THE PREVALENCE AND ASSOCIATED FACTORS FOR CYTOMEGALOVIRUS INFECTION AMONG BLOOD DONORS IN MINNA, NORTH CENTRAL NIGERIA

BY

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DECLARATION

I declare that the work in this thesis entitled "Prevalence and associated factors for cytomegalovirus infection among blood donors in Minna, north central Nigeria" has been performed by me in the Department of Community Medicine, under the supervision of Professor Aisha Indo Mamman. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

I certify that the work for this thesis entitled "PREVALENCE AND ASSOCIATED FACTORS FOR CYTOMEGALOVIRUS INFECTION AMONG BLOOD DONORS IN MINNA, NORTH CENTRAL NIGERIA" by Musa Kalamullah Bawa meets the regulation governing the award of the degree of Masters of Public health in Field Epidemiology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I wish to dedicate this work to my loving wife, Mrs. Marwa M.K. Bawa and children for patiently bearing the long absence and whose effort facilitated the actualization of this work.

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SUMMARY

Transfusion usually is a life-saving therapeutic intervention, however many preventable errors may make this a hazardous procedure. Human cytomegalovirus, otherwise called Human Herpes Virus (HHV) type 5, has over the years attained public health significance, it is a cause of morbidity and mortality in pregnancy, immunocompromised patients like recipients of organ transplants, HIV-infected persons, cancer patients on therapy and neonates. Cytomegalovirus is transmissible via blood transfusion and other parenteral routes. It is however not routinely screened for in blood donors in Nigeria. Prevalence rates in some parts of Nigeria ranged from 92 to 95 percent. This study aimed at determining the prevalence and associated factors of cytomegalovirus (CMV) infection among blood donors in Minna, North Central Nigeria.

This is a hospital based cross-sectional study. A total of 345 consenting blood donors were recruited into the study. Screening for CMV antibodies (IgM and IgG) was done using ELISA kit (DIALAB® Austria). Qualitative methods were employed through key informant interviews. As for the quantitative aspect, each blood donor was administered a questionnaire to obtain information regarding socio-demographic and socio-economic characteristics and practices. The data obtained was subjected to descriptive and analytical statistical analyses using Epi Info version 3.5.4. Chi square was used to determine associations. Significant associations were presumed if $p<0.05$.

Majority were males 336 (97.4%), with a mean age of 32.4± 8.5 years 139 (40.3%), married 230 (66.7%), had post-secondary education 146 (42.6%), whose monthly earning ranged from ₦18000 to ₦ 35000 136(40.6%). Also, majority were interviewed at the General Hospital Minna 290 (84%). The prevalence of CMV infection was found to be 96.2%. The prevalence of anti-CMV IgG and IgM antibodies were found to be 96.2% and 2.6% respectively. Of the CMV seropositive blood donors, 55.6% were anaemic (PCV<36%), but 66.7% and 77.8% had normal WBC and platelet counts respectively. Majority of the blood donors had had contact with blood in the past.

The seroprevalence of CMV is high in this environment with a significant proportion of individuals who are capable of transmitting CMV infection to blood recipients. The prevalence rate is consistent with published data from other parts of Nigeria and across the globe. Anaemia is significantly associated with CMV infection (P=0.02). Prospective blood donors for immunocompromised recipients should be screened for CMV.

Key words: Prevalence, Cytomegalovirus, blood donors, Minna

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LIST OF ACRONYMS

AIDS	-	Acquired Immuno-deficiency Syndrome
ALL	-	Acute Lymphoblastic Leukaemia
BT	-	Blood Transfusion
CDC	-	Centers for Disease Control and Prevention
CMV	-	Cytomegalovirus
GH	-	General Hospital
Hb	-	Haemoglobin
HCT	-	Haematocrit
HIV	-	Human Immuno-deficiency Virus
IBBSH	-	Ibrahim Badamasi Babangida Specialist Hospital
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
MCH	-	Mean Cell Haemoglobin
MCHC	-	Mean Cell Haemoglobin Concentration
MCV	-	Mean Corpuscular Volume
NHL	-	Non Hodgkin's Lymphoma
PCV	-	Packed Cell Volume
PLT	-	Platelet
PRCA	-	Pure Red Cell Aplasia
RBC	-	Red Blood Cell
RCDW	-	Red Cell Distribution Width
WBC	-	White Blood Cell
WHO	-	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Transfusion is usually a life saving therapeutic intervention, however many preventable errors may make this a hazardous procedure.[1] The WHO recommends that blood donation should in all cases be absolutely voluntary.[2,3] However, in Nigeria, voluntary donors are relatively scarce, hence family replacement and commercial donors have become alternative sources of blood.[4,5,6] Healthy persons who are between the ages of 18 and 65 years with haemoglobin (Hb) levels of not less than 13.5 g/dl in males or 12.5 g/dl in females are acceptable as donors if they test negative for transfusion transmissible infections which include HBV, HCV, HIV, malaria, syphilis, Chagas disease. However, females are only accepted as donors if they are not pregnant or breast-feeding.[7] Human cytomegalovirus, otherwise called human herpes virus type 5, over the years, has come to assume an important public health problem as it is a significant cause of morbidity and mortality in pregnancy, and among immunocompromised patients like recipients of organ transplants, HIV-infected persons, cancer patients on therapy and neonates. Cytomegalovirus is transmissible through blood transfusion, among other parenteral routes.[8,9] It is however not routinely screened for in blood donors in Nigeria. Other viruses that can be transmitted through blood transfusion are Hepatitis B and C and human retroviruses such as human immunodeficiency virus (HIV) and human T-lymphotropic viruses types 1 and 2 .It has been reported that most adults across the globe are seropositive for CMV.[9,10] Between 50% and 80% of people in the United States have had a CMV infection by the time they are 40 years old and about 1% of infants are infected with CMV before birth.[11,12] Prevalence rates in some parts of Nigeria are from 92 to 95 percents.[8,13]

CMV is mainly a problem for certain high-risk groups, these are unborn babies whose mothers become infected with CMV during the pregnancy and children or adults whose immune systems have been weakened by disease or drug treatment, such as organ transplant recipients or people infected with HIV. Once a person has had a CMV infection, the virus usually lies dormant (or inactive) in the body, but it can be reactivated. The virus is more likely to be reactivated and cause serious illness in people who have weakened immune systems due to illness.[12]

The symptoms of a CMV infection vary depending upon the age and health of the person who is infected, and how the infection occurred. Infants who are infected before birth usually show no symptoms of a CMV infection after they are born, although some can develop hearing, vision, neurological, and developmental problems over time. In a few cases, there are symptoms at birth, which can include premature delivery, being small for gestational age, jaundice, enlarged liver and spleen, microcephaly (small head), seizures, rash, and feeding difficulties. These babies are also at high risk for developing hearing, vision, neurological, and developmental problems. [12] Newborns also can contract CMV infection during or soon after birth by passing through the birth canal of an infected mother, consuming breast milk from a mother with the virus, or receiving a transfusion of blood donated by a person infected with CMV. Most of these infants show no symptoms of CMV infection, however, a few may develop pneumonia or other symptoms. Premature and ill full-term infants who are infected soon after birth are also at risk for neurological and developmental problems over time.[12,14] Transfusion transmitted-cytomegalovirus (TT-CMV) is a significant cause of morbidity and mortality in the immunocompromised host. The risk of TT-CMV from seropositive donors is reported to be 0.4 to 12%.[15]

1.2 Statement of the Problem

The residual risk of transfusion of CMV in some parts of Nigeria has remained steadily high with a frequency of about 0.14% to 10% per unit of blood in the last 5 years resulting in high CMV morbidity and mortality most particularly in pregnancy, and among immunocompromised patients like recipients of organ transplants, HIV-infected persons, cancer patients on therapy and neonates.[9] Children typically become infected in early childhood, especially those in childcare and preschool settings, all these are followed by serious attendant consequences of premature delivery, infants being small for gestational age, jaundice, enlarged liver and spleen, microcephaly (small head), seizures, rash, and feeding difficulties. These babies are also at high risk for developing hearing, vision, neurological, and developmental problems. It has been reported that most adults across the globe are seropositive for CMV.[10] CMV prevalence in some parts of Nigeria are 92% and 95% IgG, 3.1% IgM.[8,13] In developed countries such as the US, implementation of stringent donor eligibility criteria and more sensitive methods of viral genome detection have virtually eliminated TT-CMV infection; however in developing countries like India and Nigeria, the risk is as yet considerable.[8,16] Prevalence in some parts of Nigeria are from 92[8] to 95[13] percents. A CMV donor prevalence of between 92 and 95 percent translates to at least 92% of BT recipients being at risk of infection with only about 8% of our donors being negative; there is the need to determine the potential impact of CMV on donor safety. Yet CMV is never part of the routine screening for prospective donors for transfusion in Minna, Niger State and indeed Nigeria.

1.3 Justification

Reports show that the risk of transmission of other transfusion transmissible viruses which include hepatitis B and C, and human retroviruses such as human immunodeficiency virus (HIV) and human T-lymphotropic viruses types 1 and 2 has drastically been reduced by screening for their antibodies before transfusion.[17] Currently, routine screening for HBV, HCV and HIV have reduced their residual risk of transfusion related spread to less than 1/2,500,000. Residual risk of transfusion-transmitted HIV, HCV, and HBV were found to be 2, 17 and 69 per million donations in Italy[18] compared to sub-Saharan Africa where the risks were 1,3 and 4 per thousand donations respectively.[19] Post-transplant survival is increased by immunosuppressive agents and in like manner, cancer survival is increased. Interventions to increase neonatal and early childhood survival while reducing maternal morbidity and mortality include blood transfusion for this group. However immaturity of the immune system makes them susceptible to CMV. Cancer, organ transplant and paediatrics patients together form a group of highly susceptible hosts for CMV infection.[9] The current algorithm for donor screening is restricted to the aforementioned viruses thus making them (donors) possible vehicle for CMV spread for which they are not being screened.[20]

There is the need to determine the prevalence and access the associated factors of CMV infection among blood donors. This study will further provide bases for the implementation of donor safety strategies in health care service delivery.

1.4 Research Questions

- What is the prevalence of CMV among blood donors in Minna, North Central Nigeria?

- What is the association between practices and socioeconomic characteristics of the blood donors and their CMV serological status?
- What are the patterns of haematological indices among CMV seropositive blood donors in Minna, North Central Nigeria?

1.5 General and Specific Objectives

1.5.1 General objective

To determine the prevalence and associated factors of Cytomegalovirus (CMV) infection among blood donors in Minna, North Central Nigeria.

1.5.2 Specific objectives

1. To determine the seroprevalence of CMV antibodies among blood donors in Minna.
2. To identify those with recent CMV infection (CMV IgM) who are potentially capable of transmitting CMV to donor recipients.
3. To identify possible associations between the practices, socioeconomic characteristics of the blood donors and their CMV serological status.
4. To describe the pattern of haematological indices among CMV seropositive blood donors.

