

## DETECTION OF CYTOMEGALO VIRUS ANTIBODY AND ANTIGEN AMONG PREGNANT AND REPEATED ABORTION WOMAN IN KHARTOM STATES

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### ABSTRACT

**Background:** Cytomegalovirus is the most common cause of congenital infection. Clinical features range from Asymptomatic to symptoms that resemble infectious mononucleosis, including sore throat and prolonged fever. **Aim.** Eighty eight serum sample collected from pregnant and repeated abortion women to carry out seroprevalent study, ELISA use to detect IgM and IgG for Cytomegalovirus. Patients, categorised into those with repeated abortion and those with pregnancy. **Results.** The 88 patients included IgM 2 (52%) and IgG 86 non-BA patients; Among 32 case of repeat abortion, IgM was detected in (2) cases 6.3%) which is significant rate (p.value 0.05), while the IgG was detected in 31 case 96.9% (p.value 0.6) Out of 13 abnormal birth child case cytomegalovirus IgM was detected in 2 cases (15.4%) and all cases show positive for IgG 96.9% (p.value 0.5) only 2 patients

were CMV-positive (IgM/IgG) **Conclusions.** Our results suggest a correlation between CMV infection and repeat abortion furthermore CMV is one of the evidence in abnormal birth child.

## 1. INTRODUCTION

### 1.1. Background

Cytomegalovirus is the most common cause of congenital infection. Clinical features range from Asymptomatic (about 90%).<sup>[1]</sup> to symptoms that resemble infectious mononucleosis,

including sore throat and prolonged fever. And even severe physical and mental problems to the newborns. Maternal Sexual behavior and contact with young infected children were a known source of infection.<sup>[2]</sup> As well as through body fluids such as urine, saliva and breast milk. In utero transmission can occur and congenitally when the baby passes through delivery Canal.<sup>[3]</sup> CMV is endemic worldwide. And Africa shows the highest titers. E.g. in Egypt seroprevalence is up to 96%.<sup>[4]</sup> In Sudan it is up to 97%.<sup>[5]</sup> This is highly associated with poor hygiene, over crowding and illiterate individuals.<sup>[5]</sup> Signs of CMV infection that may be present at birth includes, Premature birth, Liver problems, Lung problems, Spleen problems, Small size at birth, Small head size and seizures. It also may result in permanent disabilities including Hearing loss, Vision loss, Mental disability, Small head size, Lack of coordination, Seizures, Death (in rare cases).<sup>[1]</sup>

CMV can infect all age groups usually causing mild and self-limited disease. Its seroprevalence in women of child-bearing age varies from 50% to over 80%, with inverse correlation to socioeconomic levels. Primary CMV infection during pregnancy carries a high risk of intrauterine transmission which may result in severe fetal damage, including growth retardation, jaundice, hepatosplenomegaly and CNS abnormalities.

Those who are asymptomatic at birth may develop hearing defects or learning disabilities later in life. It is now recognized that intrauterine transmission may occur in the presence of maternal immunity(6). Pre-conceptional primary infection carries a high risk identical to the risk of infection during early gestational weeks.<sup>[7]</sup>

## 1.2. RATIONAL

Cytomegalovirus is the most common cause of congenital infections disease, On the other hands; mothers are usually asymptomatic. This will increase the chance of transmitting the virus congenitally or even to her newborn through breast feeding and direct contact.

This study was aimed to detect the cytomegalovirus and its association with daily actions among miss carriage and repeated abortion women.

## 1.3. OBJECTIVES

### 1.3.1.General objective

To detect cytomegalovirus immunoglobulin among repeated abortion pregnant women in Al Shorta hospital them.

### 1.3.2. Specific objectives

1. To determine the frequency of CMV infection in pregnant women
2. To describe the relation between CMV infection and abortion.
3. To describe the relation between CMV infection and abnormal birth child.

## 1.4. LITERATURE REVIEW

### 1.4.1. Definition

Cytomegalovirus means "large cell virus". Is a genus that belongs to herpiviridae family. And specifically to Betaherpiviridae sub family. The species that infect human is commonly named as human cytomegalovirus or as Human Herpesvirus-5.

### 1.4.2. Structure of the virus

It is a double strand DNA virus; which possess an icosahedral protein capsid of a 105nm in diameter; and 162 capsomeres. Surrounded by a proteinaceous tegument and an outer lipid envelope.<sup>[8]</sup> It has the largest genetic content Of the Human Herpes virus.<sup>[3]</sup> This genome encode a large number of protein a few of them has been characterized. E.g. a cell surface glycoprotein acts as Fc receptor which binds nonspecifically to Fc portion of antibodies.<sup>[3]</sup>

Human cytomegalovirus (CMV) is an opportunistic pathogen associated with significant morbidity and mortality in susceptible populations; i.e. those with immature or immunocompromised immune systems. Numerous antiviral agents with in vitro activity against the various human herpesviruses . yet only a few have been approved for the treatment or prophylaxis of CMV diseases

