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Detection of Anti cytomegalovirus IgG and IgM among Women with History of Abortion in Khartoum State Hospitals

الكشف عن الاجسام المضادة للفيروس المضخم للخلايا وسط النساء المجهضات سابقا فى مختلف مستشفيات بولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of MSc in Medical Laboratory Science (Microbiology)

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الآيـــــــة

قَالَ تَعَالَىٰ: أَعُوذُ بِٱللَّهِ مِنَ ٱلشَّيۡطَٰنِ ٱلرَّجِيمِ

﴿ ٱللَّهُ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡحَيُّ ٱلۡقَيُّومُۚ لَا تَأۡخُذُهُۥ سِنَةٞ وَلَا نَوۡمٞۚ لَّهُۥ مَا فِي ٱلسَّمَٰوَٰتِ وَمَا فِي ٱلۡأَرۡضِۗ مَن ذَا ٱلَّذِي يَشۡفَعُ عِندَهُۥٓ إِلَّا بِإِذۡنِهِۦۚ يَعۡلَمُ مَا بَيۡنَ أَيۡدِيهِمۡ وَمَا خَلۡفَهُمۡۖ وَلَا يُحِيطُونَ بِشَيۡءٖ مِّنۡ عِلۡمِهِۦٓ إِلَّا بِمَا شَآءَۚ وَسِعَ كُرۡسِيُّهُ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضَۖ وَلَا يَـُٔودُهُۥ حِفۡظُهُمَاۚ وَهُوَ ٱلۡعَلِيُّ ٱلۡعَظِيمُ ٢٥٥ ﴾

صدق الله العظيم

سورة البقرة: الآية(٢٥٥)

DEDICATION

To my dear parents,

Brothers, sisters,

colleagues and friends.

AKNOWLEDGMENT

Firstly all thank to ALMIGHTY ALLAH to reconcile me to complete the study.

I would like to acknowledge my supervisor ***Dr. Ahmed Ibrahim*** *for close supervision, valuable advice, and without his guide this study would not appear.*

To all those who supporting me, help me, and encouraged me

To Sohair Ramadan, Mohammed Karrar and all staff of research lab at Sudan University of Science and Technology for providing me good environment for practical work.

ABSTRACT

This was a cross sectional study aimed to determine the frequency of CMV antibodies (IgG, IgM) using ELISA technique among women with history of abortion from Khartoum State hospitals from June to October (2015).

Eighty nine blood samples were collected from aborted women.The women included in this study varied in number of abortion from once (20), to two or more (69) as well as age with mean age of (31.79) years.The samples were tested by ELISAand it was found that 87 (97.8 %) were positive for CMV IgG, while all the samples were negative for CMV IgM.

In this study there was insignificant relationship between CMV IgG and number of abortions, and insignificant association between age groups and the IgG results but there was significant relationship between ag groups and number of abortions (*P*. value 0.001)

The study concluded that screening women with history of abortion for the presence of anti CMV IgG using ELISA alone is of a limited value; hence, it is necessary to evaluate CMV infection using advanced techniques such as polymerase chain reaction (PCR).

المستخلص

هذه الدراسة المقطعية هدفت الى تحديد تواتر الاجسام المضادة IgM ، IgG للفيروس المضخم للخلايا باستخدام تقنية الاليسا بين النساء المجهضات سابقا في مستشفيات ولاية الخرطوم في الفترة بين يونيو واكتوبر 2015م .

جمعت 89 عينة دم من النساء المجهضات سابقا ، النساء المدرجة في هذه الدراسة تختلف في عدد مرات الاجهاض من مرة واحدة (20) الى اثنين او اكثر (69) ومتوسط اعمراهن (31,79).

العينات التي تم اختبارها بواسطة الاليسا اظهرت 87 منها ايجابية للجسم المضاد IgG للفيروس المضخم للخلايا في حين ان جميع العينات كانت سلبية للجسم المضاد IgM .

في هذه الدراسة كانت هنالك علاقة ضئيلة بين الجسم المضاد IgG للفيروس المضخم للخلايا وعدد مرات الاجهاض و الفئات العمرية ولكن هنالك ارتباط قوي بين الفئات العمرية وعدد مرات الاجهاض (P value 0.001) .