CHAPTER TWO

LITERATURE REVIEW

2.1 CMV Prevalence

Transfusion medicine consists of practices that have evolved over the years from the time of Landsteiner's insightful observations. The provision of well-preserved cellular or plasma components of blood with predictable effects and uncommon severe side effects has become so developed that research and development efforts are advancing at a tremendous pace. This has permitted sophisticated surgery, chemotherapy, organ and bone marrow transplant. However, blood transfusion is still associated with complications such as infections, metabolic overload and immune reactions.[21] The African region of the World Health Organization (WHO), fraught with high disease burden including the high prevalence of HIV/AIDS, Hepatitis B and C and malaria; poor economies; and a lack of resources for blood transfusion in the face of poorly developed health infrastructure, faces unique challenges to blood safety. Cognizant to these facts, a number of resolutions on blood safety were passed by the regional committee. These resolutions culminated into the regional strategy for the WHO African region (AFRO). This region has 46 countries including 21 Francophone, 20 Anglophone and 5 Lusophone with a population of 738,083,000 (2006 estimation). The trends and status of the regional strategy for blood safety for Africa was reviewed using 1999 to 2006 data from the Global Database on Blood Safety (GDBS). An estimated 529,500 mothers died in the world in the year 2000 of which 247,000 died in Africa south of the Sahara.[22]

Moreover, it is estimated that the lifetime risk of death of mothers of childbearing age is 1:16 in sub-Saharan Africa compared with 1: 2,800 in the developed world. Africa has one of the highest maternal mortality ratios in the world standing at 920/100,000 live births compared to 20/100,000 live births in the developed world. Twenty five to 40 percent of maternal deaths are due to haemorrhage.[22,23]

Globally, billions of lives are saved through blood transfusion with attendant risk of acquiring transfusion transmissible infections (TTIs).[24] These include HIV, HBV, HCV, CMV, Syphilis, malaria and others. Majority of these TTIs are viruses and the mode of transmission of these viruses is similar including transfusion of infected blood and blood products, unsafe sex, use of sharps contaminated with body fluid, cultural and behavioral practices (circumcision, tattooing, etc) and mother to child. [25,26,27]

Infection with cytomegalovirus (CMV), a member of the herpes virus family, is very common. Between 50% and 80% of people in the United States have had a CMV infection by the time they are 40 years old, according to the Centers for Disease Control and Prevention (CDC).[12] Children typically become infected in early childhood, especially those in childcare and preschool settings. CMV infections are rarely serious in otherwise healthy children and adults; they usually cause only mild symptoms, if any. When symptoms do appear, they are similar to those seen in mononucleosis ("mono") and only last a few weeks.[11,12] However, infants who are infected before birth usually show no symptoms of a CMV infection after they are born, although some can develop hearing, vision, neurological, and developmental problems over time. In a few cases, there are symptoms at birth, which can include premature delivery, being small for gestational age, jaundice, enlarged liver and spleen, microcephaly (small head), seizures, rash, and feeding difficulties.[12,14]

Maternal cytomegalovirus (CMV) and rubella infections have adverse neonatal outcomes. In a cross sectional study conducted to investigate seroprevalence of CMV and rubella infections and associated possible risk factors among pregnant women, out of 231 pregnant women, 167 (72.2%) and 151 (65.3%) were CMV-IgG and rubella-IgG positive, respectively while only 6 (2.5%) and 8 women (3.4%) were CMV-IgM and rubella-IgM positive, respectively. Parity and illiteracy were found to be significantly associated with CMV seropositivity.[28]

CMV seroprevalence among blood donors in the city of Lages, Brazil was found to be 96.4% and there were no statistically significant associations between the presence of antibodies and the socio-economic characteristics of donors.[29] In a prevalence study on anti-CMV antibodies in blood donors in the Sfax region, Tunisia the anti-CMV antibody prevalence was found to be 97.1.[30] Studies in India and Ghana showed prevalence rates of 95% and 93.2% respectively[15,11] comparable to prevalence rates of 92% and 96% obtained in separate studies previously carried out in Nigeria[8,31]. Improving the supply of safe blood is a key intervention in reducing mortality and morbidity in sub-Saharan Africa (SSA) especially in young children and pregnant women.[32]

2.2 Active CMV Infection (CMV infection stage capable of transmission)

Cytomegalovirus (CMV) infection is a matter of concern for blood bank professionals and blood transfusion recipients, especially in cases of transfusions to neonates and immunocompromised patients. [29] Cytomegalovirus (CMV) is one of the commonest viral opportunistic infections in persons with Acquired Immune Deficiency Syndrome (AIDS). In a case control study involving HIV patients as cases and blood donors as controls, CMV IgG and CMV IgM seroprevalences among HIV patients were 93.9% and 11.1% respectively and among blood donors were 96.7% and 2.2% respectively. This indicating that 11.1% and 2.2% of the HIV patients and blood donors respectively had active CMV infection and were capable of transmission.[33] Also 2.5% prevalence of ongoing (IgM) CMV infection was found among pregnant women in Sudan.[28]

A rare case of progressive hearing loss after acquired CMV infection in a child with Langerhans cell histiocytosis (LCH) was reported. A 5-month-old female was diagnosed as having LCH. When she was 14 months old, she received an unrelated donor umbilical cord blood transfusion for the treatment of intractable LCH. CMV infection was confirmed after the blood transfusion. Because her own umbilical cord had no CMV, the CMV infection was not congenital. When she was 7 years old, mixed hearing loss was noted with bilateral otitis media with effusion, and the hearing impairment remained progressive over 3 years leading to bilateral profound hearing loss over 3 years. Three-dimensional fluid-attenuated inversion recovery magnetic resonance imaging with gadolinium contrast enhancement revealed a high intensity area in the inner ear that suggested bilateral labyrinthitis.[34]

Cytomegalovirus (CMV) DNA is frequently detected in plasma of newly seropositive donors. Selection of leucoreduced blood products from donors with remote CMV infection could avoid transfusion-transmitted CMV infections (TT-CMV) due to primarily infected donors. However, there are no data (in respect to the CMV DNA) about the prevalence of reactivations in long-term seropositive donors compared to the incidence of window period donations in seronegative donors. Therefore, the optimal transfusion strategy for at-risk patients is unclear.[35] It has been proved that donors with short inter-donation intervals (e.g., apheresis donors) have an increased risk of window period donations. The frequency of cytomegalovirus (CMV) window period donations is important information to decide whether selection of seronegative donors might be advantageous for patients at risk for transfusion-transmitted CMV infections (TT-CMV).[36]

Cytomegalovirus (CMV) infection is one of the most common infectious problems following kidney transplantation.[37] Infection with and reactivation of human cytomegalovirus (CMV), Epstein-Barr virus (EBV), and adenovirus (ADV) are frequent and severe complications in immuno-compromised recipients after hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT). These serious adverse events are associated with significant morbidity and mortality.[38] CMV is one of the main infectious problems for SOT and HSCT. The severity of the complications is mainly associated with the type of transplant and immune status against the virus of the transplant donor and the transplant recipient. It is important to prevent exposure, using safe blood transfusion CMV seronegative donors (B1) and/or use of blood leucocytes-depleted by filtration (Al).[39] Cytomegaloviruses (CMVs) establish lifelong infections that are in part controlled by CD4(+) and CD8(+) T cells. To promote persistence, CMVs utilize multiple strategies to evade host immunity including modulation of costimulatory molecules on infected antigen-presenting cells. In humans, CMV-specific memory T cells are characterized by the loss of CD27 expression, which suggests a critical role of the co-stimulatory receptor/ligand pair CD27/CD70.[40]

2.3 Associated Factors for CMV Infection

Epidemiology and clinical outcomes of human cytomegalovirus (HCMV) infection are known to vary between countries and are associated with socio-economic background. So far, few data are available from developing countries, where the overall burden of infectious diseases is frequently higher.[41] In 1985, Stagno and Whitley estimated the maternal risk of acquiring either primary or recurrent HCMV infection in pregnancy as well as the risk of intrauterine transmission to the offspring in two groups of women of low or high socioeconomic status. Their estimates showed that the risk of primary maternal infection was about three times higher among the higher-income susceptible women (45%), compared to 15% in the lower-income group.[42] Hamda et al[28] found parity and illiteracy to be significantly associated with CMV seropositivity.

In Nigeria millions of lives are saved through blood transfusion with the attendant risk of acquiring transfusion transmissible infections (TTIs).[43] Transfusion transmissible infections, particularly the viral agents, insufficient supply of blood/blood products and continuous patronage of paid donors are some of the challenges inimical to ensuring blood safety in most Blood Banks in Nigeria. Prevalence of transfusion transmissible viruses is still very high in Nigeria when compared with other developing countries with very similar challenges. This cannot be unconnected with the high level of poverty contributing to the emergence of paid donors that continue to form over 70% of the blood donor pool. This challenge needs to be addressed in order to ensure blood and blood product safety. [44] Previous studies from Nigeria indicated that about 12% and 5% of donors were deferred due to transfusion transmissible infections and anaemia respectively.[45,46]

CMV infection occur prenatally and perinatally, but the CMV infections that occur in children after the newborn period usually do not cause significant illness, some infants and young children may develop pneumonia, hepatitis (inflammation of the liver), or a rash. Older children and teens who become infected may have mono-like symptoms, including fatigue, muscle aches, headache, fever, and enlarged liver and spleen. These symptoms are generally mild and usually last only 2 to 3 weeks. CMV can cause serious infections in people who have received organ transplants or those whose immune systems are weakened.

In someone with AIDS or HIV, CMV infection may involve the lungs, nervous system, gastrointestinal tract, and the eye, sometimes causing blindness.[46] When symptoms of CMV do appear in an infected person, how long they last varies depending on how the infection occurred, the age and general health of the patient. For example, serious CMV infections before birth may cause developmental problems that affect a child for a lifetime. On the other hand, infection in teens may last only 2 to 3 weeks and cause no lasting problems. Fatal symptoms include fever that lasts for several days, unusual or extreme tiredness, muscle aches and headache.[46]

CMV infections spread widely. In the United States, about 1% of infants are infected with CMV before birth -usually only if the mother has developed a first-time CMV infection during pregnancy. An infected mother can pass the virus to her child before, during, or after birth. Anyone with a new or past CMV infection can transmit the virus to others, even if he or she isn't showing any symptoms. But transmission usually requires fairly close contact. The virus can be spread through saliva, breast milk, vaginal fluids, semen, urine, and stool. It is majorly present in blood products and donated organs, causing infection after a blood transfusion or organ transplantation. Among children, the virus is commonly spread in childcare centers or preschool settings, where it passes easily through indirect contact, especially through contaminated toys. Infected children may then spread the virus to their families.[12,15]

Infections of CMV are diagnosed by taking a culture from a person's throat, urine, blood, or other body tissues or fluid. Blood tests also can look for certain antibodies that are part of the immune system's response to a CMV infection, their presence can signal an active CMV infection. Special viral DNA-detecting tests are also sometimes used for diagnosis of CMV infection.[12]

Currently, no specific treatment is available or recommended for otherwise healthy people with CMV infection. Patients in whom a CMV infection can be life-threatening (newborn infants, organ-transplant patients, and people being treated for cancer or who have immune disorders such as AIDS) may be treated with intravenous (IV) antiviral medication, usually in a hospital. Oral antiviral medication also may be used at home once the infection is under control and the patient is stable. Because these antiviral medicines can have serious side effects, they are used with great caution, especially in children. In bone-marrow transplant patients, CMV-immune globulin (CMV-IVIG) and the antiviral drug ganciclovir given intravenously can be used to fight CMV infections.[47]

