CYTOMEGALOVIRUS INFECTION AND RECURRENT PREGNANCY LOSS IN JOS, PLATEAU STATE.

A DISSERTATION SUBMITTED TO THE NATIONAL POSTGRADUATE MEDICAL COLLEGE OF NIGERIA IN PART FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE FELLOWSHIP OF THE COLLEGE

BY

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MAY 2018.
DECLARATION PAGE

I, Dr Akunaeziri Uche Augustine hereby declare that this work is original. The work has not been submitted in support of an application for a fellowship/degree/diploma of this or any other institution of learning. It has also not been submitted for publication/conference presentation.

Signature: ..........................................................

Date: 23/01/2018 .................................................................
CERTIFICATION

This dissertation titled "CYTOMEGALOVIRUS INFECTION AND RECURRENT PREGNANCY LOSS IN JOS, PLATEAU STATE" was carried out by Dr. AKUNAEZIRI UCHE AUGUSTINE under the supervision of the signed Consultants in the Department of Obstetrics and Gynaecology of the Jos University Teaching Hospital (JUTH).

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RE: ETHICAL CLEARANCE/APPROVAL

I am directed to refer to your application dated 18th March, 2016 on the research proposal titled:

“Cytomegalovirus Infection and Recurrent Miscarriage in Jos, Plateau State”

Following recommendation from the Institutional Health Research Ethics Committee, I am to inform you that Management has given approval for you to proceed on your research topic as indicated.

You are however required to obtain a separate approval for use of patients and facilities from the department(s) you intend to use for your research.

The Principal Investigator is required to send a progress report to the Ethical Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Submission of final research work should be made to the Institutional Health Research Ethical Committee through the Secretary, Administration Department, please.

On behalf of the Management of this Hospital, I wish you a successful research outing.

Hajia R. Danfillo
For: Chairman, MAC
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Date of meeting when final determination of research was made: September 09, 2016.

This is to inform you that the research described in the submitted protocol, has been reviewed and given expedited approval by the Health Research Ethics Committee.

This approval dates from 09/09/2016 to 09/03/2017. Note that no participant accrual or activity related to this research may be conducted outside of these dates. You may liaise with the Hospital records department for necessary co-operation / assistance.

All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study. In multiyear research, endeavor to submit annual report to the HREC early in order to obtain renewal of your approval and avoid disruption of research. The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit your research site without previous notification.

Dr. Bitrus Matawal, MBBS, FWACS
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Our Ref: FAFEC/08/34/5

Date: 19th October, 2016

NOTICE OF FULL APPROVAL OF PROTOCOL FOR PERMISSION TO CONDUCT A RESEARCH ON
"CYTOMEGALOVIRUS INFECTION AND RECURRENT PREGNANCY LOSS IN JOS, PLATEAU STATE"

**Faith ALIVE FOUNDATION (FAF) & HOSPITAL HEALTH RESEARCH ETHICS COMMITTEE REG. NUMBER:**

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This is to inform you that the Research described in the submitted protocol and as stated above with accompanying proposal from Dr. Akunaeziri Uche Augustine has been reviewed and given full approval by the FAF Health Research Ethics Committee. (FAFHREC)

If there is delay in starting the research, please inform the FAFHREC so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside this period except with additional approval and in case of multi-year research, endeavour to submit your annual report to the FAF HREC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the FAFHREC. No changes are permitted in the research without prior approval by the FAFHREC except in circumstances outlined in the Code. The FAFHREC reserves the right to conduct compliance visit to your research site without prior notification.

Signed: Adeyani
Secretary FAF HREC

**Come over...and help us. Acts 16:9**

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LIST OF ABBREVIATIONS

ACOG  American College of Obstetrics and Gynecology
CDC  Centre for Disease Control
°C  Degree Celsius
CMV  Cytomegalovirus
DRG  Diagnosis – related Group
ELISA  Enzyme Linked Immunosorbent-Assay
HIG  High titre CMV immunoglobulin
IgG  Immunoglobulin G
IgM  Immunoglobulin M
JUTH  Jos University Teaching Hospital
mmHg  Millimetre mercury
nm  Nanometers
OD  Optical density
PCR  Polymerase Chain Reaction
%  Percentage
RCOG  Royal College Of Obstetrics and Gynaecology
WHO  World Health Organization
ABSTRACT

BACKGROUND: The foetal consequences of CMV infection have made it one of the most serious infections contracted during pregnancy. Recurrent pregnancy loss is a challenging problem for the obstetrician. Human cytomegalovirus is a major cause of congenital infection and has been implicated as a cause of pregnancy loss. Knowledge about the magnitude of this problem in our locality will help in developing methods of prevention of this infection which will eventually improve obstetric outcome.

OBJECTIVE: The objective was to compare the prevalence of CMV infection among women with recurrent pregnancy loss and normal postnatal women.

DESIGN: It was a case control study.

METHODOLOGY: The study involved 42 women presenting with recurrent miscarriage and 42 postpartum women with no adverse obstetric outcome. Subjects were recruited from JUTH, Plateau State Specialist Hospital and Faith Alive Hospital. A semi-structured researcher administered questionnaire was used to obtain socio-demographic information. Blood samples were collected from the respondents and cytomegalovirus antibodies (IgG and IgM) were assayed for in both groups. Data was analysed using Statistical Package for Social Sciences version 22. Test of association was done using t test, Fisher Exact test and chi square.

RESULTS/CONCLUSION: The seroprevalence of CMV IgG among women with recurrent miscarriage and normal postpartum women was 85.7% and 76.2% respectively. There was no significant association between CMV infection and recurrent...
miscarriage. An incidental finding was that the level of awareness of the respondents about CMV was very poor (4.8%). Due to the high seroprevalence of CMV in the study, all pregnant women should be educated about CMV and the methods of prevention of CMV infection. There’s however insufficient evidence from this study linking CMV infection and recurrent miscarriage.

**KEY WORDS:** Recurrent Pregnancy loss, cytomegalovirus, cytomegalovirus antibody, immunoglobulin G(IgG), immunoglobulin M(IgM).
CHAPTER ONE

1.0 INTRODUCTION

Human cytomegalovirus (CMV) is a member of the family herpesviridae and belong to the subfamily beta-herpesviridae. CMV has worldwide distribution and infects humans of all ages and socioeconomic group, with no seasonal or epidemic patterns of transmission.\(^1\) Spontaneous pregnancy loss occurs commonly. Many pregnancies fail prior to being clinically recognised and approximately 15% of all clinically recognised pregnancies result in spontaneous loss.\(^2\) Spontaneous pregnancy loss is a frustrating experience and can be physically and emotionally taxing for couples especially when faced with recurrent losses.\(^3\)

Recurrent pregnancy loss (RPL), also referred to as recurrent miscarriage or habitual abortion is defined as the loss of three or more consecutive pregnancies prior to the age of viability.\(^3,4\) Based on the incidence of sporadic pregnancy loss, the incidence of recurrent pregnancy loss should be approximately 1 in 300 pregnancies. However, epidemiologic studies have revealed that 1% to 2% of women experience recurrent pregnancy loss.\(^3\) It is important to note that the best available data suggests that the risk of miscarriage in subsequent pregnancies is 30% after 2 losses, compared with 33% after 3 losses among patients without a history of a live birth.\(^5\) This strongly suggests the need to evaluate patients with 2 pregnancy losses with no prior live births. It may be prudent to also evaluate the following patients earlier: if foetal cardiac activity was identified prior to the pregnancy loss, a woman older than 35 years, or a couple having difficulty in conceiving.\(^2\)
Recurrent miscarriage is a heterogeneous condition that has many possible causes; more than one contributory factor may underlie the recurrent pregnancy losses. There are different causes of pregnancy loss and these includes chromosomal, genetic, hormonal, anatomic, systemic, immunologic, infections and endocrine. Certain infections including listeria monocytogenes, toxoplasma gondii, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackie virus, are known or suspected to play a role in sporadic spontaneous pregnancy loss. However, the role of infectious agents in recurrent loss is unclear. Available reports on the role of CMV infection in recurrent pregnancy loss showed conflicting results. Some studies showed higher prevalence while others showed comparable and even less prevalence of antibodies to CMV among women with recurrent pregnancy loss compared to normal pregnant women.

