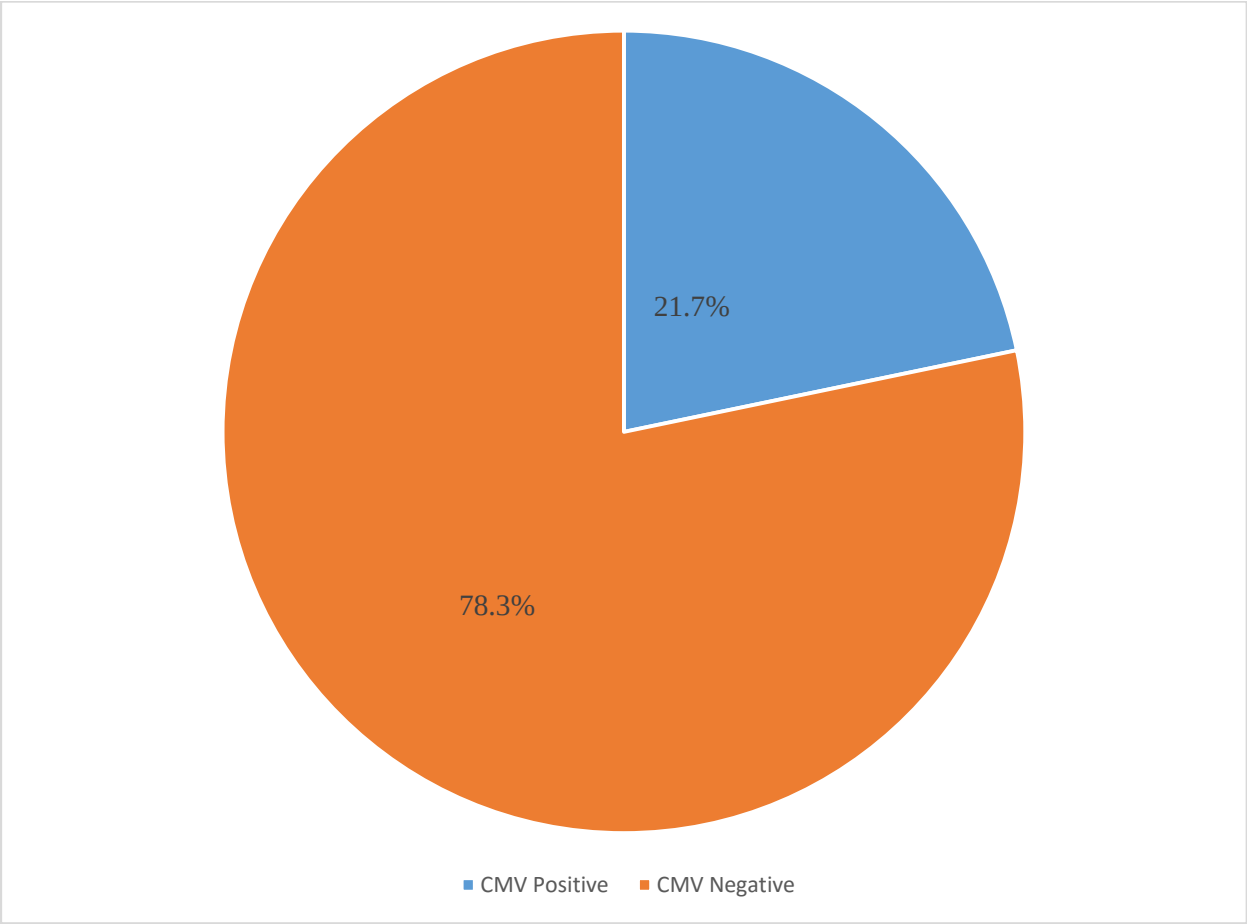


Figure 4.1: Seroprevalence of CMV among pregnant women in the study area.

Table 4.1: The Seroprevalence of Cytomegalovirus with respect to age among pregnant women in the study area.

Age(years)	No. Examined	CMV Positive (%)	Degree of Freedom (df)	Chi Square	*p*- value
15-24	32	5(15.63)	3	1.168	0.761
25-34	117	27(23.08)			
35-44	34	8(23.53)			
45-54	1	0(0)			
Total	184	40 (21.7)			

Key: df = degree of freedom

Table 4.2 presents the seroprevalence of cytomegalovirus infection in relation with educational status. The study established a 100% prevalence among women with primary education and a 40% prevalence with those who have an informal education while those with tertiary education had a prevalence of 18.2%. The *p* value was however significant for this category and the difference was statistically significant ($p = 0.035$).

For the occupational status category, the unemployed group presented a 30% prevalence while the self - employed and civil -servant groups had a 26.2% and 13.1% prevalence respectively. The *p* value was however not statistically significant ($p = 0.138$) as represented in Table 4.3.