Prevention measures in infections could include vaccination and proper screening of blood and blood products. But currently, there is no vaccine to prevent CMV infection. For those who have close contact with children, especially pregnant women or women who might become pregnant, hand washing is effective at reducing the risk of infection, as are not sharing eating utensils with young kids and avoiding intimate contact with anyone who has a CMV infection. A mother who has a CMV infection shouldn't stop breastfeeding — the benefits of breastfeeding are believed to outweigh the risks of passing CMV to the baby, who is unlikely to develop any symptoms if infected. For organ-transplant patients who are at risk of getting CMV from a transplanted organ, preventive therapies are available. Blood banks have certain screening and processing procedures that help to prevent CMV from being passed in blood products.[47]

While explanations by various researches are that there are various other routes through which CMV is transmitted in Nigeria including intrauterine (or at parturition), breastfeeding, sexual contact and spread from children. Some studies, however, suggest there is a relationship between transfusion and CMV antibody seropositivity. One such study reported that CMV can be transmitted through transfusion, with a frequency of about 0.14% to 10% per unit of blood.[15]

2.4 Pattern of Haematological Indices among CMV Infected Persons

Cytomegalovirus (CMV) infection is a well recognized cause of pancytopenia in patients of hemato-lymphoid malignancies undergoing hematopoietic stem cell transplantation (HSCT). However, there is very little information on similar problems occurring in patients of hemato-lymphoid malignancies receiving standard dose chemotherapy. Prolonged cytopenias may occur in acute lymphoblastic leukemia (ALL)/ Non-Hodgkin's lymphoma (NHL) therapy due to poor disease response, chemotherapy toxicity or viral infections. These could delay chemotherapy and increase the infection related morbidity or mortality.[25,34]

Pure red cell aplasia (PRCA) associated with cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infection is uncommon. Seven Chinese children with pure red cell aplasia characterized by anaemia, reticulocytopenia and aplasia of erythroblasts were found to be CMV DNA positive and on detection, were treated with blood transfusion, corticosteroids, and ganciclovir injection.[48] Infants, who acquire CMV after birth, especially if they are premature, may develop a sepsis-like syndrome, pneumonia, hepatosplenomegaly, hepatitis, thrombocytopenia, and atypical lymphocytosis. However, if transmission is via breast milk, the risk of severe symptomatic disease and long-term sequelae is low. Transfusion-associated perinatal CMV disease can be avoided by giving preterm neonates blood products from

CMV-seronegative donors or leucoreduced products[49,50]. Most studies on blood donors are centered on the selective evaluation of red blood cell parameters with little or no regard to white blood cells and platelets. A study by Jeremiah revealed, of the blood donors studied, 18.8% were found to be anemic (packed cell volume [PCV] <33%) while 12.5% were leucopenic (white blood cell [WBC] count <2.0 × 10(9)/L). [50] While HIV, HBV, HCV with prevalence rates of 4.1,[51] 11%, 2.2%[52] are the principal reasons for permanent blood deferral in Nigeria, the prevalence rate of malaria parasite infection among blood donors in Zaria is 29.4%,[53] but Nigeria being in malaria endemic zone of the world, blood donors are not usually screened for malaria considering the presence of premunition to malaria parasite among blood recipients in Nigeria.

Anaemia accounts for 5% of donor deferrals in Nigeria, deferral due to CMV is yet to be determined.[46] It is noted in seveal findings that screening routine screening for all blood donors is not cost effective [8, 13, 31]

CHAPTER THREE

METHODOLOGY

3.1 Study Area

Minna is the capital city of Niger State of Nigeria. The city is cosmopolitan, made up of people from all parts of Nigeria, but the major tribes are Nupe, Gwari, Hausa, Fulani, Igbo and Yoruba. The inhabitants are either business men, civil servants or farmers. The city is located in the North Central region of Nigeria (appendix 5), with Guinea Savannah vegetation. The average annual rainfall is 1,100mm. The major agricultural products are sorghum, rice, yam, cotton and ginger.[54] The city is made up of two LGAs, each having one secondary health facility which are General Hospital (GH), Minna and Ibrahim Babangida Specialist hospital (IBBSH), Minna located in Minna South and Minna East LGAs respectively. The average annual blood donor rates are 3865, 756 for GH, Minna and IBBSH, Minna respectively and the population of Minna town is 304, 113 as at 2007[54] census. Blood transfusion screening algorithm in Minna includes HIV, HBV, HBC and syphilis.[20] Malaria is endemic [53] and anaemia is a common diagnosis.

3.2 Study Design

This is a hospital based cross-sectional study with both descriptive and analytical components. The descriptive component described the occurrence of CMV among the blood donors in terms of person, place and time while the analytical component identified the factors associated with CMV infection.

3.3 Study Population

All potential blood donors that presented at the identified blood donation centers, GH Minna and IBBSH, Minna in Minna town, Niger State.

3.3.1 Inclusion criteria

- Healthy looking persons who were between the ages of 18 and 65 years with haemoglobin (Hb) levels of not less than 13.5 g/dl in males or 12.5 g/dl in females.
- Those already screened for transmission transmissible infections and found illegible to donate.

3.3.2 Exclusion criteria

Those that tested positive for HIV, HBV and HCV.

3.4 Sample Size Determination

A total of 345 blood donors were recruited into the study. The sample size was obtained using the formula as follows:

$$n = \frac{Z\alpha^2 pq}{d^2}$$ [55]

Where:

n= required sample size

z = $_{(1-a/2)}$ = 1.96 = value of the standard distribution corresponding to a significance level of α (1.96 for a 2-sided test at the 0.05 level)

p = CMV prevalence among prospective blood donors at a tertiary health facility (JUTH) in Jos, Nigeria = 92% (0.92)[8]

q = 1-p (1-0.92) = 0.08

d = absolute precision = 3% (0.03)

$$n = \frac{1.96^2 \times 0.92 \times 0.08}{0.03^2} = 314$$

10% non-response = 31

Therefore, n = 345

3.5 Sampling Technique

Minna town is made up of two LGAs, Minna West and Minna East, each made up of one secondary health facility (HF), GH, Minna and IBBSH, Minna. The study population was 1540 obtained from blood donor registers of the two HFs over a given period of time. The blood donor registers of each of the two HFs were reviewed for the number of past blood donors for a period of one year. Each was divided by 3 to obtain average blood donor number for a period of four months, same length of period for which the participants were going to be recruited. Sampling frames were obtained as 1288 and 252 (5:1) for GH, Minna and IBBSH, Minna respectively. Proportionate allocation of the participants for the HFs was done given 289 and 56 for GH, Minna and IBBSH, Minna respectively. Blood donors were systematically selected from the two HFs after obtaining an interval for selection for each, using n/N =K. The first participant was selected randomly by balloting between 1 and K, and then thereafter every Kth blood donor recruited until the required sample was obtained. The proportionate allocation and the sampling intervals were obtained as follows:

Blood donor population= 1540

Sampling frame GH, Minna = 1288

Proportion for GH, Minna = $\frac{(1288 \text{ x}345)}{1540} = 289$

Sampling interval GH, Minna (K) = $\frac{289}{1288} = \frac{1\ \ 0}{4.46} = 5$

Sampling frame for IBBSH, Minna = 252

Proportion for IBBSH, Minna = $\frac{(252 \text{ x}345)}{1540} = 56$

Sampling interval GH, Minna (K) = $\frac{56}{252} = \frac{1\ \ 0}{4.5} = 5$

3.6 Study Instruments

Laboratory forms were used for capturing laboratory data. KII guide was used as a guide for KII. Interviewer administered questionnaires made up of introduction, demography, risk factor sections were used for quantitative methods.

3.7 Data Collection Methods

The recruited blood donors were screened for CMV antibodies, IgM and IgG using blood samples collected. Blood samples, 5 mls each for serum and whole blood was collected using plain and EDTA vacuntainers respectively. The serum was used for CMV ELISA screening for CMV antibodies i.e. IgG and IgM using ELISA kit (DIALAB® Austria),[8] while the whole blood was analyzed for haematological indices using a haematological analyzer (*Abacus* junior haematology *analyzer* 2.75, manufactured in 1995 by Diatron® U.S.A). Interviewer administered questionnaire was employed to obtain information from participants regarding their socio-demographic, socio-economic characteristics and practices. Four research assistants were recruited and trained for the study.

3.7.1 Screening for CMV

Screening for CMV antibodies was done using ELISA kit (DIALAB® Austria). [8] Serum was preserved under -20^0C and analysed at ABU PEPFAR lab using ELISA. The analysis was done using automated microplate reader (*Emax* precision microplate reader), E11865 model (*Molecular Devices*® USA). ELISA procedure included the procedure attached in appendix 6.

3.7.2 Analysis for haematological indices

Blood samples for haematological analyses were analysed daily within 12 hours of collection using a haematological analyzer (*Abacus* junior haematology *analyzer* 2.75, manufacture in 1995 by Diatron® U.S.A). The analysis were done at General Hospital Minna for Hb, PCV, MCH, MCHC, WBC, RBC, MCV, neutrophils, lymphocytes, MIE (monocyte, eosinophil, basophil), platelet count, and mean cell distribution width (MCDW), mean platelet distribution width (MPDW) . The procedure included:

1. Using the EDTA blood container, whole blood was inserted into the analyzer.
2. The analyser aspirated 2 micro liters of the blood into the probe and autoanalysed.
3. The result appeared on the screen and copied.

3.7.3 Qualitative method-Key Informant Interviews (KII)

KII was conducted using semi-structured questionnaires as a guide (appendix 4) on seven (7) key informants to obtain in-depth information on the research question. These key informants were individuals found to be honest, reliable and forthcoming sources of in-depth information about topics of interest to the study which included CMV prevalence, associated factors for CMV infection, blood donation and practices of blood donors.

3.7.4 Quantitative method

Interviewer administered questionnaire method was employed using structured questionnaires (appendix 3). The questionnaires were pretested at a near by HF, General Hospital Bida, Niger State to ascertain their flexibility and acceptability. Each participant was administered a questionnaire to obtain information regarding age, sex, occupation, ethnic group, marital status, past history of transfusion and other socio-demographic, socio-economic and practices.[12]

3.8 Data Management

3.8.1 Measurement of variables

Exposure variables: The exposure variables are the socio-demographic and socio-economic characteristics of the respondents, cultural practices.

Outcome variable: The outcome variable is the CMV serological status of the blood donors in terms of IgM and IgG, which are either positive or negative

3.8.2 Statistical analysis

The data obtained was analyzed using Epi Info version 3.5.4 and Microsoft office excel 2007. Chi square was used to determine associations. Significant associations were presumed if $p<0.05$.

Results are reported accordingly, using tables.

3.9 Ethical Considerations

Ethical clearance was obtained from the GH, Minna and IBBSH, Minna research and ethical clearance committees (appendix 5). Respect to subjects' rights was observed including the right to refuse participation with explanation through participant's (appendix 1) and through provision of individual consent form (appendix 2) for the consent of the participants. There was provision for subject's safety (physical and psychological) by maintaining confidentiality.