CMV is the most frequent cause of congenital infection in humans. Infection with human cytomegalovirus is very common worldwide with seropositivity rates ranging from 40% in developed countries up to 100% in developing countries. About 10 to 20 percent of infected infants may suffer sensorineural hearing loss, ocular damage or impairment of cognitive and motor function. The common modes of infection with CMV are through saliva, urine, stool, breast milk, unscreened blood transfusion, cervical secretions and semen. For most healthy people who acquire CMV infection after birth or through blood transfusion, there are few symptoms and no long term sequelae. Therefore, for the vast majority of individuals CMV infection is innocuous. However foetal damage is more likely to be severe when maternal infection occurs early in pregnancy because of their immunocompromised
state and the risk of infection to the foetus whose immune system is not fully developed.\textsuperscript{3, 7}

The major risk factor for maternal acquisition of CMV during pregnancy is frequent and prolonged contact with a child less than three years of age.\textsuperscript{8, 9} This occurs among women with a child in the home or among women employed in child care centres or schools.\textsuperscript{10, 11} Another group of high risk women are those who are seronegative, young and poor. Even for this group, contact with a young child is an independent predictor of delivering a CMV congenitally infected infant, as is a history of frequent sexual activity.\textsuperscript{9} A recent study suggested that CMV is likely transmitted not only via the oral mucosa route, but also via the vaginal mucosal route.\textsuperscript{8}

Cytomegalovirus is mainly a problem for certain high risk groups which include unborn babies whose mothers become infected with CMV during 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.\textsuperscript{12} More children suffer disabilities caused by congenital CMV than by several better known childhood maladies such as Down’s syndrome or foetal alcohol syndrome.\textsuperscript{12} Each year about 1 in 150 babies are born with congenital CMV infection and about 8000 children develop lasting disabilities caused by congenital infection.\textsuperscript{12}

Owing to the high seroprevalence of CMV among pregnant women from recent studies\textsuperscript{12}, and the fact that some studies found high presence of CMV antigens in tissues from abortion\textsuperscript{13}, this study is aimed at determining the prevalence of CMV
infection among patients with recurrent pregnancy loss and also if there is an association between CMV infection and recurrent pregnancy loss in our environment.

The literature review was done by searching for local and international articles relevant to contemporary obstetrics and gynaecology. The key search words and phrases used include: Abortion, cytomegalovirus infection, Recurrent pregnancy loss, CMV infection and miscarriage, CMV infection and recurrent pregnancy loss, pathologic effects of CMV infection on pregnancy, CMV infection and bad obstetric history, TORCH infection and pregnancy loss, TORCH infection and pregnancy outcome, CMV and thromboembolism, CMV infection and placental insufficiency, Seroprevalence of CMV among women with recurrent miscarriage, CMV and endometritis.

Sources searched include: PubMed, HINARI, Google, Google scholar, Medline, RCOG guideline, omni, Medscape, Scopus, Publisher

Articles that were not directly related to CMV and pregnancy loss were screened out. Also some old articles were excluded.

1.1 **Justification**

Pregnancy loss is the most common complication of a pregnancy with devastating consequence for the woman and her family. It is even more frustrating when it is recurrent. For this reason, patients that present with recurrent miscarriages need to be evaluated to identify the probable causes. Viral infections during pregnancy carry a risk for intrauterine transmission which may result in miscarriage. Cytomegalovirus infection
is one of the infectious causes of pregnancy loss and its association with recurrent miscarriages has not been studied in Jos.

Previous studies aimed at determining the seroprevalence of CMV among prospective blood donors\textsuperscript{14} have been carried out in JUTH but none has tried to determine the seroprevalence of CMV infection among patients with recurrent pregnancy loss and whether CMV infection is an established cause of recurrent abortion. Other studies done in Nigeria tried to determine the seroprevalence of CMV infection among pregnant women.\textsuperscript{12, 15, 16} Due to the high seroprevalence of CMV among pregnant women and the possible link between CMV infection and recurrent miscarriages, screening for CMV infection may be considered in the management of patients with recurrent pregnancy loss.
CHAPTER TWO
LITERATURE REVIEW

2.0 Recurrent pregnancy loss and causes

Recurrent pregnancy loss (RPL), usually referred to as three or more consecutive abortions, is one of the most frustrating and difficult areas in reproductive medicine. The aetiology of recurrent pregnancy loss is still unclear and few evidence-based diagnostics and treatment approaches are available. Aetiological factors associated with recurrent pregnancy loss include anatomical, immunological, genetic, endocrine, infectious, thrombophilic, and environmental factors.

Human cytomegalovirus infection is the most common congenital viral infection worldwide and may be asymptomatic (in 90% of cases) or cause severe damage and, in rare cases, abortion.

2.1 Cytomegalovirus infection and pregnancy loss

Cytomegalovirus is a common virus that infects most people at sometime during their lives. It is a significant cause of morbidity and mortality in pregnancy, and among immunocompromised patients like recipients of organ transplants. There are many studies about the association between CMV infection and pregnancy loss. Some of them showed that human cytomegalovirus can result in abortion or still birth. A study in Sudan showed a significant association between CMV infection and frequency of abortion, age and congenital malformation in children. The study also found the
A study done in Egypt showed significant statistical correlation between positive human cytomegalovirus immunostaining of maternal endothelial lining, glandular epithelial, decidual cells and chorionic villi with necrosis and inflammation involving chorionic villi and decidua. The study indicated that in early pregnancy, cytomegalovirus can be transmitted from the decidual glands to the foetus. A seven-year prospective study of primary cytomegalovirus infection during pregnancy showed that cytomegalovirus affected approximately twice as many pregnant women as did rubella virus and foetal loss occurred in 15% of cases of early CMV infections which was seven-fold higher than the rate found in controls. Babies that were delivered were all normal at birth but two have so far developed definite intellectual impairment attributable to CMV infection. The mothers of both of these cases were infected after the foetus had become legally viable. A study conducted in Japan in which screening for vaginal shedding of CMV in healthy women using real-time PCR was done, showed that the miscarriage rates in healthy women with vaginal shedding of CMV was significantly higher than those in comparable women without CMV infection. The CMV positive women were almost seven times more likely to have a miscarriage than were CMV negative women. Previous studies conducted in London and Iran revealed statistically significant association between CMV infection and ethnic group and a high seroprevalence of CMV IgG antibodies among women with spontaneous abortion. A study conducted in South Iran showed significant association between CMV and spontaneous abortion.
Studies that have been carried out in the past to establish if there is an association between cytomegalovirus infection and recurrent pregnancy loss have produced conflicting results. A study on the diagnostic value of anti CMV antibodies in the evaluation of patients with recurrent abortions conducted in Poland showed that most of the studied women manifested presence of serum IgG class anti CMV antibodies and levels of the antibodies proved significantly higher in women following spontaneous abortions. The result suggests that in majority of the studied women, the spontaneous abortion might have resulted from foetal infection due to reactivation of chronic CMV infection in the course of pregnancy.\textsuperscript{28} The findings from this study were not in keeping with the findings from a study that assessed the immunity to cytomegalovirus in women with unexplained recurrent spontaneous abortion. A significantly lower prevalence of serum anti CMV antibodies was observed in women with recurrent spontaneous abortion compared to either their male partners or age-matched female controls.\textsuperscript{29}

2.2 \textbf{Prevalence of cytomegalovirus infection}

Different studies in Nigeria have shown a high prevalence of CMV infection among pregnant women. A study conducted in Lagos reported a prevalence of 97.2\%.\textsuperscript{30} Other serological surveys conducted in Kaduna, Sokoto and Bida found a seroprevalence of 94.8\%, 98.7\% and 84.2\% respectively.\textsuperscript{12, 15, 16} A similar study in Benue showed a lower seroprevalence of 54.30\%.\textsuperscript{31} An overall rate of 87\% of anti-CMV IgG ELISA antibodies in pregnant women was reported in Singapore and 100\% in Thailand.\textsuperscript{32} CMV infection is more prevalent in developing countries. This was reported as early as 1973 when it was reported in a
study that CMV antibodies are more prevalent in developing countries and areas of lower socioeconomic conditions in comparison to developed countries. An ethnically diverse study conducted in London showed a prevalence in Asian women at 88.2%, 77.2% in black women and 45.9% in white women. The rate of seropositivity of anti-CMV immunoglobulin (IgG) enzyme linked immunosorbent assay (ELISA) antibodies of pregnant women in Turkey was reported to be 98.5% and 84% in Spain. These rates are much higher than the typical European rate but similar to the rate obtained amongst black pregnant women. High rates of congenital CMV infection have been consistently demonstrated in populations with high CMV seroprevalence. This is because the incidence of congenital CMV infection depends on epidemiological characteristics of a population, in particular the maternal CMV seroprevalence.