### 1.4.3. CMV infection and CMV disease

Cytomegalovirus is easily transmitted, usually through contact with bodily fluids or by placental transfer. Seroprevalence rates vary by socioeconomic class and geographic location, but the overall seroprevalence in developed countries is estimated to be in the range of 30 70% (9). Primary infection in immunocompetent individuals is usually benign, with minimal or no clinical manifestations (although approximately 10% of mononucleosis syndromes are a result of CMV infection). Following primary infection, the virus establishes latency, and viremia is mainly controlled by cell-mediated immunity. Virus reactivation occurs when this protective immune surveillance fails; e.g. as a result of chemotherapy or in patients who have AIDS or who are immunosuppressed for transplantation purposes. Such reactivation or primary infection in the context of a disabled immune system can lead to overt disease. In the

case of vertical transmission of CMV to the developing fetus, adverse outcomes are most commonly associated with primary infection of the mother, although significant morbidity has also been associated with secondary infection.<sup>[10]</sup>

#### 1.4.4. Congenital CMV infection

In developed countries, congenital CMV infection occurs in approximately 1% of live births. The majority of the cases are asymptomatic, but approximately 5–10% of infants with congenital CMV will have symptomatic disease, associated with profoundly deleterious effects on the central nervous system (CNS), including microcephaly, intracranial calcifications, and ventriculomegaly. Prognosis for neonates with symptomatic disease is poor, with a high likelihood of mental deficits, hearing loss and psychomotor and perceptual handicaps.<sup>[11] [12]</sup> It is now recognized that even asymptomatic congenital CMV is associated with increased risk of sensorineural hearing loss (SNHL) (11), an observation that highlights the importance of identifying infants with congenital CMV infection and conducting periodic auditory assessments. The morbidity and mortality associated with congenital CMV infection underscores the need for a vaccine to prevent CMV infection. CMV vaccines currently in preclinical and clinical development are reviewed in.<sup>[13]</sup>

#### 1.4.5. CMV-associated disease in transplant recipients

CMV infection is the leading viral cause of morbidity and mortality facing patients who receive hematopoietic stem cell transplant (HSCT) or solid organ transplant (SOT), with both direct adverse effects resulting from viral invasion of organ systems and indirect effects on the immune systems that increase the risk of other infections and promote acute graft rejection.<sup>[14]</sup> CMV viremia is a significant predictor for organ involvement and progression to CMV disease.<sup>[15]</sup>

Risk of CMV-associated complications is increased with more potent immunosuppressive regimens, such as many of those required for HSCT, and transplant patients are at greatest risk for CMV-associated disease within the first 100 days post-transplant. For recipients of SOTs, the most vulnerable patients (“high-risk patients”) are CMV-sero negative recipients who receive an organ from a CMV-seropositive donor (D+/R-). CMV-seropositive recipients of allogeneic stem cell transplants are at risk for reactivation of latent CMV infection. In highrisk patients without symptomatic CMV disease, two common strategies of disease management are prophylactic and preemptive therapy, both of which are designed to prevent CMV disease. In the prophylactic approach, therapy is usually initiated at the time of stem

cell engraftment or solid organ transplant. The suppressive doses used for prophylaxis are generally lower than those instituted for induction treatment of active disease, and the suppression of CMV reactivation in specific transplant populations can be successfully accomplished with a less potent antiviral agent than would be used for treatment. In the preemptive approach, therapy is initiated in asymptomatic high-risk patients based on diagnostic test results indicating primary CMV infection or reactivation of latent virus to a threshold level that signals the potential for disease escalation (blood CMV DNA load by PCR or pp65 antigenemia). This latter strategy often involves intermittent therapy, creating conditions thought to pose a greater risk of selection of resistant virus. However, this risk may be balanced by the protective effect of restoration of Tcell responses to CMV afforded by the delay in treatment with potent antivirals, particularly with myelo suppressive agents. On the other hand, the longer duration of drug exposure in the prophylactic approach also poses risk of resistance emergence.<sup>[15]</sup>

#### **1.4.6. CMV retinitis in AIDS patients**

Although CMV retinitis is a relatively rare manifestation of CMV disease in other immunocompromised populations, it is the primary manifestation of CMV infection in patients with AIDS, usually resulting from reactivation of latent virus. CMV retinitis is a disease characterized by progressive, necrotizing retinitis that can lead to retinal detachment and blindness. Initial symptoms are non-specific, but may include blurred or distorted vision, floaters, light flashes, and loss of peripheral vision. CMV retinitis and other manifestations of CMV disease in individuals with HIV-1 infection are opportunistic infections, occurring when CD4+ cell counts are profoundly suppressed (e.g. <50 cells/ $\mu$ l). Since the advent of highly active antiretro viral therapy for treatment of HIV-1 infection, CMV retinitis is a condition rarely seen in developed countries, although asymptomatic CMV viremia remains a significant risk factor for death.<sup>[16]</sup>