3.10 Limitations

Private health facilities in Minna city constituted some percentage of blood donors but could not be considered in this study as their inclusion could have constituted bias as most of them were not consistent blood donation centers. Preliminary findings revealed that their exclusion may not affect the representativeness of the study.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic Characteristics

Table 1: Socio-demographic data of blood donors in Minna, North Central Nigeria

Variable	Frequency (n=345)	Percent (%)
Age		
<20	6	1.7
20-29	139	40.3
30-39	137	39.7
40-49	46	13.3
50-59	14	4.1
≥60	3	0.9
Sex		
Male	336	97.4
Female	9	2.6
Marital Status		
Married	230	66.7
Single	113	32.8
Separated	2	0.6
Occupation		
Self employed	183	53.0
Employed	95	27.5
Student	59	17.1
Unemployed	6	1.7
Retired	2	0.6
Educational status		
Post graduate	38	11.1
Tertiary (first degree)	108	31.5
Secondary	96	28.0
Primary	26	7.6
No formal education	75	21.9
Monthly income level		
Above ₦120000	3	0.9
₦70000-₦20000	16	4.8
₦35000-Below ₦70000	91	27.2
₦18000-Below ₦35000	136	40.6
Below ₦18000	89	26.6
Type of donor		
Commercial	11	3.2
Voluntary	60	17.4
Family	273	79.4

Majority of the blood donors, 139 (40.3%) were in the age group 20-29 with a mean age of 32.4± 8.5 years, 230 (66.7%) were married, 65 (19.5 %) were unemployed, 273 (79.4%) were family blood donors, 146 (42.6%) had post-secondary education, 136 (40.6%) had a monthly income of between ₦18000 and ₦35000 as shown in table 1 above.

4.2 CMV Prevalence

Table 2: Sero-prevalence of anti-CMV IgG and IgM antibodies among blood donors in Minna, North Central Nigeria

Sero-status	Anti-CMV IgG		Anti-CMV IgM	
	Frequency (n-345)	Percent (%)	Frequency (n=345)	Percent (%)
Positive	332	96.2	9	2.6
Negative	13	3.8	336	97.4
Total	345	100	345	100

Table 3 shows the prevalence of CMV, CMV IgG and CMV IgM prevalence, the prevalence of CMV infection was found to be 96.2%. The prevalence of CMV IgG and CMV IgM were found to be 96.2% and 2.6% respectively as shown in table 2 above.

4.3 Associated Factors for CMV Infection

Table 3: Socio-economic-associated factors for CMV (IgG) infection among blood donors in Minna, Northern Nigeria-2013

Variable	CMVI Gg Positive	CMVI Gg Negative	OR (95%CI)	*P-value*
Marital Status				
Married	224 (67.5)	6 (46.2)	2.42 (0.79-7.37)	0.09*
Single	108 (32.5)	7 (53.8)		
Type of marriage				
Polygamous	56	57	1.76 (0.20-15.4)	0.51*
Monogamous	169	164		
Sex				
Male	323 (97.3)	13 (100)	1.26 (0.07-22.81	0.68*
Female	9 (2.7)	0 (0.0)		
Age				
<40 years	288 (86.7)	11 (84.6)	1.19 (0.26-5.55)	0.54*
≥40 years	44 (13.3)	2 (15.4)		
Monthly income level				
<₦18000	**90 (27.1)**	**7(53.8)**	**0.32 (0.10-0.97)**	**0.04***
≥₦18000	**242 (72.9)**	**6 (46.2)**		
Educational level				
Low education	98 (29.6)	3 (23.1)	1.40 (0.38-5.20)	0.44*
High education	233 (70.4)	10 (76.9)		
Occupation				
Unemployed	63 (19.0)	4 (30.8)	0.53 (0.16-1.77)	0.23*
Employed	269 (81)	9 (69.2)		
Period of sampling				
<01/01/14	312 (94.0)	2 (92.3)	1.60 (0.16-10.51)	0.56*
≥01/01/14	20 (6.0)	1 (7.7)		

*- Fisher exact

At bivariate analysis for socio-economic factors for CMV (IgG) infection, 8 factors were analyzed. Blood donors with monthly income level less than #18000 were 68 percent less likely to be CMV positive than those with monthly income level equal to or higher than ₦18000 (P=0.04). The rest factors were not significant, as shown in table 3 above.

Table 4: Socio-economic-associated factors for CMV (IgM) infection among blood donors in Minna, Northern Nigeria

Variable	CMV IgM Positive	CMV IgM Negative	OR (95%CI)	*P-value*
Marital Status				
Married	8 (67.5)	222 (88.9)	4.10 (0.51-33.25)	0.14*
Single	1 (32.5)	114 (11.1)		
Type of marriage				
Polygamous	56	57	1.77 (0.41-7.64)	0.34*
Monogamous	169	164		
Sex				
Male	9 (100)	327 (97.3)	0.55 (0.03-10.18)	0.79*
Female	0 (0.0)	9 (2.7)		
Age				
<40 years	7 (77.8)	269 (80.1)	0.87 (0.18-4.29)	0.57*
≥40 years	2 (22.2)	67 (19.9)		
Monthly income level				
<₦18000	2 (22.2)	95 (28.3)	0.72 (0.15-3.55)	0.51*
≥₦18000	7 (77.8)	241 (71.7)		
Educational level				
Low education	5 (55.6)	96 (28.7)	3.11 (0.82-11.84)	0.09*
High education	4 (44.4)	239 (71.3)		
Occupation				
Unemployed	1 (11.1)	66 (19.6)	0.51 (0.06-4.16)	0.45*
Employed	8 (88.9)	270 (80.4)		
Period of sampling				
<01/01/14	**5 (55.6)**	**319 (94.9)**	**0.07 (0.01-0.27)**	**0.01***
≥01/01/14	**4 (44.4)**	**17 (9.1)**		

*Fisher exact

At bivariate analysis for socio-economic factors for CMV (IgM) infection, 8 factors were analyzed. Blood donors recruited, interviewed and screened before 01/01/14 were found to be 93 percent less likely to be CMV positive than those after 01/01/14 (P=0.01). The rest factors were not significant as shown in table 4 above.

Table 5: Common practices associated with CMV (IgG) infection among blood donors in Minna, North Central Nigeria

Variable	CMV IgG Positive	CMV IgG Negative	OR (95%CI)	*P-value*
Tribal marks				
Yes	71 (21.4)	4 (30.8)	0.61 (0.18-2.05)	0.64
No	261 (78.6)	9 (69.2)		
Received blood transfusion				
Yes	12 (3.6)	0 (0)	1.05 (0.06-18.73)	0.62*
No	320 (96.4)	13 (100)		
Received surgical procedure in HF				
Yes	11(3.3)	1 (7.7)	0.41 (0.05-3.45)	0.37*
No	321(96.7)	12 (92.3)		
Local circumcision				
Yes	317 (95.5)	12 (92.3)	1.76 (0.21-14.35)	0.47*
No	15 (4.5)	1 (7.7)		
Local *belubelu* (uvulectomy)				
Yes	38 (11.4)	3 (23.1)	0.43 (0.11-1.64)	0.19*
No	294 (88.6)	10 (76.9)		
Dental procedure inside HF				
Yes	19 (5.7)	0 (0.0)	1.68 (0.10-29.31)	0.47*
No	313 (94.3)	13 (100)		
Dental procedure outside HF				
Yes	28 (8.4)	2 (15.4)	0.51 (0.12-3.52)	0.31*
No	304 (91.6)	11 (84.6)		
Worked in contact with blood				
Yes	21 (6.3)	1 (7.7)	0.81 (0.10-6.53)	0.58*
No	311 (93.7)	12 (92.3)		
Sex partner not spouse				
Yes	58 (17.5)	2 (15.4)	1.17 (0.25-5..41)	0.59*
No	273 (82.5)	11 (84.6)		

*- Fisher exact

At bivariate analysis for common practices associated with CMV (IgG) infection, 9 factors were analysed. Factors like received transfusion, local circumcision, dental procedure inside HF and sex partner not spouse were positively associated with CMV (IgG) infection but none was found statistically significant as shown in table 5 above.

Table 6: Common practices associated factors for (IgM) infection among blood donors in Minna, North Central Nigeria-2013

Variable	CMV IgM Positive	CMV IgM Negative	OR (95%CI)	*P-value*
Tribal marks				
Yes	4 (44.4)	71 (21.1)	2.99 (0.78-11.41)	0.11
No	5 (55.6)	265 (78.9)		
Received blood transfusion				
Yes	0 (0.0)	12 (3.6)	1.37 (0.08-24.81)	0.64
No	9 (100)	324 (96.4)		
Had surgical procedure done in HF				
Yes	0 (0)	12 (3.6)	1.37 (0.08-24.81)	0.64*
No	9 (100)	324 (96.4)		
Local circumcision				
Yes	8 (88.9)	321 (95.5)	0.37 (0.04-3.19)	0.35*
No	1 (2.6)	15 (4.5)		
Local *belubelu* (uvulectomy)				
Yes	0 (0.0)	41 (12.2)	0.37 (0.02-6.56)	0.32*
No	9 (100)	295 (87.8)		
Dental procedure inside HF				
Yes	0 (0.0)	19 (5.7)	0.86 (0.05-15.27)	0.59*
No	9 (100)	312 (94.3)		
Dental procedure outside HF				
Yes	0 (0.0)	30 (8.9)	0.53 (0.03-9.31)	0.44*
No	9 (100)	306 (91.1)		
Worked in contact with blood				
Yes	1 (11.1)	21 (6.2)	1.88 (0.22-15.70)	0.45*
No	8 (88.9)	315 (93.8)		
Sex partner not spouse				
Yes	3 (33.3)	57 (17.0)	2.44 (0.59-10.04)	0.19*
No	6 (66.7)	278 (83.0)		

*- Fisher exact

At bivariate analysis for common practices associated with CMV (IgM) infection, 9 factors were analysed. Factors like tribal mark, received transfusion, received surgical procedure in HF, local circumcision, worked in contact with blood and sex partner not spouse were positively associated with CMV (IgM) infection but none was found significant as shown in table 6 above.

4.4 Patterns of Distribution of Haematological Indices

Table 7: Pattern of haematological indices among CMV seropositive (IgG) blood donors in Minna, North Central Nigeria

Variable	Low (%)	High (%)	Normal (%)
Hb	177 (53.3)	45 (13.6)	110 (33.1)
PCV	185 (55.7)	1 (0.3)	146 (44.0)
WBC total	32 (9.6)	46 (13.9)	254 (76.5)
Lymphocyte	98 (29.6)	119 (35.8)	115 (34.6)
Granulocyte	114 (34.3)	46 (13.9)	172 (51.8)
MIE (Mono, Eosino, Bas)	160 (48.2)	90 (27.1)	82 (24.7)
RBC	80 (24.1)	68 (20.5)	184 (55.4)
MCH	209 (63.0)	1 (0.3)	122 (36.7)
MCHC	3 (0.9)	10 (3.0)	319 (96.1)
PLT	29 (8.8)	32 (9.6)	271 (81.6)
RCDW	0 (0.0)	231 (69.6)	101 (30.4)
MPV	110 (33.3)	0 (0.0)	221 (66.7)
MCV	78 (23.5)	1 (0.3)	253 (76.2)

NB-Values of normal ranges[56]

WBC-5-10 (10^9/L), Lymphocyte count- 25-40 (%), Granulocyte count- 50-75 (%), MIE- 3-7 (%), RBC- 4-5 (10^{12}/L), HB- 12-17.4 (g/dl), HCT- 36-52 (%), MCV- 76-96 (fl), MCH- 27-32 (g/dl), MCHC-30-35 (g/dl), PLT- 150-400 (10^9/L), RDWC- 11.5-14.5 (%), MPV- 8-15 (fl)

Of the CMV seropositive (IgG) blood donors, 55.9% were anaemic (HCT<36%), 53.3% low Hb, 63.0% MCH, while 76.5% had normal WBC, 55.4% normal RBC, 81.6% normal platelet count as shown in table 7 above.