Table 4.4 presents the seroprevalence of cytomegalovirus in relation to marital status among pregnant women in Lokoja Metropolis, Nigeria. For the single category, two (2) respondents were examined which gave a zero prevalence while for the married category, one hundred and eighty two (182) respondents were examined giving a 21.7% prevalence.

Table 4.5 presents the seroprevalence of cytomegalovirus infection in respect to Type of Marriage, for the monogamy category, it presented a percentage positivity of 20.7% and 33.3% for the polygamy category. The *p* value was not significant.

The seroprevalence of cytomegalovirus infection in respect to pregnant women with scarification. The percentage positivity was 25% for respondents that had scars while 20.9% positivity was for respondents that did not have scars. The *p* valuewas not significant (Table 4.6).

Table 4.2: The Seroprevalence of Cytomegalovirus with respect to Educational status among Pregnant women in the study area.

Educational status	No. Examined	CMV Positive (%)	Degree of Freedom (df)	Chi Square	*p*- value
Tertiary	148	27(18.2)	3	8.014	*0.035
Secondary	30	10(33.3)			
Primary	1	1(100.0)			
Informal	5	2((40.0)			
Total	184	40 (21.7)			

Table 4.3: The Seroprevalence of Cytomegalovirus in relation to Occupation among pregnant women in the study area

Occupation	No. Examined	CMV Positive (%)	Degree of Freedom (df)	Chi Square	*p*- value
Civil servant	61	8(13.1)	3	5.446	0.138
Self-employed	84	22 (26.2)			
Unemployed	30	9 (30.0)			
Others	9	1((11.1)			
Total	184	40 (21.7)			

Table 4.4: Seroprevalence of Cytomegalovirus in Relation to Marital Status among Pregnant Women in Lokoja Metropolis, Nigeria

Marital Status	No. Examined	CMV Positive (%)	Degree of Freedom (df)	Chi Square	*p*- value
Single	2	0 (0.0)	1	0.526	1.000
Married	182	40 (21.7)			
Total	184	40 (21.7)			

Table 4.5: Seroprevalence of Cytomegalovirus in relation to Type of Marriage among pregnant women in the study area

Type of Marriage	No. Examined	CMV Positive (%)	Degree of Freedom (df)	Chi Square	*p*- value
Monogamy	169	35 (20.7)	1	1.290	0.325
Polygamy	15	5 (33.3)			
Total	184	40 (21.7)			

Table 4.6: Seroprevalence of Cytomegalovirus in relation to scarification with respect to Pregnant Women in the study area.

Scarification	No. Examined	CMV Positive (%)	Degree of Freedom (df)	Chi Square	*p*- value
Yes	36	9 (25.0)	1	0.280	0.653
No	148	31 (20.9)			
Total	184	40 (21.7)			

Table 4.7 presents cytomegalovirus infection in relation to some risk factors, a large significant proportion of the participants had no knowledge of cytomegalovirus before this study (176 participants) while only 8 participants had a vague knowledge of the virus bringing the prevalence to 22.2% and 12.5% respectively.

The prevalence of the group with first pregnancy had a 14.1% to those who answered "yes" while those with a "no" answer had a 27.4% prevalence and thus with a significant *p* value of (0.046).

The analysis of CMV infection in relation to trimester for the study showed no significant difference (p = 0.814). The pregnant women at the 3rd trimester showed the highest prevalence of 25% while 1st trimester showed 21.1% with the 2nd trimester having the lowest prevalence of 19.1%.

The pregnant women with a 'yes' response to having toddlers had 25.6% prevalence while those with a 'no' response had 18.4% prevalence. Mothers who gave birth to babies who were deformed had a 100% prevalence as compared to mothers who did not have deformed babies. There was a significant *p*value of 0.045.

The pregnant women who practiced frequent hand wash after using the toilet or after handling a toddler's diaper had a lower prevalence of 16.1% as compared to those who did not practice frequent hand wash with a prevalence of 30.6% thereby giving the *p* value to 0.027. The pregnant women who had undergone blood transfusion had a higher prevalence value of 57.1% as compared to those who did not have a blood transfusion with a prevalence value of 18.8%, thereby having a significant *p* value of 0.003.

The pregnant women who had an underlying disease of diabetes had a prevalence of (0%) while those that did not have diabetes as an underlying disease had a prevalence of (21.9%). this difference was not statistically significant (p = 1.000).

Note:

OR=1 Exposure does not affect odds of outcome

OR>1 Exposure associated with higher odds of outcome

OR<1 Exposure associated with lower odds of outcome

Odds ratios are used to compare the relative odds of the occurrence of the outcome of interest (e.g. disease or disorder), given exposure to the variable of interest (e.g. health characteristic, aspect of medical history). The odds ratio used is to determine whether a particular exposure is a risk factor for a particular outcome, and to compare the magnitude of various risk factors for that outcome.