2.3 Possible pathophysiological mechanisms of association of cytomegalovirus infection and pregnancy loss.

Embryo-foetal infections have been reported to cause recurrent spontaneous abortions. The proposed mechanisms for infectious causes of pregnancy loss include direct infection of the foetus or placenta, placental insufficiency, chorioamnionitis, endometritis, endocervicitis, altered immune response and toxic metabolic by-products.

2.3.1 Direct infection of the foetus or placenta:

Cytomegalovirus during pregnancy can reach the placenta by viraemia, following both primary and recurrent infection, or by ascending route from the cervix, mostly following
reactivation.\textsuperscript{38} A study on the correlates of placental viral infection with cytomegalovirus, parvovirus B\textsubscript{19} and Human Herpes Virus 7 showed that the major pathogen detected in all cases of placental infection associated with foetal death was human CMV.\textsuperscript{39} Human CMV replication in cervico-vaginal secretion can lead to placental CMV infection, and high CMV DNA loads in cervico-vaginal secretion and placental villi/decidua are associated with miscarriage.\textsuperscript{40}

2.3.2 Placental insufficiency:

Studies have attributed pregnancy loss to thrombosis of the utero-placental vasculature. There is however implantation problems as an important event.\textsuperscript{41} Cytomegalovirus has been found to be associated with thrombosis and various mechanisms have been proposed.\textsuperscript{42} IE84 a gene product of CMV binds to and inhibits P53 mediated apoptosis increasing smooth muscle proliferation and increased risk of thromboembolism. Other mechanisms involve CMV enhanced platelets and leucocyte adhesions to infected endothelial cells, effects mediated by activating factor X, factor VIII and triggering thrombin generation. The most accepted theory supported by several in-vitro studies conclude that CMV transiently induces production of anti-phospholipid antibodies which is a well-known risk factor for venous and arterial thromboembolism.\textsuperscript{42} The enhanced platelet and leucocyte adhesion to endothelial cells occurs following the direct invasion of vascular endothelial cells by the cytomegalovirus. The vascular endothelial cells are activated which leads to the increased expression of adhesion molecules. These adhesion molecules then react with platelets and leucocytes to cause a hypercoagulable state. Definitive conclusions, however, cannot be drawn concerning the effects of
human cytomegalovirus infection on the coagulation system, although the in-vitro studies are convincing and offer insight in the pathogenesis. Some studies found that the primary effect of antibodies is most likely on the placenta which, during a primary CMV infection in the mother, becomes dysfunctional and results in poor oxygenation and nourishment of the foetus in utero. Thus, many symptoms of congenital CMV infection that are present at birth may not be due to any direct effect of the virus on the foetus but rather to the infection of the placenta which impairs its capacity to provide oxygen and nutrition to the developing foetus.

2.3.3 Chorioamnionitis
This has been suggested by some studies.

2.3.4 Chronic endometritis and endocervicitis
The association between early gestational wastage and cytomegalovirus endometritis has been documented in recent tissue culture studies without the morphologic demonstration of the virus. Involvement of the endocervix by cytomegalovirus has been demonstrated histologically.

2.3.5 Altered immune response:
It has been suggested that cytomegalovirus brings about alteration in immune response. A study conducted in Japan suggested that CMV infection may cause excessive immune reactions between the mother and the foetus.

2.3.6 Toxic metabolic by-products:
Endotoxin, exotoxin or cytokines could have a direct effect on the uterus or the foetoplacental unit.
2.4 Pathogenesis of cytomegalovirus infection

CMV is acquired early in life and transmission can be vertical and horizontal. Infection can be classified as congenital if acquired before birth, perinatal at the time of delivery or as post natal if acquired later in life. Horizontal transmission is more common and most infections are acquired when there is direct close contact with individuals (especially young children less than 3 years of age) who are shedding the virus in body fluids such as saliva or urine. Other routes of transmission include blood transfusion, sexual intercourse, bone marrow transplant and solid organ transplant. Infected individuals are usually asymptomatic but once a person becomes infected, the virus remains latent with the possibility of reactivation later in life if immunosuppression occurs. The infection is associated with some obstetric complications.

2.5 Clinical features of cytomegalovirus infection

About 40 - 60% of pregnant women are susceptible to CMV infection at conception. Of these, 1-4% will acquire CMV during pregnancy (seroconversion) and about 40-50% of infected women will transmit the virus to the foetus. Transmission rate is lowest in the first trimester (about 35%), and as pregnancy progresses, the transmission rate increases to 73% for women who acquire CMV infections in the third trimester. Of infants infected in utero, about 33% of them will have symptoms or develop severe neural impairment. This neonatal disease rate is probably highest for children of women who have had a primary infection in the first half of pregnancy, but no definite data supports this.
The role of maternal immunity to CMV prior to conception is not clear. Infants born of mothers with immunity prior to conception not only give birth to infected infants but also occasionally give birth to infants with symptoms at birth that may develop long term sequelae like hearing deficit.\textsuperscript{50} It is however an established fact that the rate of congenital infection among women with preconception immunity is only between 0.5% and 2% as compared to an average of 40-50% in women who have seroconverted during pregnancy. A study showed that 3% of pregnant women that were seronegative at or before conception had congenitally infected infants compared with 1% for women seropositive before conception.\textsuperscript{45} Another study showed that the most severe infant sequelae occurred only among women with primary CMV infection during pregnancy.\textsuperscript{51} Infant hearing loss was observed in women who had a recurring infection but this was much less when compared to the children born of mothers without preconception immunity.\textsuperscript{50} The focus of serologic screening during pregnancy is to eliminate or reduce the morbidity associated with primary CMV infection.\textsuperscript{45} About 90% of congenitally infected infants are asymptomatic at birth, 5-17% of who develops symptoms such as sensorineural hearing loss, chorioretinitis or neurologic deficits usually during the first two years of life.\textsuperscript{52} Among 10% of symptomatic newborns, 20% die and 90% of survivors develop severe sequelae.\textsuperscript{52} Irrespective of the number of babies affected, CMV embryopathy (sensorineural hearing loss, chorioretinitis, mental retardation and fetal death) should be a major concern for public health.\textsuperscript{53} Abortion can also occur following CMV infection.\textsuperscript{19}
2.6 Diagnosis of cytomegalovirus infection

The gold standard of serologic diagnosis is maternal seroconversion based on the detection of IgG antibodies to CMV, i.e. the de novo appearance of virus-specific IgG in the serum of a pregnant woman who was previously seronegative. Such an approach is feasible only when seronegative women are identified and prospectively monitored. The IgG assay is nearly 100% sensitive and specific, readily available, and automated for high volume capacities in developed countries. In the absence of a universal serial serologic screening of pregnant women, diagnosis via seroconversion is seldom achieved since an initial seronegative serum is rarely available. IgM detection in a pregnant woman is likely to be a reliable marker of primary infection; as it can reveal various clinical situations as related with the acute phase of primary infection, convalescent phase of primary infection or persistence of IgM antibody. In pregnant women detection of IgM antibody may be related to a primary infection occurring during pregnancy when the IgM titre falls sharply in sequential blood samples. However the detection of IgM antibodies in maternal sera has some problems; although IgM antibodies to CMV occur in all primary infections, they may also occur after reactivations or reinfections and the assay has a high false positive rate. It has been observed that IgM usually peaks 3-6 months after a primary infection but may remain present in serum for over 12 months. Hence, finding IgM to CMV in a single serum of a pregnant woman does not alone establish a recent primary CMV infection during pregnancy.
Antibody avidity, which is an indirect measure of the tightness of antibody binding to its target antigen, increases in the first weeks after a primary infection. Low avidity IgG antibodies to CMV persist for up to 20 weeks after a primary CMV infection. These low avidity antibodies are then replaced by high avidity antibodies (>60% binding in presence of 5M urea). Currently, the combination of the presence of anti-CMV IgM antibodies and low avidity anti IgG antibodies along with maternal or foetal symptoms is used for the diagnosis of a primary maternal infection.