#### **1.4.7. How does CMV affect pregnancy?**

Pregnant women who are healthy are not at special risk from CMV infection. Pregnant women who are infected with CMV rarely have symptoms, but rather their developing baby may be at risk for congenital (meaning from birth) CMV disease. The transmission rate to the fetus is between 30-50% according to the Organization of Teratology Information Service (OTIS). Of those babies who become infected, only 10-15% show signs of congenital CMV

after primary maternal infection. Congenital CMV affects about 0.2-2.5% of babies worldwide. For a woman who has a recurrent CMV infection during pregnancy (meaning this is not a primary infection), the rate of newborn CMV infection is about 1%. Of these, only 1-10% of the babies born with the CMV infection will have symptoms at birth and another 10-15% may not show any symptoms at birth, but still may have long term affects such as hearing loss and learning disabilities. The following potential prlems can occur for infants who are infected from their mothers before (during pregnancy) birth:

- Moderate enlargement of the liver and spleen, small red spots on the skin, problems with the eyes and seizures are potential complications.
- 85-95% will have no symptoms or complications at birth and the majority will not develop problems later in life.
- 10-15% of those at birth with no symptoms, will go on to develop varying degrees of hearing and mental or coordination problems.

When CMV is transmitted at the time of delivery from contact with genital secretions or later in infancy through breast milk, these infections usually result in few, if any, symptoms or complications.<sup>[17]</sup>

#### **1.4.8. PATHOGENICITY AND PATHOGENESIS**

##### **1.4.9. Transmission**

Although the infected person shed the virus in their body fluids such as urine, saliva, blood, and semen, into the environment; the chance of getting cytomegalovirus infection through casual contact is very low. The main roots for infection are direct contact with infectious body fluids. Also it can be transmitted sexually; congenitally; through organ transplantation<sup>[3]</sup> and blood transfusion .Once the person acquires the infection it lasts for life.

##### **1.4.10. Pathogenesis in normal host**

After exposure the viral particles an incubation period of 4-6 weeks takes place in normal adults and children. CMV causes a systemic infection .Several vital organs are affected, such liver, lungs, kidneys, colon and esophagus. As well it has been isolated from monocytes, T and B lymphocytes.<sup>[3]</sup>

Although most infections are asymptomatic, CMV can cause symptoms similar to infectious mononucleosis which is caused by Human Herpes Virus. The affected person is able to shed

the virus from the pharynx and through urine for years after primary infection. CMV is capable of establishing a lifelong latency.<sup>[3]</sup>

#### **1.4.10.1 Pathogenesis in Immunosuppressed hosts**

Immunosuppressed is a large group that includes organ recipients, bone marrow recipients, patient who undergo chemotherapy and AIDS patient's primary infection is much severe than normal individuals. And recurrent infection is also associated with apparent symptoms. This increases the rates of morbidity and mortality. Several complications are reported including pneumonia, leucopenia in solid organ graft, gastroenteritis and chorioretinitis are common among AIDS patients.<sup>[3]</sup>

#### **1.4.10.2. Pathogenesis in congenital and prenatal infections**

In both cases of primary and recurrent infection the virus can be transmitted in utero. Intrauterine transmission happens in 1% ( 3) of seropositive pregnant women. This type of infection can lead to the death of the fetus in utero. Infants also can acquire the virus during delivery from genital tract of the mother and through breast feeding. Cytomegalic inclusion disease is usually associated with involvement of central nervous system and reticuloendothelial system. Symptoms include Jaundice, growth retardation, hepatosplenomegaly, microcephaly and retinitis. Mortality can be up to 20%.<sup>[3]</sup>

#### **1.4.10.3. Immunity**

Antibodies against CMV in human sera increase with age. And the reactivation of latent infection occurs in the presence of humeral immunity .The presence of antibodies in breast milk doesn't prevent transmitting the infection to breast feeding infants. But they can prevent development of a serious disease in the infant.<sup>[3]</sup>

#### **1.4.10.4. Clinical picture**

CMV infections varies according to several factors, the most significant one is age, Mode of acquisition and clinical presentation.

The clinical features according to age:

#### **1.4.10.5. Newborns**

The virus reaches the fetus through placenta leading to both morbidity and mortality. Also they can acquire the infection during delivery. New born symptoms include lethargy and neurological symptoms (Seizures and microcephaly), accompanied by jaundice.



#### **1.4.10.6. Infants**

They acquire the infection through breast feeding. This infection is asymptomatic.

#### **1.4.10.7. Young children**

Acquire the infection through direct contact with other children in schools and play grounds. Also this is asymptomatic infection.

#### **1.4.10.8. Adolescence and Early adulthood**

This group is sexually active; hence infection is acquired through kissing and sexual intercourse. Developing symptoms similar to infectious mononucleosis (Enlarged lymph nodes and Pharyngitis).