The 95% confidence interval (CI) used is to estimate the precision of the OR. A large CI indicates a low level of precision of the OR, whereas a small CI indicates a higher precision of the OR. It is important to note however, that unlike the p value, the 95% CI does not report a measure's statistical significance. In practice, the 95% CI is often used as a proxy for the presence of statistical significance if it does not overlap the null value (e.g. OR=1). Nevertheless, it would be inappropriate to interpret an OR with 95% CI that spans the null value as indicating evidence for lack of association between the exposure and outcome.

Table 4.7: Seroprevalence of Cytomegalovirus with respect to some risk factors among Pregnant Women in the study area

Risk Factors	No. Examined	CMV Positive (%)	Degree of Freedom (df)	Chi Square	*p*- value	Odds Ratio
CMV Awareness						
Yes	8	1 (12.5)	1	0.420	1.000	0.502
No	176	39 (22.2)				
First Pregnancy						
Yes	78	11 (14.1)	1	4.641	*0.046	0.436
No	106	29 (27.4)				
Trimester stage						
1st	19	4 (21.1)	2	0.391	0.814	
2nd	90	18 (19.1)				
3rd	75	18 (25.0)				
Nursing Toddlers						
Yes						
No	86	22(25.6)	1	1.401	0.283	1.528
	98	18 (18.4)				
Wash hands						
Yes	112	18(16.1)	1	5.404	*0.027	0.435
No	72	22(30.6)				
Undergone						
transfusion	14	8(57.1)	1	11.164	*0.003	5.750
Yes	170	32(18.8)				
No						
Are diabetic	1	0(0.0)	1	0.279	1.000	1.280
Yes	183	40(21.9)				
No						

CHAPTER FIVE

5.0 DISCUSSION

This study “Serological and PCR detection of cytomegalovirus among pregnant women in Lokoja metropolis, Kogi State, Nigeria, had an overall seroprevalence of 21.7%. This shows that cytomegalovirus IgM antibodies is high among the pregnant women in the study location indicating a primary or an acute infection.

Globally, primary or acute cytomegalovirus infection occurs in 1-4% of all pregnancies in which there is a 40% chances of transmission from mother to child (Aliyu *et al.,* 2016). In consequence, there is a high risk of mother to child transmission which could result to congenital defects.

In comparison of the seroprevalence obtained from this study with other studies done by Kolo *et al.*(2013) in Zaria, who had a prevalence of 18%, Edward *et al.*(2015) in Kafanchan had a prevalence of 10.5%, Aliyu *et al.*(2016) in Kaduna had a prevalence of 11.8% and Ndako *et al.*(2016)from the Northern East region of Nigeria with a prevalence of 18%. However, Anuma and Umeora (2016) recorded a high seroprevalence of 43.6%. In effect, the seroprevalence from our study location can be attributed to the fact that lower socio economic conditions and geographical locations has an increased effect of a high seroprevalence of cytomegalovirus among the study population. The selected hospitals within the study location is a strategic point bringing an influx of pregnant women seeking antenatal health care servicesboth from within Lokoja and other environs such as Obajana, Okene, Ajaokuta, Kabba among others within the metropolis to have access to better equipped health care.

Polymerase chain reaction (PCR) is a sensitive test for detecting cytomegalovirus infection among pregnant women. Early detection of patients suspected in having CMV infection is of advantage as immediate treatment commences to prevent vertical transmission of the virus to the fetus via the mother's placenta, which subsequently could result in congenital cytomegalovirus infection in the baby. A random selection of seven (7) positive CMV IgM with high optical densities were selected to run on PCRHowever, results obtained from this test showed a negative resultas shown in (appendixV1).This result canbe attributed to the sample medium being used for the Polymerase Chain Reaction.

Plasma was the sample medium used for Polymerase Chain Reaction (PCR) but according to published reports from Zhang *et al.,* (2010),their study was a comparative study monitoring CMV using DNA extracted from plasma and leukocytes separately and bydetecting CMV using PCR. Their study revealed the confirmation of more positive cytomegalovirus samples detected by using leukocytes as the sample medium than by using plasma as a sample mediumsuggesting that the use of peripheral blood leukocyte was a preferred sample medium for an improved sensitivity of cytomegalovirus. In addition, Lashini,*et al.*,(2011) who stated from his findings that plasma and serum medium detects the presence of cytomegalovirus using PCR buthowever maintained that peripheral blood leucocytes is the best sample medium of choice to use for detecting cytomegalovirus when using Polymerase Chain Reaction in immunosuppressed patients.