The preferred method for diagnosing congenital CMV is via PCR identification of CMV in amniotic fluid. Sensitivities of PCR range from 70-100%. One of the first studies observed that amniocentesis correctly identified 12 of 13 (92%) infants with congenital CMV infection. A subsequent study observed that amniocentesis was 100% sensitive in diagnosing congenital CMV infection. Data suggests that sensitivities are higher if the testing is performed after 21 weeks’ gestation and after a six-week lag time between maternal infection and the procedure. This period allows sufficient time for the virus to infect the placenta and foetus with subsequent replication of the virus in the foetal kidney followed by excretion into the amniotic fluid. Therefore if an amniocentesis is performed soon after infection and returns negative, the procedure should be repeated later in pregnancy.

Viral culture can also be used to diagnose congenital CMV infection. A more recent study observed that viral culture of amniocentesis was 77% sensitive in detecting congenital CMV infection and the specificity was 100%. False negative results in some studies are probably due to infants becoming infected in utero after the amniotic fluid
sampling. False positive results are rare and when they occur may be due to maternal contamination of amniotic fluid. For maximal accuracy, both viral culture and PCR should be obtained. A diagnosis of foetal CMV infection alone is not sufficient to predict new born disease. A large amount of virus as measured by PCR in the amniotic fluid is most likely related to gestational age and should not be used as an independent predictor of a poor foetal outcome.\textsuperscript{63, 64}

2.7 **Pathological diagnosis of CMV infection in abortuses and still births**

The diagnosis of CMV infection in abortus and still births can be done by histologic analysis to identify histopathologic changes associated with CMV in tissues, detection of CMV DNA using polymerase chain reaction or by immunostaining of viral proteins.\textsuperscript{21}

Following histopathological examination of products of conception and placenta after a miscarriage, certain histopathological changes are suggestive of cytomegalovirus infection.\textsuperscript{65} These changes include necrosis, inflammatory cellular infiltrates, cellular enlargement with or without vacuolation and fibrin as intervillous and intravascular plugs.\textsuperscript{65} Necrosis could be mild, moderate or severe. Necrosis is evidenced by erosion of the villous outlines, decreased or absent vascularity of villi, loss of double trophoblastic layering and decidual necrosis.\textsuperscript{65} Inflammation may be mild, moderate of severe. In moderate inflammation, there is a small foci of inflammatory cells ranging from 4 to 15 leukocytes per high power field. In marked inflammation, there are more than 15 leukocytes per high power field.\textsuperscript{65}

Following immunohistochemistry, positive result of anti Human CMV immunostain is evidenced by granular cytoplasmic staining in maternal glandular epithelium, vascular
endothelial lining of maternal vessels, decidual cells and or chorionic villi. Foetal autopsy is the most important diagnostic test in determining the cause of stillbirth. Postmortem examination of stillbirths affected by CMV reveals foetal thrombotic vasculopathy which is the presence of thrombi in the foetal circulation resulting in the clustering of fibrotic villi, characterized by absence or degeneration of foetal capillaries in contiguous villi. This is consistent with CMV infection of endothelial and vascular cells and may potentially result in placental damage or altered placental permeability with increased transplacental transmission.

Using molecular analytic techniques in one study, CMV DNA was detected by polymerase chain reaction (PCR) and foci of viral infection by immunostaining formalin-fixed, paraffin-embedded foetal kidney, liver, and placentas from stillbirths. When all tissues were CMV DNA-positive, the placentas had histologic evidence of stromal haemorrhage of villi, extensive vascular sclerosed villi, and vascular proliferation. Stillborn infants had extramedullary haematopoiesis and petechial haemorrhages. Foetal hydrops and IUGR were also present, but these symptoms were not exclusively related to CMV infection. In contrast, foetal thrombotic vasculopathy was significantly more likely to occur in stillbirths associated with CMV infection than in those associated with other causes.

2.8 Prevention and treatment of cytomegalovirus infection in pregnancy

Both the Center for Disease Control (CDC) and American College of Obstetrics and Gynaecology (ACOG) recommend that pregnant women be counselled on ways to
reduce their risk of CMV acquisition during pregnancy.\textsuperscript{45,67} Practices for seronegative pregnant women to reduce the risk of CMV infection are simple hygienic precautions\textsuperscript{45} which include:

- Assume that children under 3 years in your care have CMV in their urine and saliva.\textsuperscript{45}
- Thoroughly wash hands with soap and warm water after: diaper changes and handling child’s dirty laundry, feeding or bathing child, wiping child’s runny nose or drool, handling child’s toys, or toothbrushes.\textsuperscript{45}
- Do not share cups, plates, utensils, toothbrushes, or food.\textsuperscript{45}
- Do not kiss your child on or near the mouth.\textsuperscript{45}
- Do not share towels or washcloths with your child.\textsuperscript{45}
- Do not sleep in the same bed with your child.\textsuperscript{45}

Studies demonstrating the efficacy of hygienic precautions are few but compelling. Some studies demonstrated that these hygienic measures when provided to CMV seronegative pregnant women with a young child in the home were effective.\textsuperscript{45} Although seronegative health care workers do not have an increased risk, pregnant child care employees are at a significant risk for CMV acquisition.\textsuperscript{45} In 2008, the CDC website recommended that pregnant child care employees be informed they could assess their risk by serologic testing and if seronegative, avoid if possible caring for children less than 2 years age for the duration of pregnancy.\textsuperscript{45} Serial serologic screening would identify pregnant women with a primary CMV infection prior to foetal infection. For these women, prompt passive immunization may prevent
fetal infection. Prevention of foetal infection with a high titre CMV immunoglobulin (HIG) preparation was reported in 2005. After primary infection, for women with or without infected foetuses or newborns, treatment with HIG was associated with significant reductions in placental thickness, placental inflammation, and placental viral load for seroconversion for gestational weeks 12 to 36. A limitation of HIG administration is that it does not appear to affect hearing deficit. This is anticipated since the incidence of hearing deficit among congenitally infected infants is independent of preconception high titre high avidity maternal antibodies and may progress postnatally in spite of high titre neonatal antibodies.

Regarding the safety of HIG, no toxicity has been observed and immunoglobulins have been used safely in pregnancy since the 1950s. Intravenous immunoglobulins are the most purified among the blood derivatives, including albumin, and are pasteurized. For a woman with a primary CMV infection during pregnancy, legitimate safety concerns have to be weighed against the risk of an affected infant or foetus.

2.9 Complications of CMV infection in pregnancy

CMV infection in pregnancy can lead to abortion, intrauterine growth retardation, stillbirth, sensorineural hearing loss, chorioretinitis, mental retardation, hepatosplenomegaly, intracranial calcifications, jaundice, thrombocytopenia, hepatitis, microcephaly, and early neonatal death. Deaths are usually secondary to liver dysfunction, bleeding, disseminated intravascular coagulation, or secondary bacterial infections. Dental defects can also occur by the age of two.
CHAPTER THREE
OBJECTIVES OF THE STUDY

3.0 Aim
The aim of this study was to determine the seroprevalence of cytomegalovirus infection in patients with recurrent miscarriage and if there is a relationship between cytomegalovirus infection and recurrent miscarriage in Jos.

3.1 Null hypothesis
There is no relationship between CMV infection and recurrent pregnancy loss in Jos.

3.2 Alternate Hypothesis
A relationship does exist between CMV infection and recurrent pregnancy loss in Jos.

3.3 Objectives
3.3.1 General objective
To compare the prevalence of CMV infection among women with recurrent pregnancy loss and normal postnatal women.

3.3.2 Specific objectives
1) To review the recent available literature on the association of cytomegalovirus infection and recurrent pregnancy loss
2) To determine the prevalence of CMV infection amongst women with recurrent pregnancy loss.
3) To determine if there is an association between CMV infection and recurrent pregnancy loss.
4) To make recommendations concerning cytomegalovirus antibody screening for patients presenting with recurrent pregnancy loss
4.0 **Materials and methods**

4.1 **Study Area**

The study was a multi centred case control study in which respondents were recruited from the Jos University Teaching Hospital, Plateau State Specialist Hospital and Faith Alive Hospital. The Jos University Teaching Hospital (JUTH) is a 600-bed tertiary health institution located in Jos, the capital of Plateau State in North Central Nigeria. The Jos University Teaching Hospital, established in 1981, is located in the northern part of Jos metropolis. It has a well-established department of Obstetrics and Gynaecology, with now seventeen consultant Obstetricians and Gynaecologists. The department has a gynaecological emergency unit, among other service points, which offers gynaecological emergency services to patients from Plateau state and receives referrals from neighbouring states including Bauchi, Benue, Kogi, Gombe, Nassarawa, Adamawa, Taraba and parts of Kaduna and Niger states.