#### **1.4.10.9. Epidemiology**

Worldwide cytomegalovirus(CMV)is a universally distributed pathogen with approximately 40 – 100% of the world's population having CMV antibody present in blood as evidence of infection the highest prevalence in the developing world. In US >90% of healthy adult have become infected with CMV by the age of 80 years. Immunocompromised patients (AIDS patients or organ transplant recipients) premature infants, and newborns with congenital CMV are at a high risk of developing serious, life threatening illness with CMV infection. In the US approximately 30,000 children are born annually with congenital CMV infection, 20% of whom develop permanent disabilities. Transmission of infection has been reported to occur in day-care center furthermore, the majority of these congenital infection result from recurrent infection in pregnant woman.

#### **1.4.11. Laboratory assessment of CMV infection in pregnant women**

CMV was recognized as the cause of fetal stillbirth following a cytomegalic inclusion disease (CID) in the mid-1950s when it was first grown in tissue cultures in three laboratories.<sup>[18]</sup> Since then demonstration of CMV infection of the mother or fetus by laboratory testing has become an essential part of the assessment of pregnancies at risk.<sup>[19]</sup> Assessment of congenital CMV infection begins with maternal serology which should establish recent primary or secondary infection . Not all maternal infections result in fetal transmission and damage. Only 35–50% of maternal primary infections and 0.2–2% of secondary infections lead to fetal infection, out of which only 5–15% in primary infection and about 1% in secondary infections are clinically affected.<sup>[20]</sup> Therefore , following maternal diagnosis, and if early pregnancy termination was not chosen, subsequent prenatal diagnosis should take



place using methods for virus detection in AF samples. Demonstration of maternal infection relies on ELISA IgM and IgG assays and on CMV IgG avidity assay. for CMV there are currently no serological “gold standard” assays which can be used for confirmation and reassurance. Recently an attempt to find association between viral load in maternal blood and the risk for fetal infection did not yield positive results.<sup>[21]</sup>

#### **1.4.12. Prenatal assessment of congenital CMV infection**

Maternal infection during pregnancy prompts testing for fetal infection as outlined in Prenatal CMV diagnosis cannot rely on detection of fetal IgM since frequently the fetus does not develop IgM.<sup>[22]</sup> On the other hand, because CMV is excreted in the urine of the infected fetus, detection of virus in the AF has proven to be a highly sensitive and reliable method. Numerous studies have focused on the most appropriate timing for performing amniocentesis which will yield the best sensitivity for detection of fetal infection.<sup>[22][23]</sup> These studies clearly indicated that amniotic fluid should be collected on 21–23 gestational week and at least 6–9 weeks past maternal infection. If these requirements are met then the sensitivity of detection of intrauterine infection can reach over 95% while the general sensitivity is only 70–80%. One study measured the sensitivity for AF obtained at gestational weeks 14–20 and reported only 45%.<sup>[24]</sup>

#### **1.4.12. Laboratory diagnosis**

##### **1.4.12.1. Specimen:** mainly body fluids:

Diagnosis of Cytomegalovirus can be done through several ways including:

##### **1.4.12.2. Virus Isolation**

Up to now human fibroblast cells are used for the isolation of cytomegalovirus CMV is a slow growing virus. It takes it up to 4 weeks to show cytopathic effect, which appears as giant multinucleated cells. CMV infects a wide range of tissues and cell types: it has been found in salivary glands, lungLiver, pancreas, kidney, ear, eye, placenta, alimentary Tract, heart, ovaries, pituitary, brain, skin, thyroid, esophagus, prostate, testes, and adrenals.<sup>[25]</sup> Viral isolation direct detection of CMV antigen in blood, andPatients with clinical syndromes that are attributed to aberrant immunological responses triggered by the presence of CMV, rather than to tissue damage related to active replication of the virus such as the Guillain-Barre syndrome.<sup>[26]</sup>

#### 1.4.12.3. Serological techniques

Detection of IgM and IgG by:

#### 1.4.12.4. Direct detection method

#### 1.4.12.5. Direct diagnosis of viral nucleic acids

**1.4.12.6. Dot and blotting:** nucleic acids which are present in clinical samples in reasonable numbers can be readily and specifically detected by blotting and fixing to a flexible support (membrane) for subsequent hybridization of a complementary probe.

Nucleic acids are conveniently fixed to membrane (nitrocellulose and nylon) by application as dots or preferably slots using a blotting apparatus (Bio Rad) which sandwiches the membrane and allow a vacuum to be applied to the underside while specimen nucleic acids are applied through a plastic template containing an array of dots or slots. Following fixing to the membrane by backing or UV irradiation, the nucleic acids can be detected by probe hybridization.