Table 8: Pattern of haematological indices among CMV seropositive (IgM) blood donors in Minna, North Central Nigeria

Variable	Low (%)	High (%)	Normal (%)
Hb	5 (55.6)	0 (0.0)	4 (44.4)
PCV	5 (55.6)	0 (0.0)	4 (44.4)
WBC total	1(11.1)	2 (22.2)	6 (66.7)
Lymphocyte	2 (22.2)	3 (33.3)	4 (44.4)
Granulocyte	2 (22.2)	1 (11.1)	6 (66.7)
MIE (Mono, Eosino, Bas)	1 (11.1)	5 (55.6)	3 (33.3)
RBC	0 (0.0)	1 (11.1)	8 (88.9)
MCH	8 (88.9)	0 (0.0)	1 (11.1)
MCHC	0 (0.0)	0 (0.0)	9 (100.0)
PLT	0 (0.0)	2 (22.2)	7 (77.8)
RCDW	0 (0.0)	6 (66.7)	3 (33.3)
MPV	4 (44.4)	0 (0.0)	5 (55.6)
MCV	3 (33.3)	0 (0.0)	6 (66.7)

NB-Values of normal ranges[56]

WBC-5-10 (x 10^9/L), Lymphocyte count- 25-40 (%), Granulocyte count- 50-75 (%), MIE- 3-7 (%), RBC- 4-5 (10^{12}/L), HB- 12-17.4 (g/dl), HCT- 36-52 (%), MCV- 76-96 (fl), MCH- 27-32 (g/dl), MCHC-30-35 (g/dl), PLT- 150-400 (10^9/L), RDWC- 11.5-14.5 (%), MPV- 8-15 (fl). Of the CMV seropositive (IgM) blood donors, 55.6% were anaemic (HCT<36%), 55.6% low Hb, 88.9% low MCH, while 66.7% normal WBC, 88.9% normal RBC, 77.8% normal platelet count as shown in table 8 above.

Table 9: Pattern of Hb and PCV among various strata of blood donors in Minna, North Central Nigeria

Category	Type of blood donor	Parameters			
		Hb		PCV	
		Frequency	Percentage	Frequency	Percentage
Low	**Commercial**	8	3.5	6	3.1
	Family	**185**	**85.5**	**158**	**81.0**
	Voluntary	34	15.0	31	15..9
Normal	**Commercial**	3	2.5	5	3.3
	Family	89	75.4	116	77.4
	Voluntary	26	22.0	29	22.0
High	**Commercial**	0	0	0	0
	Family	0	0	0	0
	Voluntary	0	0	0	0

NB-Values of normal ranges

Hb=12-17.4 g/dl, PCV=36-52%. Hb-Low=<12g/dl, Normal=12-17.4g/dl, High=>17.g/dl.

PCV- Low=<36%, Normal=36-52%, High=>52%

Table 9 shows the pattern of Hb and PCV among various strata of blood donors as they relate to their Hb and PCV level where 56.5% have PCV below the normal range and family replacement blood donors form the majority, 158 (81.0%) of the total (195) anaemic (PCV<36%) blood donors. None of the blood donors had Hb or PCV above normal range.

Table 10: Correlation relationship between haematological indices of blood donors in Minna, North Central Nigeria

Variables	**Correlation coefficient (r^2)**	***P-value***
Hb and MCH	0.24	0.19
Hb and PCV	**0.85**	**<0.001**
Hb and WBC	0.06	0.53
Hb and Lymph	0.12	0.38
Hb and Neutrophil	0.16	0.30
Hb and MCV	0.01	0.76
Hb and MCHC	0.00	0.88
Hb and MCDW	0.16	0.20
WBC and Lymph	0.36	0.09
WBC and Neutrophil	0.24	0.19
WBC and MIE	0.07	0.48
WBC and PCV	0.00	0.91
WBC and MCHC	0.32	0.12
WBC and PLT	0.19	0.25
WBC and Age	0.13	0.30

PCV was found to be significantly correlated with Hb ($P<0.001$). Other haematological indices all respectively showed positive correlation with Hb and WBC except MCHC and PCV which showed no correlation with Hb and WBC respectively, but were all not statistically significant as shown in table 10 above.

Table 11: Correlation relationship between haematological indices of blood donors in Minna, North Central Nigeria

Variables	Correlation coefficient (r^2)	*P-value*
Lymphocyte and PCV	0.15	0.31
Lymphocyte and MIE	0.00	0.86
Lymphocyte and Neutrophil	**0.91**	**<0.001**
Lymphocyte and PLT	0.42	0.07
PCV and Neutrophil	0.23	0.20
PCV and MCV	0.00	0.91
PCV and MCH	0.33	0.11
Neutrophil and MIE	O.12	0.37
Neutrophil and PLT	0.23	0.19
Platelet and MPV	0.15	0.32
Platelet and RBC	0.23	0.19
Platelet and MPDW	0.26	0.17
MPV and MPDW	**0.59**	**0.02**
MCV and MCDW	**0.65**	**0.01**

Lymphocyte and Neutrophil count (P<0.001), MCV and MCDW (P=0.02), MPV and MPDW (P=0.01) respectively showed significant correlation relationship (P<0.05) All other indices showed various levels of positive relationship except lymphocyte and MIE, PCV and MCV that showed no relationship, but are all not statistically significant as shown in table 11 above.

Table 12: Variations and statistical significance of means of CMV seropositive and CMV seronegative groups of blood donors in Minna, North Central Nigeria

Variable	Means and standard deviation				*P-value*
	CMV seropositive group		CMV seronegative group		
	Means	Standard deviation	Means	Standard deviation	
Hb	**11.31**	**0.08**	**11.38**	**1.67**	**0.02**
Lymphocyte	33.72	13.17	31.41	12.80	0.59
WBC	7.10	2.56	6.81	2.34	0.72
PCV	**34.85**	**5.32**	**34.88**	**2.74**	**0.03**
Platelet	286.44	107.28	251.36	89.08	0.25
RBC	4.63	0.48	4.36	0.74	0.27
MCH	25.61	1.90	25.73	2.99	0.91
Neutrophil	57.18	13.99	57.50	14.56	0.95
MIE	8.7	3.58	10.86	5.56	0.25
MPV	8.81	2.25	8.06	1.37	0.10
MCV	81.22	6.36	79.26	7.26	0.42
MCHC	32.23	0.97	32.45	1.42	0.64
MCDW	15.76	1.58	15.23	2.19	0.47
MPDW	**32.37**	**8.30**	**36.02**	**5.229**	**0.04**

Testing for variations and statistical significance of means of CMV seropositive group and CMV seronegative group on haematological indices showed CMV seropositive group are more likely to have depleted Hb (P=0.02), PCV (P=0.03) and MPDW (P=0.04) than those of CMV seronegative group.

In the qualitative methods, seven interviews were conducted, out of which 6 were males. On work that entails contact with blood, majority had had contact with blood in the past and they considered transfusion transmissible infections, inadequate blood storage facilities, non-compatible blood, and lack of voluntary donors as the most significant health problems associated with blood transfusion in the community. But felt CMV was not a significant health problem in the community because the infection is not common in the community and it is not screened for transfusion.

On the risk associated with CMV infection in terms of socio-demographic characteristics and practices, majority felt blood recipients were at high risk of CMV infection, and practices such as local circumcision, local excision of *'belubelu'*- uvulectomy or/and tonsillectomy, body piercing, sharing of sharp objects, needle stick injury, exposure to blood, body fluids or tissues, local hair shaving, dental procedure outside health facility, tribal marks, receiving surgical procedure in health facility", are factors associated with CMV infection.

On best preventive measures against CMV infection, majority suggested CMV blood screening for transfusion, safety measures by laboratory and other health workers, provision of vaccine and proper treatment, regular screening of health workers and government should provide CMV test logistics.

CHAPTER FIVE

DISCUSSION

A 100% response reflects the level of enthusiasm of the research participants as well as the dire need for safe blood. The blood donation centers are characterized by a continuous flow of prospective donors to meet a perennial need for transfusion as an intervention for trauma related injuries, sickle cell anaemia, obstetric haemorrhage and many more. Family replacement donors constituted the majority of 79.4% while commercial donors and voluntary donors made up 3.2% and 17.4% respectively. This contrasts with the report by Chaudhari and Bindra in India,[57] and Eivazi-Ziaei and colleagues in Iran[58] who conducted similar studies on 100% voluntary donors in separate locations. Voluntary blood donors (17.4%) in this study are lower than 24% reported in Albania.[59] The low proportion of voluntary donors reflects the duo of ignorance and low national development index. The proportion of voluntary donation varies directly with the development index of a nation.[2] The modal age group in this study was 20-29 years with a mean age of 32.4± 8.5years, which is higher than 18-20 years reported by a study in Chennai, India.[60] A study in Lagos, Nigeria [31] reported a mean age of 31.3 ± 8.7 years which is identical with the mean age of 32.4 ± 8.5years obtained in this study. The mean age in this study is higher than the mean of 28.2 ± 7.2 years, obtained by Chaudhari and Bindra[57] while working on voluntary blood donors. The predominance of male donors at 97.4% is comparable with 95.4% reported by Akinbami and colleagues,[31] 98.4% by Amiwero and colleagues,[22] 91.7% by Ojide and colleagues [13] and 94.5 by Kothari and colleagues.[61] This may be linked to the belief that women do not donate blood because of menstrual flow, pregnancy and child-bearing.[62] Most of the participants

(66.7%) were married, this may be related to the culture of a part of Nigeria where females are married by 15years of age and males are married in their early 20 years of age.[62,63]

Most participants were in some of vocation or study as 80.5% and 17.1% were either employed or students respectively, retirees accounted for 0.6%. This shows that blood donation is an activity of persons less than 65years of age.[2] Aging with attendant medical challenges may contra-indicate blood donation. Unemployed donors who consist of 1.7% may be paid donors, a further reflection of the endemic poverty in the community. Majority, 40.6% had post-secondary education, while 28% had secondary education, as those without education made up 21.9%. The rate of illiteracy observed in this study is higher than 15.4% reported for the North-Central zone in the National Demographic and Health Survey (NDHS) 2008 by National Population Commission, Nigeria (NPC).[64] With a national monthly minimum wage of ₦18000 (≈$110), 26.6% were below the national poverty level. This suggests that at least a quarter of the participants live on less than $3.5 per day. This further confirms that poverty induces donation for money.