Plateau State Specialist Hospital is a specialist hospital located within Jos metropolis with a well established department of Obstetrics and Gynaecology. Referrals are received from the general hospitals and primary health clinics from every part of the state.

Faith Alive Hospital is a non-profit, non-governmental hospital that offers PMTCT services to the general populace. The hospital offers obstetrics and gynaecology services usually provided by consultants and senior registrars in obstetrics and gynaecology.
4.2 **Study Design**

A multi centred case-control study.

4.3 **Study Population**

The study population comprised of women presenting with recurrent pregnancy loss (that is, the 3rd or more consecutive spontaneous pregnancy loss) at the gynaecological emergency unit and gynaecological wards of the Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital and Faith Alive Hospital, Jos-Nigeria. The control group comprised of postpartum women with no adverse obstetric outcome.

4.4 **Data Collection**

Forty two cases and controls each were recruited for the study over a period of thirteen months, from January 2017 to January 2018. Consenting women who presented to the gynaecological emergency or gynaecological ward with recurrent pregnancy loss (that is, the 3rd or more consecutive spontaneous pregnancy loss) were recruited into the case group, while the control group comprised of postpartum women presenting to the post natal clinic with no adverse obstetric outcome.

A structured questionnaire was administered and privacy was ensured while interviews were being conducted. Serial numbers were assigned to each patient in both groups to protect her identity.

The information that was collected included; demographics, parity, gestational age, obstetric history, gestational age at pregnancy loss, number of pregnancy losses.
Awareness about CMV was sought and knowledge on possible ways of acquiring and preventing the infection were asked.

Blood samples were collected for cytomegalovirus antibody screening for both cases and controls. The sample bottles were assigned serial numbers to match the patients. The patients’ phone numbers were collected to enable me get in touch with them after analysis of the blood samples.

4.5 **Sample handling:**

Three millilitres of blood was collected from the antecubital fossa of each subject. The samples were allowed to clot and then centrifuged at 4000 revolutions per minute for 3 minutes. Serum was extracted and stored at –20°C until a reasonable number of samples were obtained and then, analysis was done.

4.6.0 **Cytomegalovirus antibody assay:**

The CMV IgG and IgM antibodies were measured using CMV IgG and IgM DRG enzyme-linked immunosorbent assays (ELISA) kits. The laboratory analysis was carried out by a consultant medical microbiologist in conjunction with a consultant chemical pathologist in JUTH according to the kit manufacturer’s specifications. The consultants were blinded to the number coding used to identify the patients so as to eliminate bias.
4.6.1 **Principle of the test**

The DRG Cytomegalovirus IgG and IgM ELISA Kits are solid phase enzyme-linked immunosorbent assays. Microtiter wells as a solid phase are coated with inactivated cytomegalovirus (CMV) antigen. Diluted sample specimens and ready-for-use controls are pipetted into these wells. During incubation CMV-specific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control materials, horseradish peroxidase conjugated anti-human IgG (or IGM) antibodies are dispensed into the wells. During a second incubation this anti-IgG (or IgM) conjugate binds specifically to IgG (or IgM) antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed are detected by incubation with TMB substrate and development of a blue colour. The blue colour turns into yellow by stopping the enzymatic indicator reaction with sulphuric acid. The intensity of this colour is directly proportional to the amount of CMV-specific IgG (or IgM) antibody in the sample specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

4.6.2 **Test Procedure**

Prior to commencement of the assay, the Wash Solution was diluted, and the samples prepared and mixed well before pipetting. The distribution and identification plans for specimens and control were carefully followed as supplied in the kits. The following steps were followed as per manufacturer’s manual:
1. The required number of microtiter strips or wells were selected and inserted into the holder.

2. 100 μL of the substrate blanks, low control, high control, calibrators and the samples were dispensed into appropriate wells.

3. The wells were covered with foil supplied in the kit and incubated for 60 minutes at 37 °C.

4. The contents of the wells were shaken briskly and rinsed 5 times with diluted Wash Solution (300 μL per well) and the wells were striken sharply on absorbent paper to remove residual droplets.

5. 100 μL Enzyme Conjugate was dispensed into each well, except A1.

6. Incubation for 30 minutes at room temperature (20 °C to 25 °C) was done.

7. The contents of the wells were shaken briskly and rinsed 5 times with diluted Wash Solution (300 μL per well) and the wells striken sharply on the absorbent paper to remove residual droplets.

8. 100 μL of Substrate Solution was added into all wells.

9. Incubation was done for exactly 10 minutes at room temperature (20 °C to 25 °C) taking care not to expose to sunlight.

10. The enzymatic reaction was stopped by adding 100 μL of Stop Solution to each well and any blue colour that developed during the incubation turned into yellow.

11. The optical density was read at 450 nm with a microtiter plate reader within 30 minutes after the Stop Solution was added.
4.6.3 **Measurement:**

The ELISA microplate or microstrip reader was adjusted to zero using the substrate blank in well A1. The absorbance of all wells was measured at 450 nm. The absorbance values for each control and sample in the distribution and identification plan was recorded.

4.6.4 **Calculation of Results:**

Samples were considered positive if the absorbance value was higher than 10% above the cut-off otherwise they were considered negative.

\[
\text{OD (optical density) of specimen} \times 10 \\
\text{OD of calibrator}
\]

Interpretation: Positive= > 11 Negative= ≤ 11

4.6.5 **Quality control**

Quality control was ensured by using commercial control and standards for comparison of results as well running some samples in duplicate. All reagents, samples and controls were brought to room temperature before starting the test. The stored serum specimens were thawed and inverted several times prior to testing. The kit manufacturer’s instructions were strictly adhered to.

4.7 **Inclusion Criteria**

1. Women presenting with recurrent pregnancy loss less than 28 weeks of gestation (age of viability in our environment). These were the cases.
2. Postpartum women with no adverse obstetric outcome (examples: Miscarriage, history of intrauterine fetal death, still birth, early neonatal death, congenital anomalies, mental retardation, blindness, deafness). These were the controls.

4.8 **Exclusion Criteria**

1. Women who refused to give consent.
2. Women with previous pregnancy losses attributable to other causes.
3. Women with induced abortion.

4.9 **Estimate of Sample Size**

The sample size was estimated using the formula;

\[
n = \frac{(P_1(1-P_1) + P_2(1-P_2)) \times (Z_\alpha + Z_\beta)^2}{(P_1-P_2)^2}
\]

Where:

- \(n\) : number of sample size in each of the group
- \(P_1\) = proportion of positive anti-CMV antibody among controls (0.65 in a similar study)
- \(P_2\) = proportion of positive anti-CMV antibody among cases (0.35 in the same study)
- \(Z_{-\alpha/2}\) = value of standard normal distribution corresponding to a significance level of alpha (1.96 for two-sided test at the 0.05)
- \(Z_{-\beta/2}\) = value of standard normal distribution corresponding to the desired level of power (0.84 for a power of 80%)

\[
n = \frac{0.65(1-0.65) + 0.35(1-0.35)}{(0.65 - 0.35)^2} \times (1.96 + 0.84)^2
\]

\[
n = \frac{0.2275 + 0.2275}{(0.30)^2} \times (2.8)^2 = 39.64
\]

The sample size was adjusted to compensate for an attrition rate of 5% giving approximately 42 subjects.
Therefore, 42 cases and 42 controls were recruited for the study.

4.10 **Sampling technique**

Sampling for the case and control groups was done using simple non-random consecutive sampling. Control subjects were healthy age-matched postpartum women with no adverse obstetric outcome. Fourteen controls were recruited from each of the three centres.

4.11 **Data Analysis**

All statistical analysis was performed using SPSS software version 22. Frequencies and percentages were computed for demographic and educational characteristics of cases and controls and presented in tables; test for association was done using t test, Fisher’s exact test and Chi square. A P value of less than 0.05 was taken as significant.