خلصت الدراسة الى ان فحص النساء المجهضات سابقا لوجود الاجسام المضادة IgG للفيروس المضخم للخلايا باستخدام تقنية الاليسا وحدها له اهمية محدودة ومن ثم فإن من الضروري تقييم العدوى بالفيروس المضخم للخلايا باستخدام تقنيات متطورة مثل تفاعل البلمرة المتسلسل PCR.

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LIST OF ABBREVIATIONS

BOH Bad obstetric history

CMV Cytomegalovirus

CDC Center of Disease and Control

DNA Deoxyribo Nucleic Acid

ELISA Enzyme Linked Immune Sorbent Assay

HCMV Human Cytomegalovirus

HSV Herpes simplex virus

IgG Immunoglobulin G

IgM Immunoglobulin M

NK Natural Killer

PCR Polymerase Chain Reaction

RPL Recurrent Pregnancy Loss

RSA Recurrent Spontaneous Abortion

SPSS Statistical Package for Social Sciences

TORCH Toxoplasma Rubella Cytomegalovirus Herpes simplex virus

CHAPTER ONE
INTRODUCTION

1. Introduction

Maternal infections play a critical role in pregnancy wastage and their occurrence in patients with bad obstetric history (BOH) is a significant factor (Chopra *et al*., 2004).

Recurrent pregnancy wastage due to maternal infections transmissible in utero can be caused by wide array of pathogenic organisms mostly belonging to the torch group (Kishore *et al*., 2011).

Torch infections in pregnant women may be transferred to the fetus inside the womb or during the birth, leading to spontaneous abortion or occurrence of disorders accompanied with symptoms in the neonate (Ariani and Chaichi, 2014)

These infections usually occur before the woman realizes that she is pregnant or seeks medical attention. The primary infection is likely to have a more important effect on fetus than recurrent infection (Sadik *et al*., 2012).

All these pathogens infect and affect the fetus at various stages of gestation and can cause various types of feto-maternal complications (Kishore *et al*., 2011).

Cytomegalovirus (CMV) infects high percentage of human during their life and after recovery of disease; it hides in a group of leukocytes. Although this virus is not considered as a hazardous agent for health, in pregnant women it is a major factor that threatens the health of neonates (Ariani and Chaichi, 2014) and a leading infectious cause of congenital malformation in developed countries, with a mean global incidence of 0.64%. Up to 32% of mothers with a primary CMV infection will vertically transmit the virus. Congenital CMV may results in the development of serious clinical squeal (Hamilton *et al*., 2012). Its clinical manifestations range from asymptomatic forms (90% of cases) to severe fatal

damage, and rare cases of death Following miscarriage latency a primary infection may be punctuated by periodic reactivations that gives rise to recurrent infection, and in utero transmission may occur during either primary or recurrent infections (Khairi *et al*., 2013).

Cytomegalovirus infection during pregnancy is far more complex than other infections, due to the ability of the virus to be frequently reactivated during child bearing age and be transmitted to the fetus in spite of maternal immunity (Jasim *et al*., 2011).

Reactivation of latent CMV results in at least as many adverse outcomes as primary infections. Infection of the placenta with transplacental movement of CMV across the materno-fetal interface is a pre-requisite for infection of the fetus. Fetal injury results from direct viral cytopathic damage to CMV infected fetus. However; infection can also be restricted to the placenta, and there is increasing evidence that indirect effects of CMV through placenta infection contribute to adverse pregnancy outcome (Hamilton *et al*., 2012).

Rationale

CMV is a common virus that infects people of all ages and can cause disease in immune compromised people and babies can infect before birth. About 1 in 150 children is born with congenital CMV infection and about 1 of every 5 children born with congenital CMV infection will develop permanent problems such as hearing loss or developmental disabilities due to the infection (CDC, 2010).

Recurrent spontaneous abortions occur by different etiological causes including viral infections. Cytomegalovirus and rubella infections can cause or promote the recurrent fetal loss. Cytomegalovirus virus is one of the important viral infections which may play a role in recurrent spontaneous abortion (Ebadi *et al*., 2011), the risk of intrauterine infection largely depends on the time of maternal infection during pregnancy (Bagheri *et al*., 2012).