The seroprevalence of CMV, 96.2% is high which corroborates findings of a study in India (95%),[16] US (80%),[12] Sfax region, Tunisia (97.1%),[30] Jos, Nigeria (92%),[8] and Lagos, Nigeria (96%),[31] this indicates CMV virus infection is widely spread among human population but not commonly known as most CMV infections are asymptomatic[11] and may remain asymptomatic without been diagnosed. The high prevalence of anti-CMV IgG antibodies (96.2%) found in this study corroborates findings of other studies in Southern Brazil (96.4%),[29] Ghana (93.2%),[14] Lagos, Nigeria (96%),[31] Benin, Nigeria (95.8%),[13] and Ilorin, Nigeria (96.7%).[33]

The prevalence of anti-CMV IgM antibodies is 2.6% which is much lower than 85%, 5.5%, and 3.1% reported in Iran,[58] Albania[59] and Benin, Nigeria[13] respectively. Studies from Brazil,[29] Sudan[28] and Ilorin[33] reported nearly comparable anti-CMV IgM antibody rates of 2.3%, 2.5% and 2.2% respectively. The high anti-CMV IgM prevalence recorded in Iran contrasting the findings of this study is attested to by the fact that numerous studies in some regions of Iran have had different results with high prevalence regarding the prevalence and seropositivity of anti-CMV IgM,[58] as reported by Sepehrvand[65] and Amin-Zadeh.[66] This high prevalence of anti-CMV IgM may not be unconnected with certain cultural practices in Iran such as kissing children to show love and bonding. Most children are infected very early in life and reinfection continuous in addition to vertical infection from mother to child.[58]

Combined CMV IgG and CMV IgM antibodies were detected in 9 (2.6%) a figure that is higher than 1.6% reported by Chaudhari and Bindra.[57] A significant portion of CMV infections are symptomatic, their ability to find sanctuary in peripheral blood monocytes coupled with episodes of recrudescence makes re-infection and/or activation a common event. Therefore re-activation like de-novo infection is associated with a CMV IgM response. Individuals who suffer a re-activation have previously mounted a CMV IgG response. Therefore anti-CMV IgG prevalence rates are considered to reflect the overall prevalence for epidemiologic purposes.[8,9,14,31]

The odds of infection is doubled with marriage but is not statistically significant, to this end polygamy did not significantly increase the risk. Males were more at risk of infection but not statistically significant, similar observations were reported in India.[67] This is contrary to the report in California[68] in which females had a higher infection rate. This difference may be due to the study populations, while findings in India were from blood donors aged 18 to 60 years, findings in California were from community-dwelling Mexican Americans aged 60 to 100 years at baseline. The prevalence of CMV raises with donor age but not significantly, a pattern similar to the observation by Seferi and colleagues,[59] Chaudhari and colleague.[57] Low educational level did not significantly increase the risk for CMV infection, but the odds are increased three-folds. A similar pattern was seen in the report of the study in California.[68]

A monthly earning of ₦18000 and above was significantly associated with CMV antibody positivity. Blood donors with monthly income level less than ₦18000 were 68 percent less likely to be CMV positive than those with monthly income level equal to or higher than ₦18000. This finding on individuals with higher monthly income being more at risk corroborates the findings of a study by Revello and Giuseppe [42] where they found that the risk of primary maternal infection of CMV was about three times higher among the higher-income susceptible women (45%), compared to 15% in the lower-income group. This is also identical with the report in Poland[69] which professional education like medicine and surgery, haematology, anatomy, medical laboratory sciences, nursing and a very good financial status are associated with increased risk of CMV infection in a cohort of pregnant polish women. But this finding is contrary to the report in California[68] in which persons who earned less than $1000 had a risk of 43.5% more than individuals with higher income. This difference may be associated with the study population, while the findings in Poland were from a cohort of pregnant women aged 16-45 years, the findings in California were from a community-dwelling Mexican Americans aged 60–100 years at based line. Most predisposing characteristics like professional practice like surgery, phleombotamy, social practices like ability to attract women for sex with parners not spouse that go with higher income status may have reduced at this age level.

Low educational level did not significantly increase the risk for CMV infection, but the odds are increased three-folds. A similar pattern was seen in the study by Dowd and colleagues.[68] This study indicates that the prevalence of CMV antibody is inversely proportionate to the level of educational attainment which is statistically insignificant but the contrary was observed in a similar trend reported in California[68] and Sudan[28] in which individuals with lower educational level had significant higher CMV IgG antibody levels.[68] A study in Paris among French and immigrant populations[70] also observed that children of low socio-economic immigrant families had a higher CMV antibodies level. Therefore, poor education and subsequent poverty are facilitators of CMV spread. Employment was associated with higher likelihood of CMV infection, this is not far from the fact that most employed are at higher risk of engagements with exposure tendencies in terms of occupational hazards. The increased positivity of CMV infection among those employed can also be linked to the fact that those employed are better placed to belong to higher economic levels which was also found to be associated with increased likelihood of CMV infection.[42,70]

There was a statistically significant association on periodicity of CMV infection where blood donors recruited, interviewed and screened before 01/01/14 were found to be 93 percent less likely to be CMV positive (IgM) than those after 01/01/14, this is in agreement with the findings of Orlikowski[71] who found annual periodicity of CMV infection between months of July and October which coincides with the winter of the Northern Hemisphere. CMV infection is generally known to be non seasonal,[72] the finding in this study may be peculiar cases which call for further research to verify the hypothesis. The relationship between practices and CMV infection was not statistically significant for practices like tribal mark, received blood transfusion, received surgical procedure in health facility, local circumcision, dental procedure in health facility, dental procedure outside health facility, local *belubelu* (uvulectomy), worked in contact with blood, sex partner not spouse, though all showed association with CMV infection.

Many of the blood donors in this study, 55.9% were found to be anaemic (PCV <36%). This corroborates findings of other studies which include tertiary health facility in Port Harcourt, Niger Delta region of Nigeria[50] and south India.[25] This is also identical with findings of Xu and colleagues,[48] Gordon-smith and associates[64] and Taglietti and colleagues.[73] This high level of anaemia may be associated with the finding in the study that 81.5% of the anaemic donors were family replacement donors and family replacement donors constituted 79.4% of all the donors (345) in the study. Comparison of the difference of the means of PCV and Hb between CMV seropositive and CMV seronegative donors was statistically significant with *P value* less than 0.05 ($P<0.05$), as the CMV seropositive donors had a lower PCV and Hb, this implicates CMV in anaemia as CMV has been found to predispose infected individuals to anaemia. PCV and Hb were strongly and positively significantly correlated (($r^2=0.85$; $p<0.001$)). Therefore CMV seropositivity with its attendant risk predisposes to anaemia. Suffice to say that CMV causes infection of bone marrow suppression which is a risk factor for aplastic anaemia.[74,75,76]

The high percentage of CMV IgG leucopenia observed in this study is in tendem with the high proportion of blood donors with low percentage of percent lymphocyte count (29.6%) and percent monocyte count (48.2%) in this study. The CMV seropositive donors had a lower but not statistically significant different WBC count. This may be due to bone marrow suppressive effect of CMV. It may also explain the relative high prevalence of leucopenia found in so many percent blood donors. Leucopenia has also been reported in CMV infected persons in India,[25] US,[75] and Portugal.[77] CMV is also a cause of marrowcytosis and lymphocytosis, neutropenia.[75,76,78] The statistically significant positive correlation between lymphocyte and neutrophil count (r^2=0.91; P<0.001) shows that CMV induces an inflammatory response that results in lymphocytic lymphocytosis initially with subsequent neutrophilia following secondary bacteria infection as CMV infection worsens background immunosurpression.[74]

Majority, 81.6% and 66.7% have platelet count and MPV within normal ranges (150-400 * 10^9/L) and (8-15 fl) respectively, this is in line with findings by the study among blood donors in Port Harcourt, Nigeria[50] where no thrombocytopenia was observed. However in comparison of the means of platelet count between CMV seropositive and CMV seronegative donors, the CMV seropositive donors had a higher platelet count but without a statistically significant difference. Thrombocytopenia is a feature of CMV infection due to its marrow suppression effect.[75,79] Paradoxically this study recorded a higher platelet count in the CMV seropositive group of donors. Platelet increase may be as a result of persistent inflammatory responses. The mean platelet distribution width (MPDW) for CMV seropositive and CMV seronegative 32.37 and 36.02 respectively, the difference which is statistically significant is lower among the CMV seropositive donors. This may be as a result of marrow suppression, a harsh haemophoeitic microenvironment and subsequent platelet fragmentation (a form of CMV induced dysplasia).[74,75] The overall MPDW is far greater than normal of the established normal value of 12%, this is indicative of severe platelet anisocytosis, a feature of iron deficiency.[73,78] Mean corpuscular volume (MCV) and mean cell distribution width (MCDW) were also correlated and recorded mild anisocytosis.[75,78]

Findings in qualitative study showed majority feel CMV is not a significant health problem in the community because the infection is not common in the community. This is because it is not screened for in transfusion. This feeling by most of the participants is because they are not familiar with the infection and CMV infection is mostly asymptomatic. [72,75,78] Where as CMV infection is a very common disease in the community as indicted by the high prevalence, people seem to generally feel the infection is not common in the community, as they do not hear people get sick because of CMV. This is basically because CMV is not been commonly screened for even in the case of transfusion for immunocompromised patients. The high prevalence in the study shows the ubiquitous nature of CMV despite its low recognition in the community. The absence of specific symptoms limits community recognition,[11] hence the poor perception by the local. Some said they have never had of a disease called CMV infection nor do they know anybody afflicted by it. Majority of the key informants opined that blood transfusion recipients are at risk from CMV infection and CMV screening should be part of screening for transfusion.

Despite the non recognition of CMV infection in the community, routine screening of donors is mandatory [8,67] as well as screening of the potential recipients of the transfusion products with a view to identifying CMV negative recipients as suggested by Alao and colleagues in Nigeria and Radhiga and colleagues in India in their various studies, [8,67] even though that will not be cost effective [58]. To this end, CMV screening will be aimed at identifying CMV negative recipients as leucodepletion of CMV positive blood and irradiation will protect such recipients.[80,81,82] In the event that CMV negative donors are scarce, leucodepletion and irradiation[58,80] are suggested interventions for the CMV negative immunocomprommised, premature or neonatal recipients.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The seroprevalence of CMV is high in this environment but consistent with published data in other parts of Nigeria and across the globe. There is a significant proportion of individuals who are capable of transmitting CMV infection to blood recipients. But Since up to 96.2% of blood donors in this study (Minna) are seropositive for CMV, it would seem superfluous to screen blood donors for CMV for all transfusions, as few seronegative blood units would be available for transfusion.

CMV negative donors remain the best for CMV negative recipients and immuno-compromised persons but in their absence as they are scarce, leucodepletion and irradiation are suggested interventions.

The study showed there is a statistically significant association between monthly income level of participants, period of recruitment and screening and CMV infection. There is high level of anemia most especially among family replacement donors, leucopenia and a significant difference in the haemoglobin level between CMV seropositive and CMV seronegative groups.

6.2 Recommendations

1. Prospective blood donors should be screened for CMV most especially for immunocompromised recipients.
2. Prospective blood donors should be properly screened for anaemia based on the minimum required Hb/PCV levels in accordance with WHO guidelines.
3. Leucoreduced products and other blood products from CMV seronegative donors should be given to preterm neonates and infants as this will help prevent transfusion-associated perinatal CMV disease.
4. There should be further study on seasonality of CMV infection.
5. There should be more campaign and awareness on provision of voluntary blood donation.
6. There should be awareness in the community on the significant impact of CMV infection on health.