The prevalence of CMV infection among women with recurrent pregnancy loss (in percentage) was the number of patients with recurrent pregnancy loss and positive cytomegalovirus antibody assay divided by the total number of patients with recurrent pregnancy loss, multiplied by hundred.

The prevalence of CMV infection in postnatal women (in percentage) was the number of postnatal women with positive cytomegalovirus antibody assay divided by the total number of postnatal women, multiplied by hundred.

According to the serological tests, women were classified as follows:

- CMV seronegative: both CMV-IgM and -IgG negative.
• CMV seropositive: CMV-IgM negative and CMV-IgG positive.\(^8\)
• Primary CMV infection: CMV-IgM positive and CMV-IgG negative.\(^8\)

4.12 Ethical Consideration

Ethical clearance was obtained from the Ethical Committees of the Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital and Faith Alive Hospital, Jos. The nature, aim and objectives of the study were explained to each woman and consent obtained before recruitment into the study. The participants were assured of confidentiality of their information based on the Principles of Bioethics. The women were offered the option to opt out of the study, bearing in mind such action would not in any way compromise the quality of care they would receive at any service point.

4.13 Benefits to the participants

The women were counseled about CMV infection, it’s possible effects and ways of preventing infection.

They also had free CMV antibody screening.

Women that were seropositive for CMV IgM were referred to the consultant medical microbiologist for further evaluation.
## RESULTS

Table 1: Background characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study group</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>n=84(%)</td>
</tr>
<tr>
<td></td>
<td>n=42(%)</td>
<td>n=42(%)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>32.33±5.4</td>
<td>31.88±6.55</td>
<td>32.11±5.9</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.24±1.21</td>
<td>2.62±2.07</td>
<td>1.93±1.64</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal</td>
<td>7(16.7)</td>
<td>2(4.8)</td>
<td>9(10.7)</td>
</tr>
<tr>
<td>Primary</td>
<td>3(7.1)</td>
<td>7(16.7)</td>
<td>10(11.9)</td>
</tr>
<tr>
<td>secondary</td>
<td>10(23.8)</td>
<td>20(47.6)</td>
<td>30(35.7)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>22(52.4)</td>
<td>13(31.0)</td>
<td>35(41.7)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>15(35.7)</td>
<td>17(40.5)</td>
<td>32(38.1)</td>
</tr>
<tr>
<td>Trading</td>
<td>9(21.4)</td>
<td>8(19.1)</td>
<td>17(20.2)</td>
</tr>
<tr>
<td>Teaching</td>
<td>7(16.7)</td>
<td>3(7.1)</td>
<td>10(11.9)</td>
</tr>
<tr>
<td>Others</td>
<td>11(26.2)</td>
<td>14(33.3)</td>
<td>25(29.8)</td>
</tr>
</tbody>
</table>

*Fishers Exact derived value
The overall mean age was 32.11±5.9 years. The mean parity of the controls was higher than that of the cases.

More of the cases (52.4%) had tertiary education than the controls (31.0%). Thirty eight percent of these women (case and control) were housewives and 20.2% were traders.

Figure 1 shows that most (97.60%) of the pregnancy losses are first trimester pregnancy losses.

Figure 1: Gestational age at current pregnancy loss (Cases only)
Figure 2 shows the past obstetric history of the patients with recurrent miscarriage. Sixty nine percent have had normal deliveries in the past while 11.9% have had intrauterine foetal death.
Table 2: CMV infection among the cases and controls

<table>
<thead>
<tr>
<th>CMV infection</th>
<th>Study Group</th>
<th>Total n=84</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases n=42</td>
<td>Control n=42</td>
<td></td>
</tr>
<tr>
<td><strong>IGG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>36(85.7)</td>
<td>32(76.2)</td>
<td>68(81.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>6(14.3)</td>
<td>10(23.8)</td>
<td>16(19.0)</td>
</tr>
<tr>
<td><strong>IGM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4(9.5)</td>
<td>2(4.8)</td>
<td>6(7.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>38(90.5)</td>
<td>40(95.2)</td>
<td>78(92.9)</td>
</tr>
</tbody>
</table>

*Fishers Exact derived values

The overall prevalence of CMV using IgG was 81.0%. The prevalence of CMV (IgG seropositivity) among cases was 85.7% and 76.2% among controls. With CMV IgM, more subjects among the cases (9.5%) were seropositive compared to the controls (4.8%). However these differences were not statistically significant.
Table 3: Various combinations of the CMV serostatus of the cases and control

<table>
<thead>
<tr>
<th>CMV</th>
<th>Case n=42</th>
<th>Control n=42</th>
<th>Total n=84</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG(+) IgM(-)</td>
<td>32(76.2)</td>
<td>30(71.4)</td>
<td>62(73.8)</td>
<td>0.804*</td>
</tr>
<tr>
<td>IgG(-) IgM(+)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td>IgG(+) IgM(+)</td>
<td>4(9.5)</td>
<td>2(4.8)</td>
<td>6(7.1)</td>
<td>0.433*</td>
</tr>
<tr>
<td>IgG(-) IgM(-)</td>
<td>6(14.3)</td>
<td>10(23.8)</td>
<td>16(19.0)</td>
<td>0.254*</td>
</tr>
</tbody>
</table>

*Fishers Exact derived values

Table 3 shows that no respondent was seropositive for CMV IgM only. Most of the respondents (73.8%) were seropositive for only CMV IgG.

Table 4: Distribution of the number of pregnancy losses among the cases and their serostatus

<table>
<thead>
<tr>
<th>CMV</th>
<th>Recurrent Miscarriage</th>
<th>Total n=42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>&gt;3</td>
</tr>
<tr>
<td>IgG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29(69.0)</td>
<td>7(16.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>4(9.5)</td>
<td>2(4.8)</td>
</tr>
<tr>
<td>IgM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1(2.4)</td>
<td>3(7.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>32(76.2)</td>
<td>6(14.3)</td>
</tr>
</tbody>
</table>
Most of the cases had 3 pregnancy losses (78.6%) while 21.4% had greater than 3 pregnancy losses.

Table 5: Awareness about cytomegalovirus infection among the respondents

<table>
<thead>
<tr>
<th>Aware</th>
<th>Study group</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case n=42(%)</td>
<td>Control n=42(%)</td>
<td>n=84(%)</td>
</tr>
<tr>
<td>Yes</td>
<td>1(2.4)</td>
<td>3(7.1)</td>
<td>4(4.8)</td>
</tr>
<tr>
<td>No</td>
<td>41(97.6)</td>
<td>39(92.9)</td>
<td>80(95.2)</td>
</tr>
</tbody>
</table>

*Fishers exact derived value

Only 4.8% of all the respondents were aware about cytomegalovirus (2.4% among cases and 7.1% among controls). There was no significant association between awareness of CMV infection and recurrent miscarriage, (P > 0.05).
Table 6: Knowledge about mode of transmission of CMV, susceptible group of people, complications of CMV infection and methods of prevention of infection

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study group</th>
<th>Total n=84(100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case n=42(%)</td>
<td>Control n=42(%)</td>
</tr>
<tr>
<td><strong>Mode of transmission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unprotected sexual intercourse</td>
<td>1(2.4)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>0(0.0)</td>
<td>2(4.8)</td>
</tr>
<tr>
<td><strong>Susceptibility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant women</td>
<td>1(2.4)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Unborn babies</td>
<td>1(2.4)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Daycare workers</td>
<td>0(0.0)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Health care workers</td>
<td>0(0.0)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td><strong>Complication</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUFD</td>
<td>1(2.4)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>0(0.0)</td>
<td>3(7.1)</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular and thorough handwashing</td>
<td>0(0.0)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Avoid kissing children on or near the mouth</td>
<td>0(0.0)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Avoid sharing towels or washcloths with your child</td>
<td>0(0.0)</td>
<td>1(2.4)</td>
</tr>
</tbody>
</table>
From table 6 above, Only two respondents (2.4%) had knowledge about unprotected sexual intercourse as a mode of transmission of CMV infection. Two respondents (2.4%) were aware that pregnant women are susceptible to CMV infection. No respondent was aware that miscarriage could be a complication of CMV infection. One respondent (1.2%) was aware of thorough hand washing as a method of prevention of CMV infection. One respondent (1.2%) knew that acquiring the infection could be prevented by avoiding kissing children.