Since, CMV can remain in the body without treatment in subsequent pregnancy it may lead to recurrent abortion (Meybodi and Taheripanah, 2000). Hence; it is important to screen pregnant women for the presence of CMV to protect them against recurrent abortions and protect future children from congenital CMV infection.

General objectives:

To detect CMV IgG and IgM antibodies in women with history of abortion

Specific objectives:

1. To determine anti CMV IgM and IgG in women with history of abortion

2. To determine the association between the CMV infection, number of abortion and age

CHAPTER TWO
LITERATURE REVIEW

2.1 Cytomegalovirus

Cytomegalovirus belongs to the family herpesviridae of betaherpesvirnae subfamily. It is also known as HHV-5 (Abdul wahab, 2012). Compared to other human herpes viruses, it is the largest, with a genome of 235 kb encoding 165 genes. The virion consists of a double- stranded linear DNA core in an icosahedral nucleocapsid enveloped by a proteinaceous matrix, which are enclosed in a lipid bilayer envelope that is derived from the nuclear membrane (Hama and Abdurahman,2013).

The virus is highly species-specific and only human strains are known to produce human disease. Initial infection with HCMV commonly occurs during childhood and depending on geographic location and socioeconomic group (35-90%) of the population have antibody against the virus by adulthood(Hama and Abdurahman, 2013).

The virus is ubiquitous virus infection with worldwide distribution and association with opportunistic disease that has been recognized in more highly developed areas of the world and it covers larger areasin developing countries and in communities with lower socioeconomic status (Elamin and Omer, 2015).

2.2. Transmission of CMV

The different modes of transmission are: close interpersonal contact (body fluids like urine and saliva), sexual activity, breastfeeding, blood transfusions and organ transplantation. After the initial infections, the virus stays in saliva, tears, semen, urine, cervical secretions, and blood for month to years. Seroconversion occur at mucosal surfaces via infected urine, saliva, or other body fluids, making children an excellent host for the virus, especially in day care settings. However; all

transmission modes are not equivalent in terms of risk. The most dangerous transmission mode is the congenital one, in which the pregnant mother transmits the virus via placenta to the fetus (Nassikas and Tsaples, 2013).

There are two possible routes of transplacental transmission of CMV to the fetus: across syncytiotrophoblasts with subsequent infection of the underlying cytotrophoblasts, or via invasive cytotrophoblasts within the uterine wall. Mode of perinatal transmission include ingestion or aspiration of cervicovaginal secretions at the time of delivery and ingestion of breast milk after delivery (McCarthy *et al*., 2009).

Transplacental transmission of CMV may occur even in seropositive women after non primary infection. The non primary infection consist of two different routes, those are reinfection with a newly acquired CMV strain and reactivation of a latent CMV infection (Nagamori *et al*., 2010).

2.3 Pathogenesis

In primary maternal infection, the antiviral immune response begins proximate to virus transmission to the fetus, whereas in the case of recurrent infections, the virus transmission occurs in the presence of both humoral and cell mediated immune responses (Revello and Gerna, 2002).

Multiple mechanism of immune evasion for HCMV could relate to the pathogenic role of the virus. It has been reported that about 15% of women undergoing primary infection during the first months of pregnancy abort spontaneously, showing placental but not fetal infection. Subsequently in the course of pregnancy, placental infection has been shown to be consistently associated with fetal infection (Revello and Gerna, 2002).

2. 4 Signs and symptoms

CMV is rather benign disease, whose worse symptoms are similar to those of a mild flu. While acquired infection is harmless for children, CMV can have serious consequences for immune system deficient patients as well as for congenitally infected infants. The latter develop serious permanent impairments which mostly affect the central nervous system and include progressive hearing loss, spastic tetraplegia, mental retardation and visual impairments (Nassikas and Tsaples, 2013). Apart from major clinical findings observed in HCMV mononucleosis (such as fever, cervical adenopathy, sore throat, splenomegaly, hepatomegaly, and rash) which are not commonly detectable. In addition, a slight increase in serum levels of liver enzymes (alanine transaminase, aspartate transaminase) may help in dating the onset of infection (Revello and Gerna, 2002).