**1.4.12.5. In-situ hybridization:** Principle, specific RNA and or denatured DNA sequences in tissue can be detected by hybridization to labeled probe. In-situ hybridization (ISH) is the most suitable for the detection of nucleic acids that are not-uniformly distributed in tissue or cell, and consequently, examination of virus-infected samples, where the infection is often focal, can provide rewarding results. The detection of viral nucleic acids by ISH is conceptually analogous to detection of antigen by immunochemistry and must satisfy the same primary objective; it must reflect accurately the distribution of target molecules in the sample. Since genomes can be double or single-strand, DNA or RNA molecules the protocols must be designed accordingly, and a denaturation step for double-strand genomes is necessary. Fresh frozen or formalin-fixed tissue can be used.<sup>[27]</sup>

#### 1.4.12.6. Radioimmunoassay (RIA)

This method offers the advantages of specificity and high sensitivity. The principle of the method is identical to that of enzyme-labelled anti-globulins. Used to measure either antibody or antigen and detection of antigen. It includes radioimmuno precipitation test which is measurement of antibody undertaken in solution or in microtitre plate. Radioimmuno precipitation test the radiolabelled antigen is reacted with test serum in which the antibody is to be detected and antibody/antigen complex is precipitated and separated from the unbound

labeled antigen by treatment either with anti-globulin or by ammonium sulphate precipitation.<sup>[28]</sup>

#### **1.4.12.7. Enzyme linked immunosorbent assay (ELISA)**

ELISA techniques are becoming increasingly used in the diagnosis of microbial infections. They are specific and sensitive and require only a small amount of specimen. A quantitative result is possible from a single dilution of serum. The results of qualitative ELISA techniques can be read visually and large numbers of specimens can be tested at one time and can be easily automated for use in epidemiological surveys. The principle of this test is the enzyme linked immunosorbent assay uses an enzyme system to show the specific combination of an antigen with its antibody. It has two types direct and indirect.<sup>[29]</sup>

#### **1.4.12.8. Molecular techniques**

##### **1.4.12.8.1. Polymerase chain reaction (PCR)**

Until recently polymerase chain reaction was widely regarded as research tool with limited application as the diagnostic workbench. In principle polymerase chain reaction is a simple procedure that uses a heat-stable DNA polymerase to amplify target molecules prior to their detection. Identification of polymerase chain reaction product is commonly done by (1) Dot blot hybridization using a probe specific for the amplified sequences or immunological (2) southern blot analysis.<sup>[26]</sup>

##### **1.4.12.8.2. Western blots technique**

Western blots (WB) employing viral polypeptides separated from purified viral particles has repeatedly been shown to be a reliable and sensitive method to detect HCMV-specific IGM. Description: the protein antigen of cytomegalovirus are separated by gel electrophoresis, and then transferred to nitrocellulose membrane (i.e. to the respective WB strips).

If specific antibodies are present in the sample, they will bind to respective antigen, the complex is labeled with conjugate and detected through a colour reaction with substrate.<sup>[29]</sup>

#### **1.4.12.9. Immunofluorescent technique**

In general, antigen can be detected by three different methods:<sup>[1]</sup> by direct conjugate method,<sup>[2]</sup> by indirect conjugate method,<sup>[3]</sup> by multilayer method.

In direct method procedure the antibody is conjugated directly to fluorochrome or enzyme for fluorescence, fluorescein isothiocyanate (FITC) is the most commonly used,

tetramethylrhodamineisothiocyanate(TRITC) is the next most popular. The advantage of direct conjugate includes speed and high specificity but is inconvenient to prepare a conjugate from each individual antibody used. The direct procedure is particularly useful if human convalescent serum is the only available antibody with activity for particular antigen and especially when clinical sample contain immunoglobulin formed by patient .direct conjugates are also useful in double-labelling experiments. A limitation of method is that conjugation is not infrequently accompanied by some loss of antibody potency. thus, as the serum is only of moderate potency at the start ,a direct conjugate may be of limited value. in this circumstance the indirect procedure may be more satisfactory approach here ,the primary antibody (1-commonly raised in rabbits ) is generally detected with a species –specific conjugated antibody.<sup>[2]</sup> consequently ,this procedure allows a single conjugated antibody. Multilayer method are generally used to increase sensitivity .thus ,a multilayer peroxidase – antiperoxidase complex is often used to detect viral antigen ,particularly in formalin-fixed tissue in which antigen immunoreactivity has been reduced by the fixation process .the avidin-biotin complex(ABC) system is a satisfactory alternative to PAP system.<sup>[26]</sup>

#### 1.4.13. Treatment

Maternal CMV infections may be treated with immune boosting drugs to help decrease the risk of the baby being born with symptoms of CMV infection. There are no treatments for prenatal or postnatal therapy of the infection. Vaccines for treatment are still in the research and developmental stages.<sup>[30]</sup>

#### 1.4.14 Prevention and control

If you are pregnant or considering it, you might want to get tested. If you've already been infected with CMV and its dormant, that's safer for your unborn child than if you become infected for the first time while pregnant; although it's possible to become infected with more than one strain or to reactivate your sleeping infection, and this could affect the baby. You want to avoid all infection while pregnant, so just use a little more caution. Don't share forks or cups or kisses with kids under six, be wearing when dealing with diapers or cleaning up blood or other fluid and wash your hands. That can't be emphasized.<sup>[31]</sup>

#### 1.4.14. Control

There's no vaccine at this time.