Note: Respondents were allowed to give multiple answers.
CHAPTER SIX

DISCUSSION

The most common cause of congenital viral infection is human cytomegalovirus which can cause miscarriages.\textsuperscript{18} This study set out to determine the seroprevalence of CMV in patients presenting with recurrent miscarriage and if there is an association between CMV infection and recurrent miscarriage in Jos.

SEROPREVALENCE OF CMV IN WOMEN WITH RECURRENT PREGNANCY LOSS

The seroprevalence of anti-CMV IgG in women with recurrent miscarriage in this study was 85.7%. The implication of this high seroprevalence is that most of the patients that presented with recurrent miscarriage have been previously exposed to the virus. Bearing in mind the risk factors for CMV infection transmission, the possible reasons for this high seroprevalence may include low standard of hygiene in our environment, low socio-economic status and cultural practices that aid in the propagation of the infection. However, the presence of IgG is not enough to determine if seroconversion occurred during the recent miscarriage since the CMV serostatus of the women were unknown before conception. Although individuals that are CMV IgG positive are said to be ‘protected’ or ‘immune’, it is worth noting that the presence of CMV IgG antibodies is not completely protective because an individual can be infected primarily with a different strain or have a reactivation of a latent virus. Thus vertical transmission can still occur in pregnant women who are CMV IgG seropositive. The chances of transmission are however less than in women that seroconvert in pregnancy.
The finding in this study is similar to the finding in the study carried out by Sherkat et al where the CMV IgG seroprevalence among women with recurrent miscarriage was 90.6%. Similar findings were obtained in other studies: Kafi et al found a seroprevalence of 97.8%, Odland et al found a seroprevalence of 78% and Hammed et al found a seroprevalence of 92.9% among women with recurrent pregnancy loss. The finding of a high seroprevalence of CMV IgG in patients with recurrent pregnancy loss in this study differs from the finding in a study done by Johnson et al where the seroprevalence rate for CMV IgG was 35%. These conflicting results may be due to different study population, variation in sample size and differences in the interpretation of the various diagnostic kits used to assay for CMV antibodies.

The seroprevalence of anti CMV IgM in patients with recurrent pregnancy loss in this study was 9.5%. This may suggest that these women had primary infection. The implication of a primary infection is that CMV may have been responsible for the pregnancy loss in these patients. However, the interpretation of a positive CMV IgM result can be problematic since CMV IgM persists in some individuals for one or more years following primary infection. The seroprevalence of CMV IgM in women with recurrent pregnancy loss obtained in this study is higher than that obtained in studies by Sherkat et al and Ariani et al who found CMV IgM seroprevalence to be 2.3% and 1.3% respectively. From the study by Kafi et al, a higher CMV IgM seroprevalence (38.3%) was obtained for women presenting with pregnancy loss. Possible explanations for the differences include different study populations with varying socio-demographic
characteristics, differences in the number of women recruited for the studies, the high false positive rate of the IgM CMV assay and variations in the interpretation of results of different kits.

In Nigeria, studies aimed at determining the seroprevalence of CMV infection in women presenting with recurrent pregnancy loss are lacking.

**SEROPREVALENCE OF CMV IN THE CONTROL GROUP**

The seroprevalence of CMV IgG in the control group in this study was 76.2%. This is similar to the findings in studies by Abdolreza et al (78%)\textsuperscript{27} and Odland et al (81.1%)\textsuperscript{71} but higher than the seroprevalence obtained by Johnson et al (65%).\textsuperscript{70} These values are high and are similar to the findings in numerous studies done in Nigeria among pregnant women (94.8% in Kaduna\textsuperscript{12}, 98.7% in Sokoto\textsuperscript{15}, 84.2% in Bida\textsuperscript{16} and 97.2% in Lagos\textsuperscript{30}). Seroprevalences obtained from studies in Nigeria are higher than that obtained in developed countries like Australia (56.8%)\textsuperscript{74} and Italy (68.3%).\textsuperscript{75} The high seroprevalence in this study and other studies within the country could be due to: low socio-economic status, failure to adhere to simple hygienic practices like regular handwashing, large family sizes of many homes in developing countries which ensures that frequent and prolonged contact with children less than 3 years of age will occur and that CMV is not screened for in our environment like in some developed countries (Spain, Isreal, Netherlands, Portugal and Austria).\textsuperscript{76,77}

About 4.8% of the controls were seropositive for CMV IgM. This is similar to the findings in the study by Sherkat et al\textsuperscript{3} which showed that 2.3% of the controls were
seropositive to CMV IgM. Emovon et al.\textsuperscript{48} conducted a study in Southern Nigeria and found that only 4\% of normal pregnant women were positive for IgM which is similar to the finding among controls in this study. The finding in this study is however not in keeping with a study by Hameed et al in which no control was found to be seropositive to CMV IgM.\textsuperscript{72} The variations in the seroprevalences of IgM in different studies may be attributed to different sample sizes for various studies, the high false positive rate for IgM, differences in assay methods and time of collection of the blood samples. If an individual has a negative CMV IgM result, this does not completely rule out a primary infection with CMV. This is because the sample may have been collected too early in the course of the primary infection and IgM levels may have not reached detectable levels. The high false positive rate of CMV IgM may be attributed to cross-reactivity with autoimmune diseases and some viral infections like influenza, Epstein barr virus, measles and Herpes Simplex.\textsuperscript{78}

The seroprevalence of CMV IgM among the normal population is generally lower than IgG since the presence of CMV IgM connotes a primary infection. A possible explanation for this is that majority of women would have recovered from primary infection with the loss of IgM by the time they reach child bearing age.\textsuperscript{48}

**ASSOCIATION BETWEEN CMV INFECTION AND RECURRENT PREGNANCY LOSS**

In this study, more cases were seropositive for IgG and IgM compared to the controls but these differences were not statistically significant (P=0.405 for IgG and P=0.676 for
IgM). Thus, even though more cases were seropositive than controls, there is insufficient evidence to associate CMV infection and recurrent miscarriage in this study. The implication of the finding in this study is that we cannot draw up a conclusion associating CMV and recurrent pregnancy loss. Radcliffe et al assessed CMV infection in women with recurrent miscarriage and found a significantly lower seroprevalence of CMV in women with recurrent miscarriage compared to their male partners and female controls.\textsuperscript{29} Cook et al used Polymerase Chain Reaction (PCR) to detect cytomegalovirus in retained products of conception of women with recurrent spontaneous abortions and none of the specimens contained evidence of CMV DNA. His finding suggested that CMV infection of gestational tissue is not a common direct cause of recurrent miscarriages.\textsuperscript{79} The finding from this study is not in agreement with other studies that found significant association between CMV and recurrent miscarriage. Sherkat et al found that previous exposure to CMV was significantly higher in patients with recurrent pregnancy loss than the control group.\textsuperscript{3} Also, Kafi et al found significant association between CMV infection and frequency of abortion.\textsuperscript{19} Possible reasons for the conflicting reports from different studies include different study populations, different sample sizes and variations in the sensitivity and specificity of various employed serological techniques used to make diagnosis.
INCIDENTAL FINDINGS

PARITY
The mean parity of the controls (2.62±2.07) is higher than that of the cases (1.24±1.21) and the difference is statistically significant (P <0.001). This finding is expected since all the controls are women who have had normal deliveries compared to some of the cases that have not carried any pregnancies beyond the age of foetal viability.

AWARENESS ABOUT CMV INFECTION AND METHODS OF PREVENTION
The cornerstone of efforts to limit the burden of congenital CMV infection globally is by prevention of infection. Awareness about CMV infection is an important step in the prevention of CMV infection. An incidental finding in this study is that only 4 (4.8%) of all the respondents know about CMV. Only 1(2.4%) of the women with recurrent miscarriages and 3(7.1%) of the controls are aware about CMV. This clearly shows that most women in this study are not aware of CMV and its effects on pregnancy. Possible reasons for this low level of awareness include: the respondents have never been educated about CMV by health care providers, the health care providers maybe, do not appreciate the devastating effects of CMV infection in pregnancy or health messages about CMV are not usually given on the media. Emovon et al in Nigeria also reported a low level of awareness (3%). Awareness is generally higher in developed countries (15% in Canada, 39% in Geneva, 34% in France).80-82
Two respondents were aware that pregnant women are susceptible to CMV infection. No respondent was aware that miscarriage is a possible complication of CMV infection and only one respondent knew that a method of prevention of CMV infection was by regular and thorough handwashing. In the study by Willame et al, 74.6% of the women that were aware of CMV answered correctly to more than five preventive measures. This clearly shows that knowledge about CMV is lacking in our environment. Therefore, there is need for health education about CMV and methods of preventing infection among susceptible women. Since the level of awareness about CMV and methods of preventing infection is very low, the high seroprevalence of CMV IgG from this study in both cases and controls is not surprising. Thus, the effects of CMV infection in pregnancy and the methods of prevention of the infection should be incorporated into the health talk given to pregnant women during antenatal clinic visits.