Infants who acquire CMV after birth, especially if they are premature, may develop a sepsis-like syndrome, pneumonia, hepatosplenomegaly, hepatitis, thrombocytopenia, and atypical lymphocytosis. However, if transmission is via breast milk, the risk of severe symptomatic disease and long-term sequelae is low (Caserta, 2013).

2.5 CMV and abortion

Pregnancy loss has been attributed to several factors involved in human reproduction such as genetic and uterine abnormalities, endocrine and immunological dysfunction infectious agents, environmental pollutants, psychogenetic factors and endometriosis (Hussan, 2013).

Many infectious factors are interfering most of them are effective only spontaneous abortion but their role on recurrent abortion aren’t demonstrated (Meybodi and Taheripanah, 2000).

These entire infectious agents induce a shift of immune response during pregnancy from Th2 to Th1 and apoptosis which can be observed clinically as an abortion process (Hussan, 2013).

Human cytomegalovirus is important as it causes abortion and in more cases congenital damage fetal death and mental retardation (Abdul Wahab, 2012).

Infection by CMV can cause pregnancy loss or spontaneous abortion which is defined as any natural abortion occurring at or before 28th week of gestation with the fetus weighing less than 1000g (Elamin and Omer, 2015)but if it is repeated 2 or 3 times , it called recurrent pregnancy loss (RPL)(Ariani and Chaichi, 2014).

CMV, which can produce chronic or recurrent maternal infection and exhibits a high tropism for cervical mucosa, is the most implicated virus as a potential cause of recurrent spontaneous abortion (RSA). During pregnancy CMV can reach the placenta from the cervix or by viremia following both primary and recurrent maternal infection, with subsequent vascular insufficiency, tissue damage, and transmission to the fetus. Abortion could follow both placental detachment and fetal death (Nigro *et al*., 2011). CMV infection of the gestational tissue is not the only likely pathogenic mechanism in the CMV induced recurrent abortion, an immune mediated process could also occur, CMV down regulate cytotrophoblast molecules, which are potentially critical for placental invasion of the maternal uterine tissue and fetal development. The interaction between CMV and decidual NK cells may cause NK cell activation and fetal death or miscarriage. NK cell activity may be increased in the uterine decidua of patients with anembryonic pregnancies or RSA, while is typically depressed in early normal pregnancy (Nigro *et al*., 2011).

2.6Laboratory diagnosis of CMV

Detection of HCMV specific antibodies is the most common approach used to identify HCMV infected individuals. Many types of assay are available for the

determination of the anti HCMV antibody titer in serum with different degree of sensitivity; the most widely used procedure is the ELISA (Hama and Abdurahman, 2013).

The presence of CMV specific Immunoglobulin M (IgM) may not be indicative of primary infection, since it is also produced during reactivation and re-infection (Khalf *et al*., 2012).

Serological diagnosis of primary CMV infection during pregnancy can be difficult as CMV immunoglobulin M, while suggestive of recent infection, can remain positive for many months and can also represent reactivation of past infection (McCarthy *et al*., 2009).

Detection of increasing HCMV IgG levels over time is an unreliable approach for distinguishing primary from non primary HCMV infection (Hama and Abdurahman, 2013).Another method of determining the timing of maternal CMV infections is to measure antibody avidity, which refers to strength of antibody binding to a target antigen. As the immune response to a particular antigen mature over time, avidity increases (Carlson, 2010) .The IgG avidity assay can help distinguish primary infection from past or recurrent infection and can assist in determining when infection occurred (Yinon *et al*., 2012).Cytomegalovirus specific immunoglobulin G avidity may assist in timing the infection but it is only performed in reference laboratories and it is often unreliable (McCarthy *et al*., 2009) . Patients who are acutely infected also will usually test positive by culture or PCR for virus in the blood and urine (Duff, 2010).

The single best test for the diagnosis of congenital infection is detection of virus in the amniotic fluid by culture or PCR (Duff , 2010).Quantitative PCR to detect high CMV viral load in the amniotic fluid has also been used to determine fetuses at risk of clinical sequelae (McCarthy *et al*., 2009).

CHAPTER THREE

3. MATERIALS& METHODS

3.1 Study design

This was a cross sectional study.