Sociodemographic and risk values from our study indicated that the age range of 35-44 years had the highest seroprevalence closely followed by the age range of 25-34 years this can be explained that both age ranges are individuals who are sexually matured and active individuals. This agreed with the works of Okwori *et al.*(2008), Akinbami *et al.*(2011), Yeroh *et al.*(2015) and Umelo *et al.*(2017).

Educational status was shown to be a risk factor as indicated from the studies, this is in agreement with the works of Umelo *et al.* (2017) that individuals of low educational background are at a higher risk of contracting CMV due to lower enlightenment/awareness and inadequate/poor hygienic practices.

There was no significant associations between marital status, type of marriage, occupation and scarification with CMV infection. This could be due to the limited number in sample size.

There was a significant association between CMV infection and women who were pregnant for the first time with CMV infection.Thewomen with multiparous birth from our study have been sexually active and may have contracted the virus either from sex activity or poor hygienic practices or possibly from the salivary secretions of their toddlers. This is similar with the studies of Okwori *et al.*(2008) and Umelo *et al.,* (2017) who gave similar explanation.

There was a significant association between women who do not wash their hands andCMV infection which could be that the hand are vehicles of transmission of pathogens andwhen not frequently washed, it may likely transmit the virus(Akinbami*et al.*(2011), Edward*et al.* 2015 and Umelo*et al.*(2017).

From our study, it was observed that women who had had blood transfusion recorded the highest prevalence of CMV infection. Similar studies conducted by Matos *et al.* (2010), Akinbami*et al.*(2011), Nasir *et al.*(2015) and Prennap *et al.* (2016)stated that, in an infected patient, CMV could be present in a latent state.Therefore, following blood transfusion, a seropositive blood donor can transmit the latent virus to the recipient and the latent virus is reactivated which may cause active CMV infection in the recipient. In addition, laboratory

screening of CMV is not a requisite in blood transfusion services, hence blood is given to most womenin this form posing a great risk of infection to the recipient.

The study is similar with Duff(2007) who posited that the rate of transmission is highest when maternal infection occurs in the third trimester; however, the risk of serious fetal injury is greatest when maternal infection develops in the first trimester or early in the second trimester. In addition, most pregnant women register for antenatal classes in their second and third trimester which could account for the positive high number of pregnant women who were screened. Studies done by Edward *et al.*(2015) and Balegamire *et al.* (2021) posited that for all the stages of pregnancy, there is a great risk of transmission to the fetus however, those at higher risk are those infected within the first 20 weeks of pregnancy and babies born in their second trimester are at risk of congenital infection.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

This study has ticked off the objectives drawnfrom the beginning of the study. From this study, the seroprevalence was 21.7%. It has demonstrated from this study that cytomegalovirus IgM among pregnant women in the study location is present and the likelihood for transmission from mother to child is possible. It therefore calls the need for sensitization of the populace and diagnosis to aid in timely intervention as to reduce the risk of spread among pregnant mothers and initiate adequate treatment and management.

The study could not detect cytomegalovirus from the Polymerase Chain Reaction as it was negative. The reason for this finding was attributed to the choice sample medium used.

The risk factors that were found to be associated with the study location was geographical location, educational status, first pregnancy, wash hands and transfusion.

The study showed no statistical association between the infection and occupation, marital status, type of marriage andscarification. Other factorsthat showed no statistical association were trimester, past history of transfusion and diabetes as an underlining disease.

Based on the research, the following recommendations are stated thus:

1. The populace need to be sensitized about the virus especially for pregnant women when they register for their ante - natal classes. Thisact is relevant as awareness about the virus was poor as observed from the responses on the questionnaires administered to the respondents.
2. Screening for cytomegalovirus IgG and IgM test is recommended for future study to have a wider picture of cytomegalovirus in the study area.

3. Further studies need be carriedout to ascertain the use of peripheral blood leukocytes as a better medium of choice compared to plasma for Polymerase Chain Reaction for detecting cytomegalovirus.
4. Seropositive mothers should have their babies screened for the virus in order to start early management for cases of positivity.
5. Blood banks should include screening of CMV to the list of viruses been screened before blood transfusion is done.

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AppendixI

Questionnaire

A research on: “Serological and Polymerase Chain Reaction detection of Cytomegalovirus infection among pregnant women in some selected hospitals in Lokoja metropolis, Kogi State, Nigeria”.

SECTION A: BIO/SOCIO-DEMOGRAPHIC DATA

1. Reference number …….
2. Age …….
3. Educational level: Tertiary (), Secondary ( ), Primary (), Informal ()
4. Marital status: single(), married ( )
5. Type of marriage: Monogamy ( ), Polygamy ( )
6. Occupation: Civil servant ( ), Self-employed ( ), Unemployed ( ) please specify…….
7. Scarification/tatoos ……
8. Place of residence ……..