#### 1.4.15. Previous study

in study done in Sudan a total of ninety serum specimens of pregnant ladies attending antenatal care at Omdurman Maternity Hospital(32), their age range (15-45 years old). Out of them 67 ladies (74.4%) were CMV IgG positive while 13 (14.4%) were positive for both antibodies (IgG, IgM), and 10 ladies (11.1%) were negative. The results showed that the highest anti-CMV IgG seropositivity rate was among those in 25-35 years age range 3 ladies (4.5%) were reported on having past history of congenital abnormalities, Present of history abortion in 20 ladies (62.5%), Also high positive results were observed among third trimesters group and among multigravidae ladies. According to chi-square analysis there was insignificant correlation between age, abortion, trimester, gravidity and presence of CMV antibodies ( $P > 0.05$ ).

in study done in Kaduna state, Nigeria in (2014).363blood sample attending antenatal clinic in Kaduna state(33). The prevalence of Cytomegalovirus was found to be 94.8% (313/330) among pregnant women, and all (100%: 33/33) the non pregnant women had antibodies to CMV. There was no significant difference in the prevalence of CMV infection between the pregnant and non-pregnant women ( $\chi^2=1.784$ ,  $df=1$ ,  $P=0.182$ ) Further analysis of the data based on geographical zoning, showed that there was a very strong Significant difference in the distribution of CMV among the pregnant women by zonal location in Kaduna State ( $\chi^2=15.381$ ,  $df=2$ ,  $P=0.000$ ). Women Attending HGSH, Zaria from the North zone had the highest prevalence (99.1%: 109/110) while those attending YDMH, Kaduna from the Central zone had the lowest prevalence (88.2%: 97/110). in study done in Sudan in (2013) a total of 200 pregnant women were included in this study. The ages of all women tested ranged from 18 to 43 years.

Overall prevalence of anti-CMV IgG antibodies in pregnant women attending Omdurman Maternity Hospital for Delivery was 97.5%. 195 women out of 200 women studied were positive for CMV IgG, while only 6% were CMV IgM positive(34). The results showed that the highest anti-CMV IgGseropositivity rate was among those with 40 years and more, while the lowest rate was among women less than 20 years old. CMV seropositivity was analyzed with respect to parity. No statistically significant difference was found between primigravidas and multiparous women on CMV infection .Out of the 195 women who were CMV IgG positive, 8 women (4.2%) were reported on having child with congenitalN abnormalities, while no one from the negative group was reported on having congenitally formed child. In

this study, no significant difference ( $P > 0.05$ ) was found between working and non working women in CMV seropositivity. The study demonstrates that the level of education of pregnant women had no effect on CMV seropositivity.

## **2. MATERIALS AND METHODS**

### **2.1. Approach**

The current study was include both qualitative and quantitative variable, qualitative by screening the CMV IgM and IgG antibodies in human serum, quantitative which the percent of the causative agent was calculated.

### **2.2. Study Design**

Cress sectional study design.

**2.3. Study area:** The study area is hospital base study in police Hospital during the period from April to march 2016.

### **2.4. Study population**

Pregnant women and repeated abortion who were attending to police hospital and Wafaa clinic for care and consultation.

### **2.5. Variables**

Age of the woman, previous miscarriages, repeated abortion and number pregnancies.

**2. 6. Ethical consideration:** verbal consent was taken from the participant.

### **2.7. Sample size**

Eighty eight samples were including into study.

### **2.8. Sampling technique**

Cases were convince non random sampling selected from the women whom were present in the hospital at the time of the study.

### **2.9. Methods & tools**

### **2.10. Data collection tool**

By using a structural questionnaire, to collect the satiable information to carry the study

### 2.11. Processing

By using Enzyme-Immuno assay (EIA) for the detection of IgM, IgG antibodies to CMV in human serum or plasma, CMVACON Laboratoties, Inc EIA test kit for EIA IgM and IgG.