3.2 Study area

This study was conducted in Khartoum State hospitals.

3.3 Study population

Pregnant women with history of abortion

3.4 Study duration

Study was carried out during 4 month period between June and October 2015

3.5 Ethical consideration

Permission to carry out the study was obtained from the College of Graduate Studies, Sudan University of Science and Technology. All the hospitals were informed about the purpose of the study before the collection of the samples.

3.6 Sample size

Atotal of eighty nine subjects were enrolled in this study.

3.7 Data collection

Personal and clinical data were collected from hospitals' laboratory data records.

3.8 Sample collection

Under a septic conditions after wearing the gloves, alcohol antiseptic (70% ethanol) was used to clean the skin. Blood samples were collected by vein puncture and hemolysis was avoided. Samples were collected into plain container and centrifugated at 3000 rpm for 5 minutes to get serum and they were kept at – 20ºC till used.

3.9 Laboratory methods

3.9.1. Detection of CMVIgG antibodies by Enzyme Linked Immunosorbent Assay (ELISA) (appendix)

The CMV IgG EIA test skit is an immunoassay for the qualitative and quantitative detection of the presence of IgG antibodies to CMV in serum or plasma specimen. Purified CMV antigens were used to selectively detect antibodies to CMV in serum or plasma.

3.9.1.1. Principle of ELISA: The CMV IgG EIA Test Kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative and quantitative detection of IgG antibodies to CMV in human serum or plasma. The microwell plate is coated with CMV antigens. During testing, the specimen diluent and the specimens are added to the antigen coated microwell plate and then incubated, if the specimens contain IgG antibodies to CMV,it will bind to the antigens coated on the microwell plate to form immobilized antigen-CMV IgG antibody complexes. If the specimens do not contain IgG antibodies to CMV, the complexes will not be formed. After initial incubation, the microwellplate is washed to remove unbound materials. The enzyme conjugated anti human IgG antibodies are added to the microwell plate and then incubated. The enzyme conjugated anti-human IgG antibodies will bind to the immobilized antigen –CMV IgG antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of CMV IgG antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of CMV IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

3.9.1.2. Storage and stability

Unopened test kits should be stored at 2-8 ºC upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8ºC. All reagents are stable for up to 3 months after the first opening if stored between 2-8ºC

3.9.1.3. Procedure of the test

The working wash buffer was prepared by diluting the concentrated wash buffer 1:25, A1 was leaved as blank well.

Step1 100µl of calibrator 1,2,3,4 was added to well B1to E1 respectively.

Step2 100 µl of specimen diluent was added to each wells starting F1 then 5µl of specimens was added.

Step3 the microwell plate were covered with the plate sealer and incubated at 37ºC for 30 min

Step4 the plate sealer was removed and each well was washed 5 times with 350µl of working wash buffer.

Step5 100 µl of conjugate were added to each well except for the blank well.

Step6 the microwell plate was coverd with the plate sealer and incubated at 37ºC for 30 min.

Step7 the plate sealer was removed and each well was washed 5 times with 350µl of working wash buffer.

Step8 50 µl of substrate A and 50µl 0f substrate B were added to each well, a blue color was developed in wells containing positive specimens.

Step9 the microwell plate was covered with plate sealer and incubated at 37ºC for 10 min.

Step10 the plate sealer was removed then 50µl of stop solution was added to each well.

3.9.1.4. Reading of the result

Within 30 minutes the absorbance was read at 450/630-700 nm

3.9.1.5. Calculation of cut-off value

The cut-off value was calculated by subtracting the blank absorbance from the mean absorbance of calibrator 2.

3.9.1.6. Validation requirements and quality control

Results of an assay are valid if the following criteria are checked:

Blank well: The blank absorbance should be < 0. 050 if read at 450\630-700 nm

Calibrator1: mean absorbance after subtraction of blank absorbance should be < 0.150

Calibrator2: mean absorbance after subtraction of blank absorbance should be > 0.150 and< 0.400

Calibrator3: mean absorbance after subtraction of blank absorbance should be > calibrator 2 and < calibrator 4

Calibrator4: mean absorbance after subtraction of blank absorbance should be > 1.200

The test results are considered invalid if the above validation requirements are not met.