SECTION B: RISK FACTORS

1 Have you ever heard of cytomegalovirus? Yes (), No ()

2 Is this your first pregnancy? Yes ( ), No ( ).

3 How old is your pregnancy?……..(months)

4 Nursing toddlers? Yes(), No()

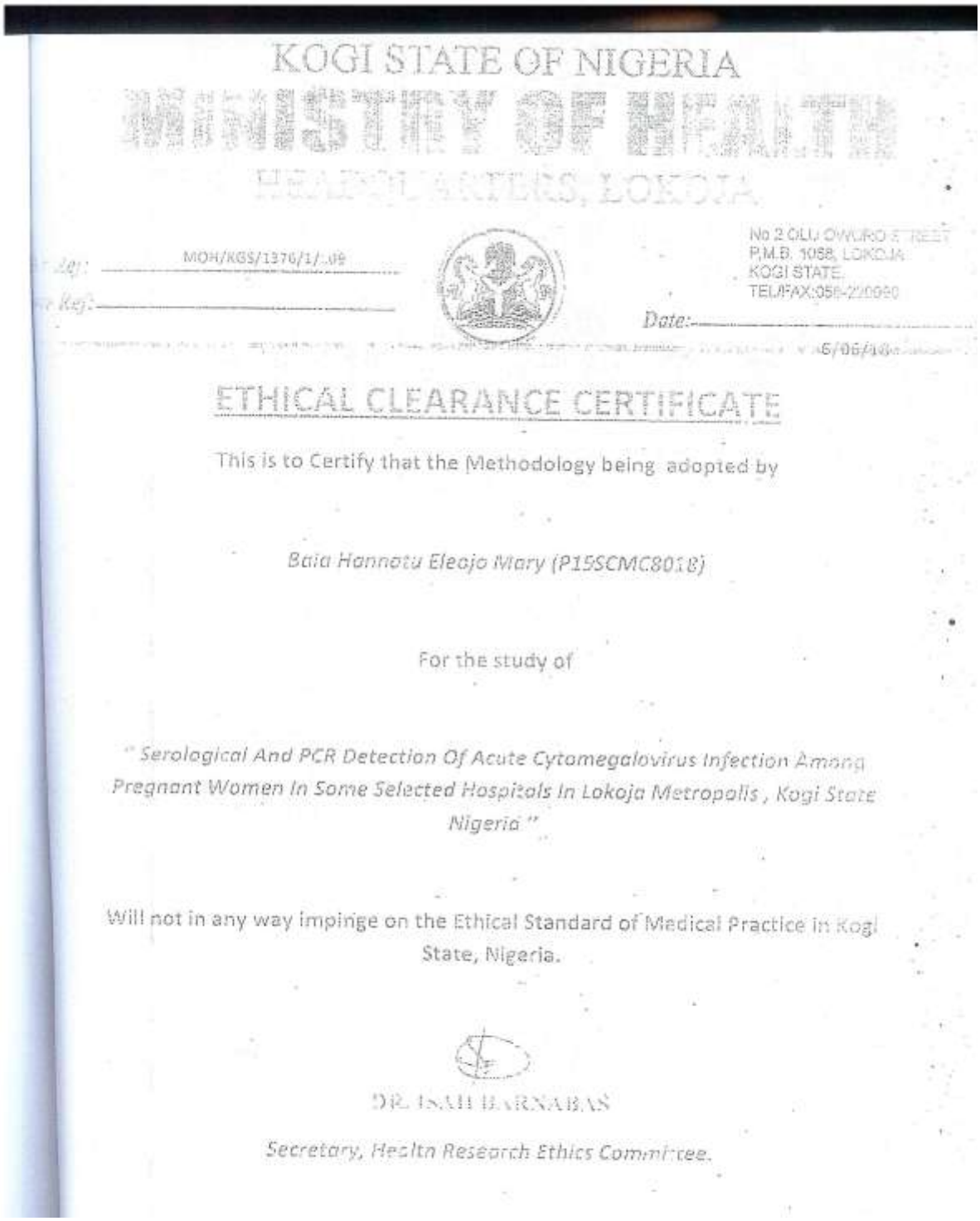
5 Do you often wash your hands? Yes(), No()

6 Have you been transfused before? Yes( ), No ( )

7 Are you diabetic? Yes ( ), No ( )

Appendix II

Ethical Clearance Certificate from Kogi State Ministry of Health



KOGI STATE OF NIGERIA

MINISTRY OF HEALTH

HEADQUARTERS, LOKOJA

Ref: MOH/KGS/1376/1/..09

Your Ref:

No 2 OLU OWURO STREET
P.M.B. 1068, LOKOJA
KOGI STATE.
TEL/FAX:058-220960

Date: 6/06/18

ETHICAL CLEARANCE CERTIFICATE

This is to Certify that the Methodology being adopted by

Baia Hannatu Eleojo Mary (P15SCMC8018)