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APPENDICES

Appendix 1

Participant's information form

My name is Mr. Musa K. Bawa, a Resident Laboratory Epidemiologist with the Nigerian Field Epidemiology and Laboratory Training program (N-FELTP). We are conducting a study titled: The Effect of Cytomegalovirus (CMV) in Determining Blood Donor Safety in Minna, North Central Nigeria**.** I will be grateful if you can participate as a respondent.

Blood transfusion has become an important component of patient management as it safes life but recently viral infection like HIV, HBV and HCV have limited the safety and advantages of blood transfusion. Consequently, screening test for HIV, HBV and HCV were introduced to protect blood transfusion recipients, improving the treatment of individuals with weak immune systems like new born, the very elderly, patients on treatment for cancer, organ transplant recipients, individuals on life-long immunosuppressive therapy and HIV infected individuals. These individuals are particularly prone to CMV infection which can be transmitted by blood transfusion, organ transplantation, from mother to child and sexually.

In most individuals, infection with CMV is not manifested clinically for several weeks or months; it only becomes obvious if for any reason, the immunity of the affected individual goes down. This therefore means that CMV may influence the safety of blood in the individuals mentioned above. In order to protect this special group of individuals, there is a need to determine the prevalence of CMV in our society so that we can accurately map out strategies and policies that will further enhance transfusion safety.

I will therefore be grateful if you kindly give me sometime to respond to some questions regarding your knowledge on CMV in addition to assessing your behavior and your ability to identify those factors that will cause individuals to have CMV. You have a right to refuse participation at any stage of the research and your refusal will not affect you in any way, your family or employment. However, if you participate, you will have the benefit of knowing the factors that influence the spread of CMV and the relationship between CMV and common viruses in the environment like chicken pox virus, herpes virus and EBV. This will help you take appropriate measures for enhancing your health.

You will also permit me to take 5mls of your blood in order to test for the CMV, malaria parasite (MP) and to count your blood cells. Your participation is generally harmless apart from a slight pain from a prick of the needle which will however subside within 24 hours. Your privacy will be respected and confidentiality upheld. All information obtained will be stored anonymously in pass word-protected soft ware packages and will only be disseminated in scientific fora.

Name of participant: ……………………………….… Sign/Thumb print ……...

Date:………………………….

Name of researcher:…………....................................Sign/Thumb print……….

Date:…………………………….one Number…………………………………

House address...………………………………………...

………………………………………………………………………………………

Appendix 2

Consent Form

I have read and have understood, I voluntarily declare my intention to participate in the research without any coercion or compulsion and I also understand that if I decline to participate there will be no adverse outcome to myself, family, employment or community.

Name of participant: ……………………………….…   Sign/Thumb print ……...

Date:………………………….

Name of researcher:…………......................................Sign/Thumb print……….

Date:…………………………….

Appendix 3

Questionnaire

QUESTIONNAIRE FOR THESIS RESEARCH ON THE EFFECT OF CYTOMEGALOVIRUS (CMV) IN DETERMINING BLOOD DONOR SAFETY IN MINNA, NORTH CENTRAL NIGERIA, 2013

QUESTIONNAIRE NO…………..

Date…………………..

Good day. My name is Mr Musa K. Bawa, a Resident Laboratory Epidemiologist with the Nigerian Field Epidemiology and Laboratory Training program (N-FELTP). We are conducting a study titled: The Effect of Cytomegalovirus (CMV) in Determining Blood Donor Safety in Minna, Niger State, Nigeria to find out proportion of blood donors infected with the virus and identify possible means of transmission of infection, create awareness as well as advocacy for effective prevention of the disease. You are not compelled to participate, however we would be grateful if you could kindly oblige us a few minutes of your time. All information supplied would be treated as strictly confidential.

SECTION 1

DEMOGRAPHIC DATA

1. Age in years at last birthday: ______________________
2. Sex: Male ☐ Female ☐
3. Marital status (Tick whichever applies)
   a) Single ☐
   b) Married ☐
   c) Separated ☐
   d) Divorced ☐
   e) Widowed ☐
   f) Others (specify)__________________________

4. TRIBE: ______________________________

5. OCCUPATION:

a) Employed for wages ☐

b) Self employed ☐

c) Retired ☐

d) Student ☐

e) Unemployed ☐

6. If employed/self-employed, what do you do? ____________________________

7. Do you know your blood group before now? Yes No ☐ ☐

8. If yes, what is your blood group?

a) O Rh positve ☐

b) A Rh positve ☐

c) B Rh positve ☐

d) AB Rh positve ☐

e) O Rh Negative ☐

f) A Rh Negatitve ☐

g) B Rh Negative ☐

h) AB Rh Negative ☐

9. Currently or in the past have you ever done work where you had contact with blood or body fluids?☐Yes ☐No If yes, what job was that? ______________

10. What is your present personal Income level per month:

- ☐ Below N18,000
- ☐ N18,000 – Below N35,000
- ☐ N35,000 – Below N70,000
- ☐ N70,000 – N120,000
- ☐ Above N120,000

11. What is your highest educational attainment

a) No formal education ☐
b) Primary ☐
c) Secondary ☐
d) Tertiary ☐
e) Postgraduate ☐

SECTION 2

RISK FACTORS

12. Have you ever heard of Cytomegalovirus (CMV)? Yes ☐ No ☐

If yes, what do you think it causes ? (Check all that apply as appropriate).

a) Birth deffect ☐
b) Congenital infection ☐
c) Congenital malformation ☐
d) Premature delivery ☐
e) Enlarged liver and spleen ☐
f) Microcephaly (small head) ☐
g) Feeding difficulties ☐
h) Malaria ☐
i) Anemia ☐
j) Others…….

13. What type of blood donation are you going to do? (Check all that apply as appropriate).

Voluntary ☐ Family ☐ Commercial ☐ Can't say ☐

14. Please indicate from the list below if you have undergone/have any of the following (Check all that apply as appropriate). If yes, when?

	NO	YES	WHEN (YEAR)
a) Scarification/Tattoo on your body	☐	☐	--------------------
b) Tribal marks	☐	☐	---------------------
c) Received blood transfusion	☐	☐	---------------------

d) Received surgical procedure in health facility ☐ ☐ -------------------

e) Local circumcision ☐ ☐ -------------------

f) Local ‘belubelu’- uvulectomy or/and tonsillectomy ☐ ☐ ------------------

g) Delivery of child at home ☐ ☐ ------------------

h) Dental procedure in health facility ☐ ☐ …………………

i) Dental procedure outside health facility ☐ ☐ -------------------

j) Blood oaths ☐ ☐ ---------------------

k) Body piercing ☐ ☐ --------------------

l) Intravenous drug use (IDU) ☐ ☐ --------------------

m) Living with and Sharing facilities and materials with IDU ☐ ☐ --------------------

n) Sharing of sharp objects ☐ ☐ -------------------

o) Cupping/blood letting (kaho) ☐ ☐ --------------------

p) Organ transplant ☐ ☐ --------------------

q) Parents with CMV infection ☐ ☐ ----------------------

r) Needle stick injury ☐ ☐ ----------------------

s) Exposure to blood, body fluids or tissues ☐ ☐ ----------------------

t) On dialysis treatment ☐ ☐ ----------------------

u) Sharing of toothbrush/chewing stick No ☐ sometimes ☐ always ☐

v) Local hair shaving No ☐ sometimes ☐ always ☐

w) Local manicure/pedicure No ☐ sometimes ☐ always ☐

x) Others ……. ……………………………..(Specify)

SECTION 3

CMV TEST HISTORY

15. Have you ever been tested for CMV? Yes ☐ No ☐ Not sure ☐

If answer is No, go to question 20

16. If yes, when did you get the last CMV Test (Year)…………………..

17. What was the result?

Reactive (Positive) ☐ Non Reactive(Negative) ☐ Not Sure ☐

18. Where was the tests done? (Tick all that applies)

(A) Government Hospital/Medical center ☐

(B) Community screening exercise ☐

(C) Private Hospital/Laboratory ☐

(D) Commercial mobile test ☐

(E) In school ☐

(F) Self Test/Home ☐

19. What was the reason for the testing? (Tick all that applies)

- Screening for blood transfusion ☐
- Pre-marital screening ☐
- Ante-natal screening ☐
- Medical examination for health/fitness/travel/job ☐
- Clinical diagnosis ☐
- For research ☐
- Dialysis ☐
- Surgery ☐
- Others ………………………….. (specify)

VACCINATION HISTORY

20. Have you ever received CMV vaccine?

Yes ☐ No ☐ Not Sure ☐

21. If yes, which year did you receive the vaccine (year)? …………

22. How many doses of the vaccine did you receive?

- One dose ☐
- Two doses ☐
- Three doses ☐
- More than 3 doses ☐
- Not sure ☐

23. Where did you receive the vaccine(s)?

- Ministry of Health Immunization centre ☐
- Health centre ☐
- Government hospital ☐
- Private Health Facilities ☐
- Community immunization center ☐
- Others ___________________(Specify)

SECTION 4

Household information

24. Type of marriage

Monogamous ☐ Polygamous ☐

25. If Polygamous; how many wives are in your household_____________

26. Have you had a sexual partner who was/is not your spouse(s) or your regular partner within the last 6 months? Yes ☐ No ☐ Not sure ☐

27. If yes, how many were they?___________________

28. How regularly do you use condom when having sex with someone who isn't your spouse or regular partner? Always ☐ Sometimes ☐ Not at all ☐

Phone contact: ____________________

Participant's name ………………………………………

____

Questionnaire administered by ___________________________________

Code Label written on Blood Sample: ________________________

Appendix 4

KII Guide

QUESTIONAIRE FOR THESIS RESEARCH ON THE EFFECT OF CYTOMEGALOVIRUS (CMV) IN DETERMINING BLOOD DONOR SAFETY IN MINNA, NORTH CENTRAL NIGERIA, 2013

KII GUIDE NO…………..

Date…………………..

Good day. My name is Mr. Musa K. Bawa, a Resident Laboratory Epidemiologist with the Nigerian Field Epidemiology and Laboratory Training program (N-FELTP). We are conducting a study titled: The Effect of Cytomegalovirus (CMV) in Determining Blood Donor Safety in Minna, Nortn Central Nigeria to find out proportion of blood donors infected with the virus and identify possible means of transmission of infection, create awareness as well as advocacy for effective prevention of the disease. You are not compelled to participate, however we would be grateful if you could kindly oblige us a few minutes of your time. All information supplied would be treated as strictly confidential.

.

Name of respondent ____

Address/location information _______________________________________

Name of the LGA ____

Name of State____

Name of interviewer____

Date of Interview____

SECTION 1

DEMOGRAPHIC DATA

4. Age in years at last birthday: ____________________
5. Sex: Male ☐ Female ☐
6. Marital status (Tick whichever applies)
   a) Single ☐
   b) Married ☐
   c) Separated ☐
   d) Divorced ☐
   e) Widowed ☐
   f) Others (specify)________________________

4. TRIBE: ______________________________

5. OCCUPATION:

a) Employed for wages ☐

b) Self employed ☐

c) Retired ☐

d) Student ☐

e) Unemployed ☐

f) Pupil ☐

g) Below School age ☐

6. If employed/self-employed, what do you do? ____________________________

7. Currently or in the past have you ever done work where you had contact with blood or body fluids?☐Yes ☐ No If yes, what job was that? ______________

8. What is your present personal Income level per month:

- ☐ Below N18,000
- ☐ N18,000 – Below N35,000
- ☐ N35,000 – Below N70,000
- ☐ N70,000 – N120,000
- ☐ Above N120,000

9. What is your highest educational attainment

a) No formal education ☐
b) Primary ☐
c) Secondary ☐
d) Tertiary ☐
e) Postgraduate ☐

CYTOMEGALOVIRUS UNDERSTANDING

What are the most significant health problems associated with blood transfusion in your community? (*Record all answers and list the most common ones.)*

_______________ __________________ ___________________

(If Cytomegalovirus not mentioned)

Is Cytomegalovirus (*use local name*) a significant health problem here in (*name of community*)?"