3.9.1.7. Interpretation of the result

Negative results: Samples gave an absorbance less than cut-off values were considered negative.

Positive results: Samples gave an absorbance more than cut-off values were considered positive.

3.9.2. Detection of CMV IgM antibodies by Enzyme Linked Immunosorbent Assay (ELISA) (appendix)

The CMV IgM EIA test skit is an immunoassay for the qualitative detection of the presence of IgM antibodies to CMV in serum or plasma specimen. The test utilizes

recombinant CMV antigens to selectively detectIgM antibodies to CMV in serum or plasma.

3.9.2.1. Principle of ELISA

The CMV IgM EIA Test Kit is a solid phase enzyme immunoassay based on immunocapture principle for the qualitative detection of IgM antibodies to CMV in human serum or plasma. The microwell plate is coated with anti-human IgM antibodies. During testing, the specimen diluent and the specimens are added to the antibody coated microwell plate and then incubated. If the specimens contain IgM antibodies to CMV, it will bind to the antibodies coated on the microwell plate to form immobilized anti-human IgM antibody-CMV IgM antibody complexes. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme conjugated recombinant CMV antigens are added to the microwell plate and then incubated. The enzyme conjugated recombinant CMV antigenwill bind to the anti-human IgMantibody-CMV IgM antibody complexes present .If the specimen do not contain IgM antibodies to CMV , the complexes will not be formed. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of CMV IgM antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of CMV IgM antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

3.9.2.2. Storage and stability

Unopened test kits should be stored at 2-8 ºC upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8ºC. All reagents are stable for up to 3 months after the first opening if stored between 2-8ºC

3.9.2.3. Procedure of the test

The working wash buffer was prepared by diluting the concentrated wash buffer 1:25, A1 was left as blank well.

Step1 100µl of negative control was added to well B1andC1 , 100 µl of cut-off calibrator was added to well D1and E1,100µl of positive control was added to well F1 and G1

Step2 100 µl of specimen diluent was added to each wells starting H1 then 5µl of specimens was added.

Step3 the microwell plate were covered with the plate sealer and incubated at 37ºC for 30 min

Step4 the plate sealer was removed and each well was washed 5 times with 350µl of working wash buffer.

Step5100µl of conjugate were added to each well except for the blank well.

Step6 the microwell plate was covered with the plate sealer and incubated at 37ºC for 30 min.

Step7 the plate sealer was removed and each well was washed 5 times with 350µl of working wash buffer.

Step8 50 µl of substrate A and 50µl 0f substrate B were added to each well, a blue color was developed in wells containing positive specimens.

Step9 the microwell plate was covered with plate sealer and incubated at 37ºC for 10 min.

Step10 the plate sealer was removed then 50µl of stop solution were added to each well.

3.9.2.4. Reading of the result

Within 30 minutes the absorbance was read at 450/630-700 nm

3.9.2.5. Calculation of cut-off value

The cut-off value was calculated by subtracting the blank absorbance from the mean absorbance of cut-off calibrator.

3.9.2.6. Validation requirements and quality control

Results of an assay are valid if the following criteria are checked:

Blank well: The blank absorbance should be < 0. 050 if read at 450\630-700 nm

Negative control: mean absorbance after subtraction of blank absorbance should be < 0.150

Cut-off calibrator: mean absorbance after subtraction of blank absorbance should be > 0.150 and < 0.400

Positive control: mean absorbance after subtraction of blank absorbance should be > 0.500

The test results are considered invalid if the above validation requirement are not met.

3.9.2.7. Interpretation of the result

Negative results: The samples which gave an absorbance less than cut-off values were considered negative.

Positive results: The samples which gave an absorbance more than cut-off values were considered positive.

3.10. Data analysis

SPSS version 16 was used for data analysis

CHAPTER FOUR

4. RESULTS

According to age difference, the women were grouped into three groups 20-28, 29-37, and 38-46 with a mean of age (31.79) (Table 4.1).