For the study of

" Serological And PCR Detection Of Acute Cytomegalovirus Infection Among Pregnant Women In Some Selected Hospitals In Lokoja Metropolis , Kogi State Nigeria "

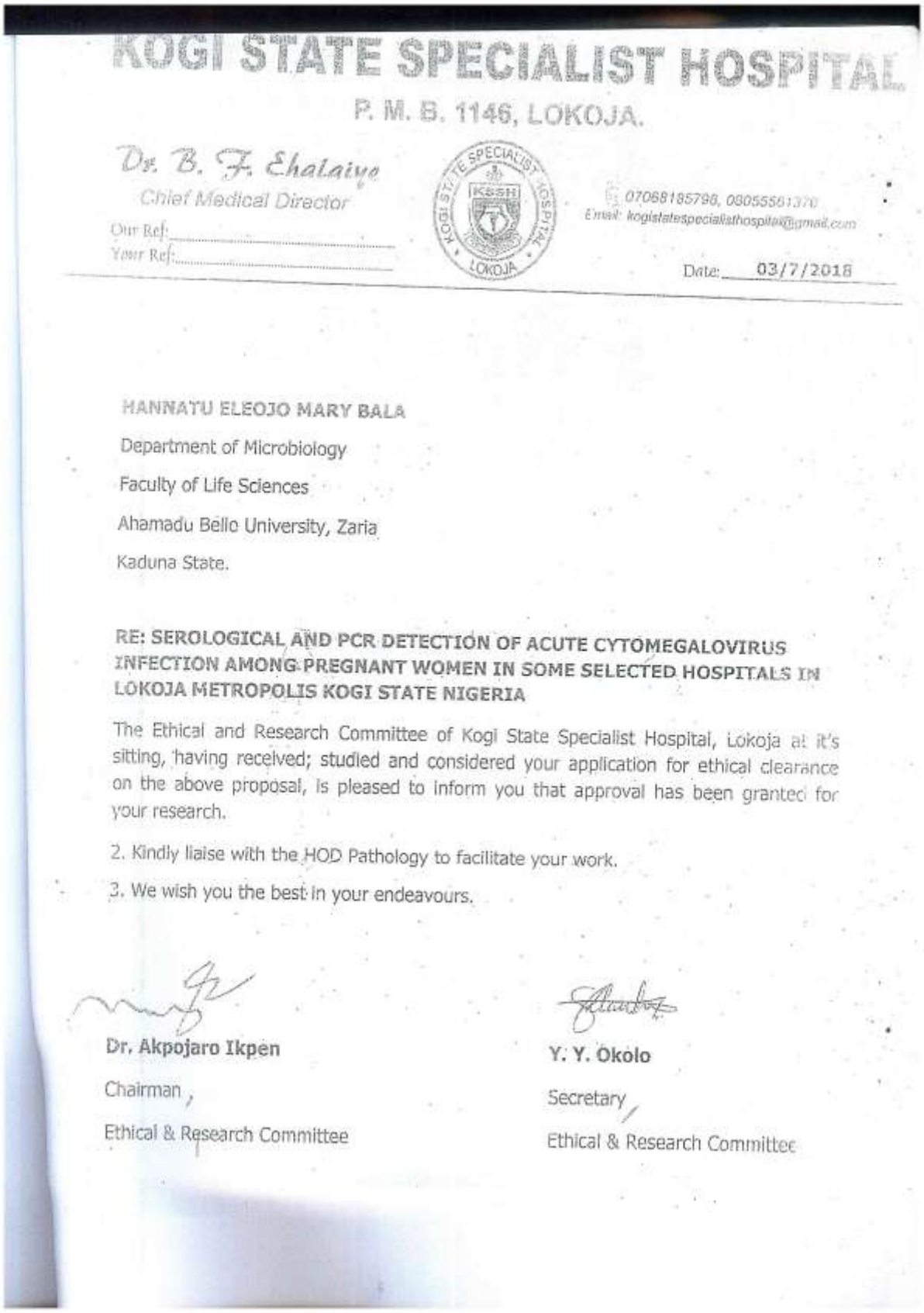
Will not in any way impinge on the Ethical Standard of Medical Practice in Kogi State, Nigeria.

DR. ISAH BARNABAS

Secretary, Health Research Ethics Committee.

Appendix III

Ethical Approval for Kogi State Specialist Hospital



KOGI STATE SPECIALIST HOSPITAL

P. M. B. 1146, LOKOJA.

Dr. B. F. Ehalaiye
Chief Medical Director

07068185798, 08055581370
Email: kogistatespecialisthospital@gmail.com

Our Ref:
Your Ref:

Date: 03/7/2018

HANNATU ELEOJO MARY BALA
Department of Microbiology
Faculty of Life Sciences
Ahamadu Bello University, Zaria
Kaduna State.