#### Enzyme linked immune sorbent assay

**Principle of the test:** the test kit contains microtiter strips each with 8 break-off reagent wells coated with CMV antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human igG enzyme-labeled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

### RESULT

#### Calculation of result

**Semiquantitative:** Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2.

Calculate the ratio according to the following formula:

Extinction of the control or patient sample = Ratio

Extinction of calibrator 2

EUROIMMUN recommends interpreting result as follows:

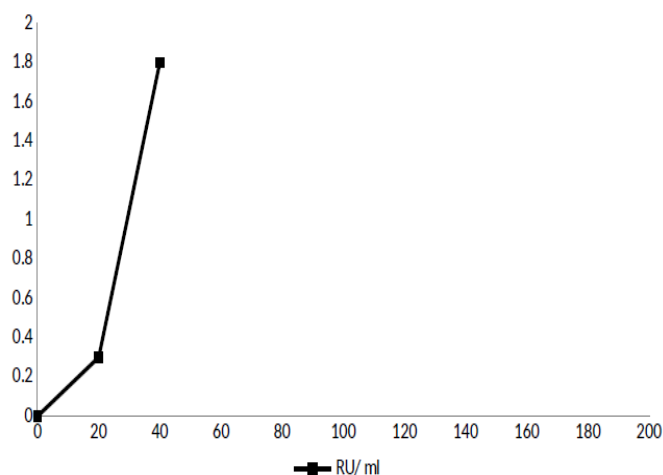
Ratio <0.8: negative

Ratio  $\geq 0.8$  to <1.1: borderline

Ratio  $\geq 1.1$ : positive

**Quantitative:** The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for 3 calibration sera against the corresponding units (linear/linear. Use “point-to-point” plotting for calculation of the standard curve b computer. The following plot is an example of typical calibration curve. Please do not use this curve for determination of antibody concentrations in patient samples.





If the extinction for a patient sample lies above the calibrator 1 (200 RU/ml), the result should be reported as “>200 RU/ml”. It is recommended that the sample be retested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	negative
≥16 to <22 RU/ml:	borderline
≥22 RU/ml:	positive

For duplication determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN to retest the samples.

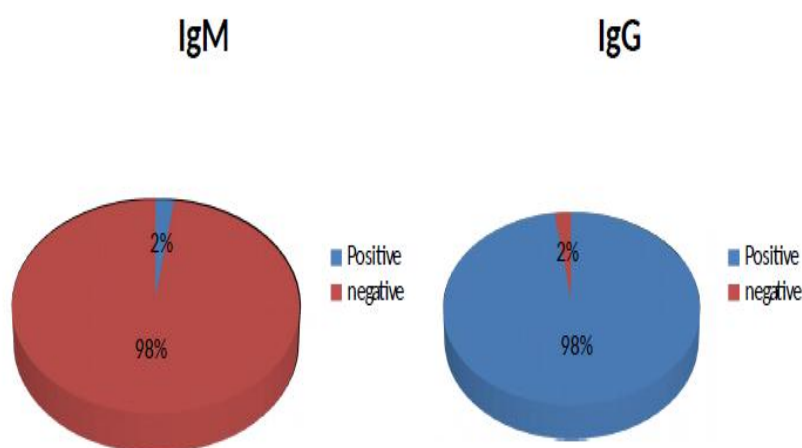
A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not be present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible if there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive finding. Significant titer increase (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7-10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should

be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

### 3. RESULT

#### 3.1. Detection of cytomegalovirus frequent infection

Eighty eight serum sample was obtained to detect Cytomegalovirus immunoglobulin among pregnant and repeated abortion women ,IgG and IgM were estimated in patient serum to check the present of virus Out of 88 sample 86 give positive result for IgG with significant rate (p. value 0.00) and only 2 sample from all sample show positive result for IgM (p. value 0.00). **chart (1)**



**Chart (1): frequency of IgG, IgM among sample**

#### 3.2. Correlation between cytomegalovirus and age

The patients was distributed into difference group of age, for IgM the group of (31-40) years has highest rate( 2) positive for IgM while other group show negative result. For IgG the positive result distributed among groups (20-30) years 32 positive cases (31-40) years 36 positive cases, (41-50) years 11 positive cases and among more than (50) years there is no positive result see **chart (2)**.

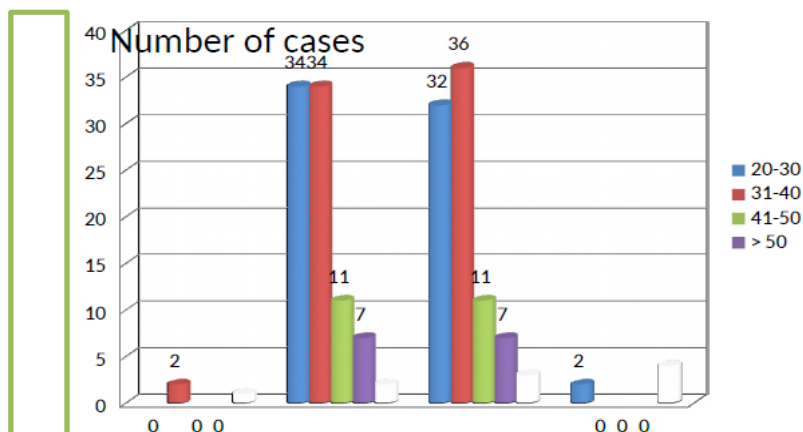


Chart (2) distribution of IgM and IgG among age groups

### 3.3. prevalence of cytomegalovirus immunoglobulin among diseases group

Among 32 case of repeat abortion, IgM was detected in (2) cases 6.3% ) which is significant rate (p.value 0.05 ), while the IgG was detected in 31case 96.9% (p.value 0.6 ) Out of 13 abnormal birth child case cytomegalovirus IgM was detected in 2 cases (15.4%) **Chart (3)** ,and all cases show positive for IgG 96.9% ( p.value 0.5 ) **Chart (4)**