CONCLUSION

Significant association between CMV infection and recurrent pregnancy loss was not established from this study. This means there is insufficient evidence linking CMV infection with recurrent miscarriage based on the findings of this study. However, the high seroprevalence of CMV in both study groups suggests that many women have been exposed to this virus. Putting into consideration the effects of CMV in pregnancy (which includes miscarriage, intrauterine foetal death, congenital anomalies and preterm delivery), pregnant women should be counselled about CMV infection and
appropriate preventive measures. Screening for CMV in women presenting with recurrent miscarriages cannot be justified from this study. A major limiting factor for screening is the cost especially in our resource constrained environment.

**RECOMMENDATIONS**

1) All pregnant women should be counselled about CMV infection and ways of preventing infection.

2) The effects of CMV infection on pregnancy should be included as part of the health talk in the antenatal clinic.

3) From this study, there is inconclusive evidence linking CMV infection and recurrent pregnancy loss. So the finding from this study does not make a case for routine screening for CMV in patients with recurrent pregnancy loss. More studies with larger sample sizes may provide better insight as regards the relationship between CMV infection and recurrent pregnancy loss.

**LIMITATIONS OF THE STUDY**

1. The sample size of this study is small. More studies with larger sample sizes may give more information about the association between CMV and recurrent pregnancy loss.

2. Antibody avidity testing will confirm primary infection of CMV but was not assayed for in this study. This would further increase cost and logistics. More studies involving antibody avidity testing are therefore needed.
3. Viral culture and Polymerase chain reaction (PCR) was not done due to the cost implication.

4. Histopathological analysis of the products of conception was not done in the study.

5. Chromosomal anomalies as a cause of pregnancy loss was not ruled out in this study.

6. The cost of the serological tests is a limitation.
References


21) Lenore P. Have We Overlooked Congenital Cytomegalovirus Infection as a Cause of Stillbirths? JID. 2011;203:1510-2.


37) Mussi-Pinhata MM, Yammato AY, Brito RMM, de Lima IM, de Carvalho e Oliveira PF, Boppana S, Britt WJ. Birth prevalence and natural history of congenital


66) Iwasenko JM, Howard J, Arbuckle S, Graf N, Hall B, Craig ME, Rawlinson WD. Human Cytomegalovirus infection is detected frequently in stillbirths and is associated with fetal thrombotic vasculopathy. JID. 2011;203:1526–1533.

67) Anderson B, Schulkin J, Ross DS, Rasmussen SA, Jones JL, Cannon MJ. Knowledge and practices of obstetricians and gynecologists regarding


INFORMED CONSENT

I, Dr Akunaeziri Uche Augustine, of the Department of Obstetrics and Gynaecology, Jos University Teaching Hospital, wish to carry out a research on Cytomegalovirus (CMV) infection and recurrent miscarriage in Jos, Plateau state.

Before you decide whether to participate in the study or not, it is important for you to know why the research is being done, what it will involve and the possible benefits.

Please take time to read the following instructions carefully.

WHAT IS THE STUDY ABOUT?

This study is designed to determine possible association between CMV infection and recurrent miscarriages in Jos, Plateau state. CMV infection has been implicated in adverse pregnancy outcomes. There has been different opinions as regards whether CMV is implicated in women with spontaneous miscarriages. No study has been done to determine the association between recurrent pregnancy loss and cytomegalovirus infection in Jos, Plateau state. This study is aimed at determining if there is an association in other to manage these patients better.

The study is in partial fulfillment of part II examinations of the National Postgraduate Medical College of Nigeria.

The test will require that 3mls of blood sample will be drawn from you only once for the analysis and I will pay for the laboratory test. You will feel a little pain while the sample is
being collected, but it is temporary. Your test results will be communicated to you at the end of the research and possible medical advice given if necessary.

You are free to decline participation in the study. Your refusal will not in any way affect your relationship with your doctors or the care you will receive (Principle of Justice).

If you decide to participate, you will be expected to sign the consent forms.

WILL THE INFORMATION BE CONFIDENTIAL (Confidentiality)?

This study is highly confidential. The information collected during the study will be stored and analyzed without including your name. The results of the study may be published in medical journals but your identity will not be revealed.

WHAT DO YOU STAND TO BENEFIT (Beneficence)?

This is an opportunity to find out if you are positive or negative for CMV infection. You will be contacted when result is out and if the result is positive you will be advised appropriately and or referred to a consultant medical microbiologist that I am working with for further care.
WRITTEN CONSENT FORM

I ………………………………………….. (Initials please), have read and understood all the information given to me about participation in this study and I have been given opportunity to discuss it and ask questions. All my questions have been answered to my satisfaction and I voluntarily agree to take part in this study. I understand that I will receive a copy of this signed, written, informed consent form.

I give permission for the release of my medical records to the investigator, regulatory authorizes and ethical committee as may be required.

Signature /thumbprint of subject

………………………………. Date ………………

Initials of subject

……………………………………

I have explained the nature and purpose of the study to the subject named above.

Signature of investigator……………………………………..

Initials of investigator…………………………………….. Date ………………………

Initials of witness………………………………………. Date ……………………….
QUESTIONNAIRE
CYTOMEGALOVIRUS INFECTION AND RECURRENT PREGNANCY LOSS IN JOS, PLATEAU STATE

DDMMYY  SERIAL NO  HOSPITAL NO

Date

CASES (A)  CONTROLS (B)

1. Age (Years)..................

2. Ethnicity (a) Hausa/Fulani (b) Igbo (c) Yoruba (d) Others (specify) ......................

3. Level of education (a) none (b) primary (c) secondary (d) tertiary

4. Occupation (a) housewife (b) student (c) trader (d) self employed (specify)
   (e) teacher (f) civil servant (specify) ..................

   NOTE: If occupation is teaching specify exactly what group of people you teach
   
   - Day care
   
   -Primary school
   
   -Secondary school
   
   -Tertiary institution

5. Parity.....................

   NOTE: Questions 6 and 7 are for the cases

6. Gestational age at current pregnancy loss (weeks)......................

7. Past Obstetric History-a) previous history of early neonatal death
b) spontaneous miscarriages

c) history of preterm delivery

d) history of intrauterine fetal death

e) history of intrauterine growth restriction

f) normal pregnancy

g) previous history of a congenitally malformed baby

8. Are you aware of a virus called cytomegalovirus (CMV)?  a) Yes  b) No

9. If Yes to 8, What are the possible modes of transmission?

   a) Contact with saliva

   b) Contact with urine

   c) Through breast milk

   d) Through blood transfusion

   e) Through unprotected sexual intercourse

   f) Others (specify)............................

10. If Yes to 8, what groups of people are more likely to become infected?

    a) Pregnant women

    b) Unborn babies

    c) Day care workers

    d) Health care workers that take care of children

    e) Others (specify)............................
11. If Yes to 8, specify some complications of CMV infection that you are aware of………………………………………………………………………………………………………………………………
……………………………………………………………………………………………………………………………………
12. If Yes to 8, what methods of preventing infection do you know?
   a) Regular and thorough hand washing after diaper changes, after feeding or bathing child, after wiping child’s nose or drool and after handling child’s toys
   b) Avoid kissing children on or near the mouth
   c) Avoid sharing towels or washcloths with your child
   d) Others (specify)…………………………………………………………………………………………………………
……………………………………………………………………………………………………………………………………
13. Cytomegalovirus antibodies tested
   a) Anti-CMV Ig G
      - Positive
      - Negative
   b) Anti-CMV IgM
      - Positive
      - Negative