RE: SEROLOGICAL AND PCR DETECTION OF ACUTE CYTOMEGALOVIRUS INFECTION AMONG PREGNANT WOMEN IN SOME SELECTED HOSPITALS IN LOKOJA METROPOLIS KOGI STATE NIGERIA

The Ethical and Research Committee of Kogi State Specialist Hospital, Lokoja at it's sitting, having received; studied and considered your application for ethical clearance on the above proposal, is pleased to inform you that approval has been granted for your research.

2. Kindly liaise with the HOD Pathology to facilitate your work.

3. We wish you the best in your endeavours.

Dr. Akpojaro Ikpen
Chairman,
Ethical & Research Committee

Y. Y. Okolo
Secretary,
Ethical & Research Committee

Appendix IV
Ethical Approval from FMC Lokoja

FEDERAL MEDICAL CENTRE LOKOJA
ETHICAL COMMITTEE

Our Ref: FMCL/MED/115/II/422 Your Ref: _____ Date: 10th August, 2018

SEROLOGICAL AND PCR DETECTION OF ACUTE CYTOMEGALOVIRUS AMONG PREGNANT WOMEN IN LOKOJA, METROPOLIS, KOGI STATE.

Name of Applicant/ Principal Investigator: **Ms. Bala Hannatu Mary Eleojo**
Address of Applicant: Depart of Microbiology, Ahmedu Bello University, Zaria, Kaduna State
Date of receipt of application: 13/06/2018
Type of Review: Committee Review
Date of Committee Decision on the Research: 10/08/2018
Date of Committee Approval: 10/10/2018

Notice of Committee Approval

I am pleased to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed by the FMCL Ethical Review Committee (ERC) and given Committee approval.

This approval dates from *10/08/2018* to 10/12/2018. Your work will be monitored by **Dr. Ojiah Ahuoiza Oba** (MBBS, FWACS) to ensure compliance with the approved protocol. If there is delay in starting the research, please inform the ERC so that the dates of the approval can be adjusted accordingly.

Note that no participant accrual or activity related to this research may be conducted outside these dates.

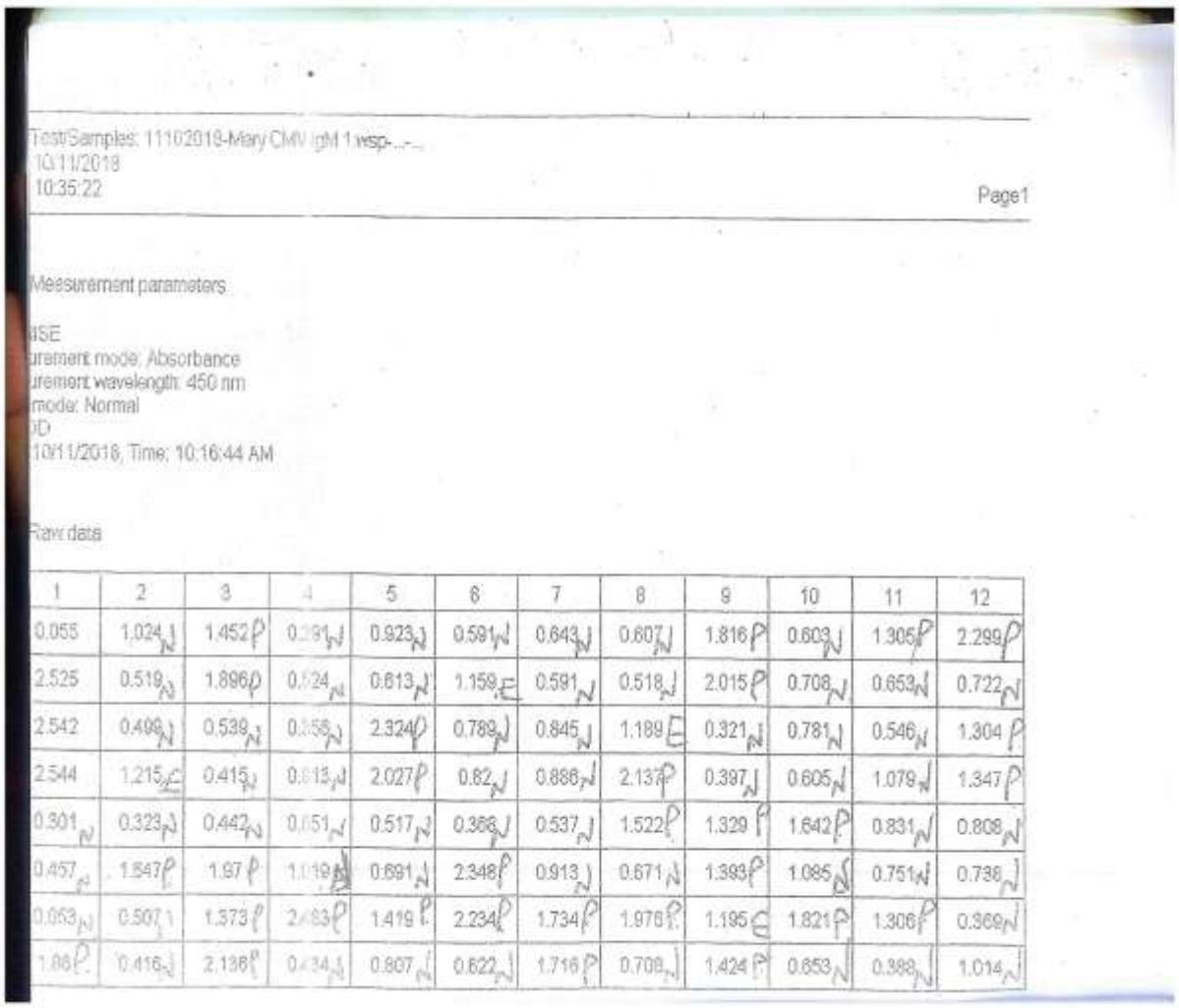
The FMCL Ethical Research Committee (ERC) requires you to comply with all the institutional guideline & regulations and ensure that all adverse events are reported promptly to the ERC. No changes are allowed in the research without prior approval by the ERC. Please note that the ERC reserves the right to conduct monitoring/oversight visit to your research site without prior notification. *This Approval acts as an Introduction of the Candidate as way.*