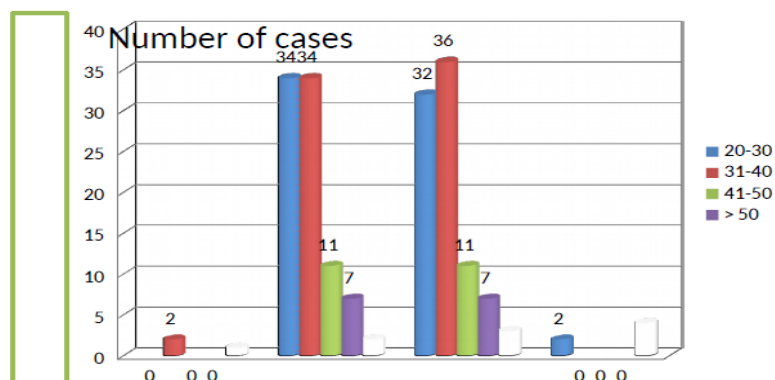


Chart (3) distribution of IgM among diseases groups

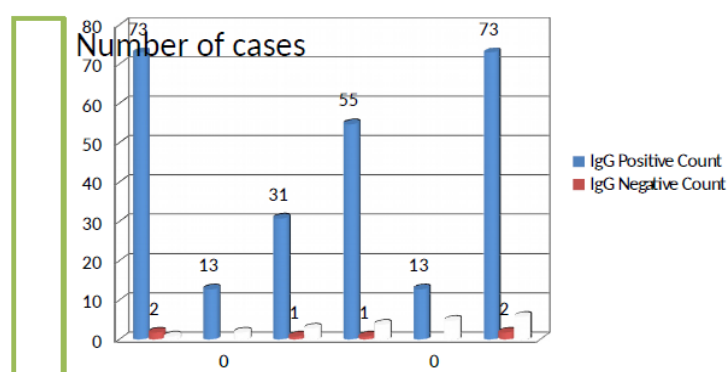


Chart (4) distribution of IgG among diseases groups

#### 4.1. DISCUSSION

Total of 88 sample was used to detect cytomegalovirus IgM and IgG these sample was collected from pregnant and repeated abortion women, IgM was detected in 2 case from 32 case of repeat abortion, while the IgG was detected in 31case 96.9% This study revealed that the serofrequency of CMV among pregnant women is high, as well as non-pregnant women, CMV IgG antibodies represent 95% of the cases, while 1.6% were positive IgM antibodies. The serofrequency of CMV among nonpregnant women (control group) was 10%, 100%for IgM and IgG respectively Positive CMV IgM results indicate a recent infection (primary, reactivation, or reinfection). IgM antibody responses in secondary (reactivation CMV infections have been demonstrated, in a few pregnant women.

The detection of CMV IgG indicated that the pregnant ladies had previously been infected with CMV or recent CMV infection. After CMV infection, IgG remains in the body for life and protects considerably against the next infections. This indicates that a negative results of CMV IgG test means that the women have not been infected with the virus. In this study These findings were similar to those obtained in Nigeria , Kaduna state by Yeroh, M(33), 94.8% among pregnant women, and all (100%) the non-pregnant women had antibodies to CMV infection Our finding also was closely related to the results obtain in study done in Sudan in 2013by Siedge Abd ELKareem.(34) which reported that the prevalence of anti-CMV IgG antibodies in pregnant women attending Omdurman Maternity Hospital for Delivery was 97.5%. while only 6% were CMV IgM positive but the difference may due to the amount of sample size included in the study. Our results indicate statistically significant difference was found between CMV infection and abortion ( $P > 0.05$ ).

#### 4.2CONCLUSION

Our results suggest a correlation between CMV infection and repeat abortion furthermore CMV is one of the evidence in abnormal birth child This a relationship that requires further investigation.

#### 4.3. RECOMMENDATIONS

1. Throughout your pregnancy practice good personal hygiene, including hand washing with soap and water.
2. If you develop a mononucleosis-like illness, you should be checked for CMV infection.
3. Refrain from sharing food, eating utensils and drinking utensils with anyone.

4. Your doctor can test the CMV antibodies to determine if you have already had CMV infection.
5. Breastfeeding benefits outweigh the minimal risk of transmitting CMV.

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