If yes, why? ____

If no, why not? ____

Who in your community is at risk for getting Cytomegalovirus?

Do you think blood transfusion recipients are at risk from Cytomegalovirus?

Yes____ No ____

If yes, (Please mention all information told by the respondent)

____

Do you know what causes Cytomegalovirus?

What are the best ways to protect against Cytomegalovirus?

Responses

1. ____

2. ____

3. ____

Do you know anyone in your community that had Cytomegalovirus and has a child with congenital problems as result?______________________

Do you know anyone in your community that had Cytomegalovirus and has been confirmed positive? ______________________

If yes, where ______________________

CMV KNOWLEDGE

Have you ever heard of Cytomegalovirus (CMV)? Yes____ No____

Do you support the idea that intending blood donors should be screened from CMV?

If yes, why? ____

If no, why not? ____

Are you aware that CMV can be transmitted through blood transfusion?

If yes, from where? ____

TANSFUSION AND OTHER RISK FACTORS

What do you understand by transfussion____

Have you ever donated blood? _____________

If yes, where you screened for CMV? Yes _______ No _____ Don't know_______

What are the other factors you think can contribute to the transmission of CMV

Tick all that apply as mentioned by the respondent

a) Scarification/Tattoo on your body
b) Tribal marks
c) Received blood transfusion
d) Received surgical procedure in health facility
e) Local circumcision
f) Local 'belubelu'- uvulectomy or/and tonsillectomy
g) Delivery of child at home
h) Dental procedure   in health facility

i) Dental procedure outside health facility

j) Blood oaths

k) Body piercing

l) Intravenous drug use (IDU)

m) Living with and Sharing facilities and materials with IDU

n) Sharing of sharp objects

o) Cupping/blood letting (kaho)

p) Liver transplant

q) Parents with Hepatitis infection

r) Needle stick injury

s) Exposure to blood, body fluids or tissues

t) On dialysis treatment

u) Sharing of toothbrush/chewing stick

v) Local hair shaving

w) Local manicure/pedicure

x) Others ……. ……………………………..(Specify)

COMMENTS

What is your general comment on CMV among blood donors and ways to go about prevention?

What would you suggest to make the preventive measures a huge success?

Are you aware of any plan in the country to include CMV screening among donor screening algorithm?

Do you think that's a good idea?

Yes ____ No ____

If No, why not ____________________________

Interviewer's comments

Appendix 5

Map of Nigeria highlighting Niger State and map of Niger state highlighting Minna metropolis

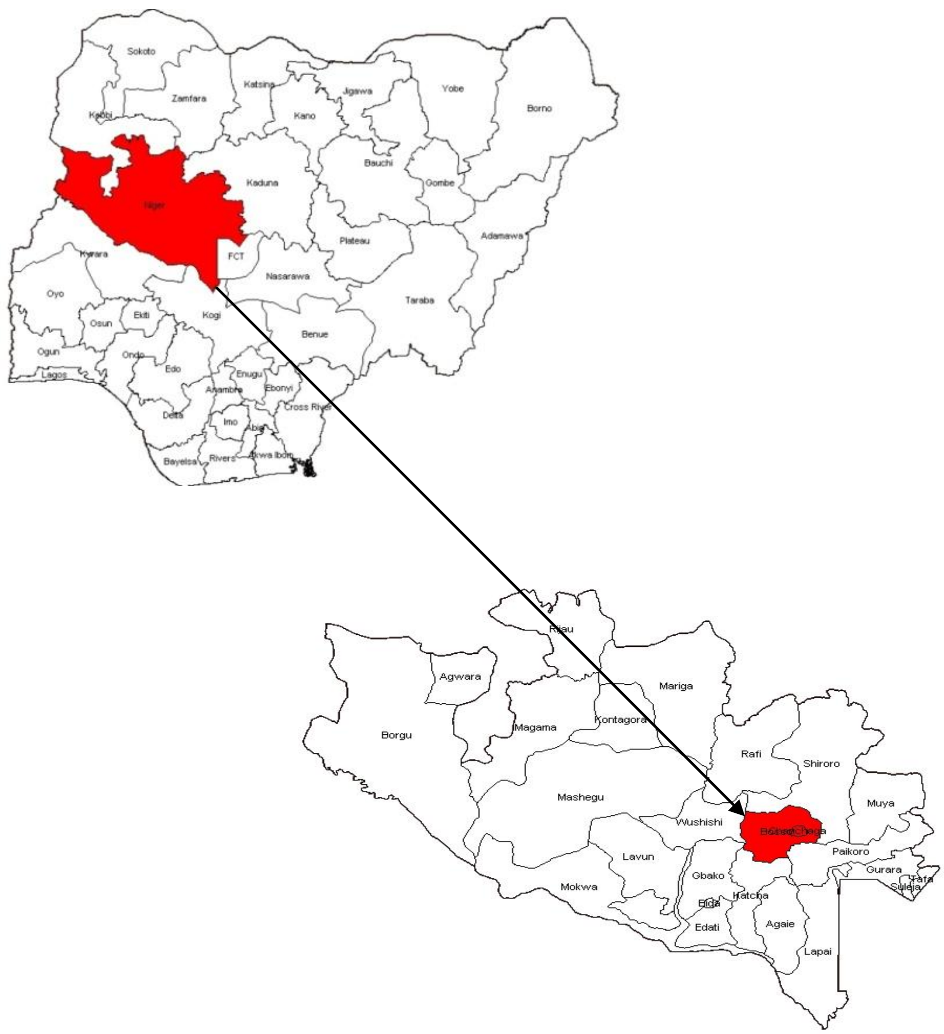

Fig: 3.1: Map of Nigeria highlighting Niger State and map of Niger state highlighting Minna metropolis

Appendix 6

Procedure for ELISA technique using ELISA kit (DIALAB® Austria) and *Emax* ELISA plate reader, E11865 model (*Molecular Devices*® USA)

1. All reagents were allowed to reach room temperature for 15 minutes before use.
2. A 1:40 dilution of Wash Buffer Concentrate with distilled water was prepared.
3. Sample diluent (100 ul, 92 drops) was added into the appropriate wells except the blank well, negative well (one well each for blank and positive control well and 2 wells for negative control)
4. 10ul specimen was added to the well, beating by pipettor repeatedly until liquid turn blue, 50ul of negative and positive control were dispensed into the negative and positive well separately. No liquid was added to the blank control well.
5. Microtiter wells were flicked for 30 seconds and allowed to mix well. The plate was affixed to sealing template and incubated at 37^0C for 20 minutes.
6. The plate was then taken out wash Buffer added to each well and absorbed after 20 seconds. It was repeated 5 times until each well was dried.
7. 1 drop (50ul) of HRP conjugate was dispensed to each well except the blank well. Gently vibration mixtured, mixed well, affixed to sealing template. Then incubated at 37^0C for 20 minutes
8. Wash Buffer was added to each well and absorbed after 20 seconds. It was repeated 5 times until each well was dried.
9. 1 drop (50ul) of Substrate A and 1 drop (30ul) of Substrate B were dispensed into every well except blank well. Incubated at 37^0C for 10 minutes. Gently vibration mixtured, mixed well and incubated at 37^0C for 10 Minutes.
10. Then, the plate was taken out, 1 drop (50ul) of Stop solution added except the blank well, mixed well, and then read with a microwell reader for the result.

Appendix 7

Work plan

S/N	TASK TO BE PERFORMED	2013 J F M A M J J A S O N D	2014 J F M A
1	Finalize research proposal and submit for clearance.		
2	Typing of questionnaires, print and multiply		
3	Identify research assistants.		
4	(After obtaining clearance), inform DMS and Health institutions where tests will be carried out.		
5	Train research assistants		
6	Pre test study and finalize procedure/ tools.		
7	Collection of data.		
8	Process data + make preliminary interpretation.		
S/N	TASK TO BE PERFORMED	2013 J F M A M J J A S O N D	2014 J F M A
9	Analysis data + write report.		
10	Feedback, discuss and disseminate research finding and recommendations to policy makers, staff and community members		
11	Draft preliminary plan of action.		
12	Meet with policy makers to discuss action plan for implementing the recommendation.		
13	Followed up implementation		

.

Appendix 8

Ethical Clearance

NIGER STATE HOSPITALS MANAGEMENT BOARD

GENERAL HOSPITAL, MINNA

ADDRESS:
No. 1 Hospital Road,
P.M.B. 2 Minna
Niger State, Nigeria.
Tel: 08169999223

E-Mail: genhosp.minna@yahoo.com
Website:www.generalhospitalminna.com

The Head of Department

Community Medicine

Faculty of Medicine

Ahmadu Bello University

Zaria,

Kaduna State.

8th October, 2013

Through: MUSA KALAMULLAH BAWA

(Nigerian Field Epidemiology and Laboratory Training Program-NFELTP, ABUJA)

SIR,

RE: MUSA KALAMULLAH BAWA MPH/MED/313658/11-12

RESEARCH, ETHICS AND PUBLICATION COMMITTEE'S APPROVAL TO CONDUCT RESEARCH

The bearer, a student of your department sought for permission to conduct research on "**The effect of Cytomegalovirus (CMV) in Determining Blood Donor Safety in General Hospital Minna, Niger State**"

The committee after going through his proposal has given him the approval to conduct the research.

The copy of his final finding must be submitted to the committee as a requisite to this approval.

Thank you for your cooperation

Dr Ndagi Umar
(B pharm. M pharm.pharm.D)
Secretary REPC
For: chairman REPC
CC: medical Director

GOVERNMENT OF NIGER STATE
IBRAHIM BADAMASI BABANGIDA SPECIALIST HOSPITAL
MINNA

Dr. Umar Isah A.
Chief Medical Director

Dr. H.I. Gbodati
Director Clinical Services

KM 10, Paiko Road,
Chanchaga P.M.B. 169
Minna, Niger State, Nigeria.

Our Ref: IBBSH/SUB/ 549/I Your Ref: Date: 21/07/2014

The Programme Director
Nigeria Field Epidemiology
And laboratory training program

ETHICAL APPROVAL TO CONDUCT RESEARCH ON THE EFFECT OF CYTOMEGALOVIRUS IN DETERMINING DONOR SAFETY IN MINNA

With reference to your letter dated 16th August, 2013 requesting for ethical approval on above subject matter. The committee after critical review of the proposal has granted him approval to conduct the research.

As part of the hospital policy, a copy of the final thesis should be submitted to the committee.

Thank you.

Dr. Umar Isah A (FWACP)
Consultant Nephrologist
Chairman, ethics committee

Tel: 07032415295, 08166558113, 0806569839, 08063802858 e-mail: ibbsh-ng@yahoo.com