Thank You.

Dr. Osawe Osayande Osa (MBBS, FMCOPHTH)
Chairman, FMCL Ethical Research Committee (ERC)

Chairperson

Appendix V
ELISA Plate 1



TestSamples: 11102018-Mary CMV IgM 1.wsp-...-...
10/11/2018
10:35:22 Page1

Measurement parameters

BSE
urement mode: Absorbance
urement wavelength: 450 nm
mode: Normal
D
10/11/2018, Time: 10:16:44 AM

Raw data

1	2	3	4	5	6	7	8	9	10	11	12
0.055	1.024	1.452	0.281	0.923	0.591	0.643	0.607	1.816	0.603	1.305	2.299
2.525	0.519	1.896	0.724	0.613	1.159	0.591	0.518	2.015	0.708	0.653	0.722
2.542	0.499	0.539	0.358	2.324	0.789	0.845	1.189	0.321	0.781	0.546	1.304
2.544	1.215	0.415	0.813	2.027	0.82	0.886	2.137	0.397	0.605	1.079	1.347
0.301	0.323	0.442	0.151	0.517	0.368	0.537	1.522	1.329	1.642	0.831	0.808
0.457	1.847	1.97	1.119	0.691	2.348	0.913	0.671	1.393	1.095	0.751	0.738
0.053	0.507	1.373	2.183	1.419	2.234	1.734	1.978	1.195	1.821	1.306	0.369
1.86	0.416	2.136	0.434	0.807	0.622	1.716	0.708	1.424	0.653	0.388	1.014

Appendix VI
ELISA Plate II

Plate/Test/Samples: 11102018- Mary CMV IgM 2 нер-...-...
Date: 10/11/2018
Time: 11:09:59

Measurement parameters

SUNRISE
Measurement mode: Absorbance
Measurement wavelength: 450 nm
Read mode: Normal
Unit: OD
Date: 10/11/2018, Time: 11:09:37 AM

Raw data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.455	0.447	1.121	0.871	0.532	0.354	0.823	1.463	0.649	0.606	0.522
B	2.523	0.513	1.822	0.352	0.914	0.604	0.54	0.533	0.598	0.613	0.494	0.356
C	2.32	0.941	0.615	1.02	0.418	0.448	1.284	1.048	0.286	0.29	0.296	0.526
D	2.494	0.74	1.318	0.449	1.475	1.288	0.885	0.41	0.546	1.791	0.569	2.192
E	0.409	1.152	0.34	2.425	0.898	0.397	0.753	0.619	1.668	0.193	0.188	0.815
F	0.605	0.872	0.782	0.7	1.018	1.607	0.397	0.977	0.415	0.445	0.629	0.559
G	0.836	0.44	0.51	0.874	0.467	0.358	0.558	0.672	0.29	0.281	0.196	0.288
H	1.356	0.633	0.349	0.449	0.414	0.973	0.883	0.498	1.877	0.481	0.498	0.334

Appendix VII

Polymerase Chain Reaction of Cytomegalovirus