Table 4.1 Distribution of the women according to age groups

Age group	Frequency	Percent
20-28	25	28.1
29-37	51	57.3
38-46	13	14.6
Total	89	100.00

The number of abortions varied between the women from once to recurrent abortions (Table 4.2)

Table 4.2 Distribution f the women according to the number of abortions

Number of abortion	Frequency	Percent
Once	20	22.5
Two and more	69	77.5
Total	89	100.00

When the samples tested by ELISA technique 87(97.8%) of them were positive for CMV IgG (Table 4.3), while CMV IgM was not detected in any sample.

Table 4.3 Results of CMV IgG test among women with history of abortion using ELISA technique

CMV IgG results	Frequency	Percent
Positive	87	97.8
Negative	2	2.2
Total	89	100.00

Chi square test was used to analyze the association between the number of abortions and CMV IgG result (Figure 4.1), no statistical difference found with (0.441) *P*. value which was > 0.05

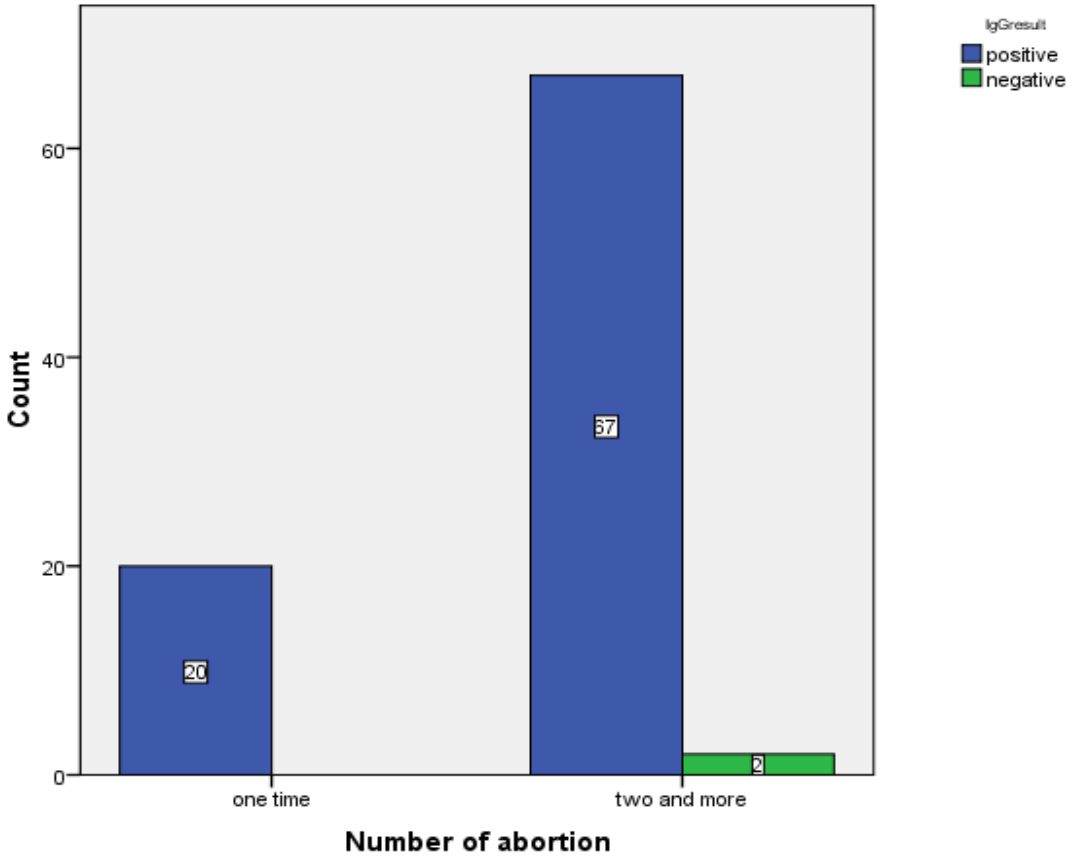

Fig 4.1 Frequency of CMV infections among women with history of abortion according to number of abortion *P*. value (0.441)

Chi square test was also used to compare the age groups with CMV IgG results, no significant statistical difference were found between them (Figure 4.2) with *P.* value (0.716) but, there was significant statistical relationship between age groups and number of abortion with (Figure 4.3) *P.*value (0.001).

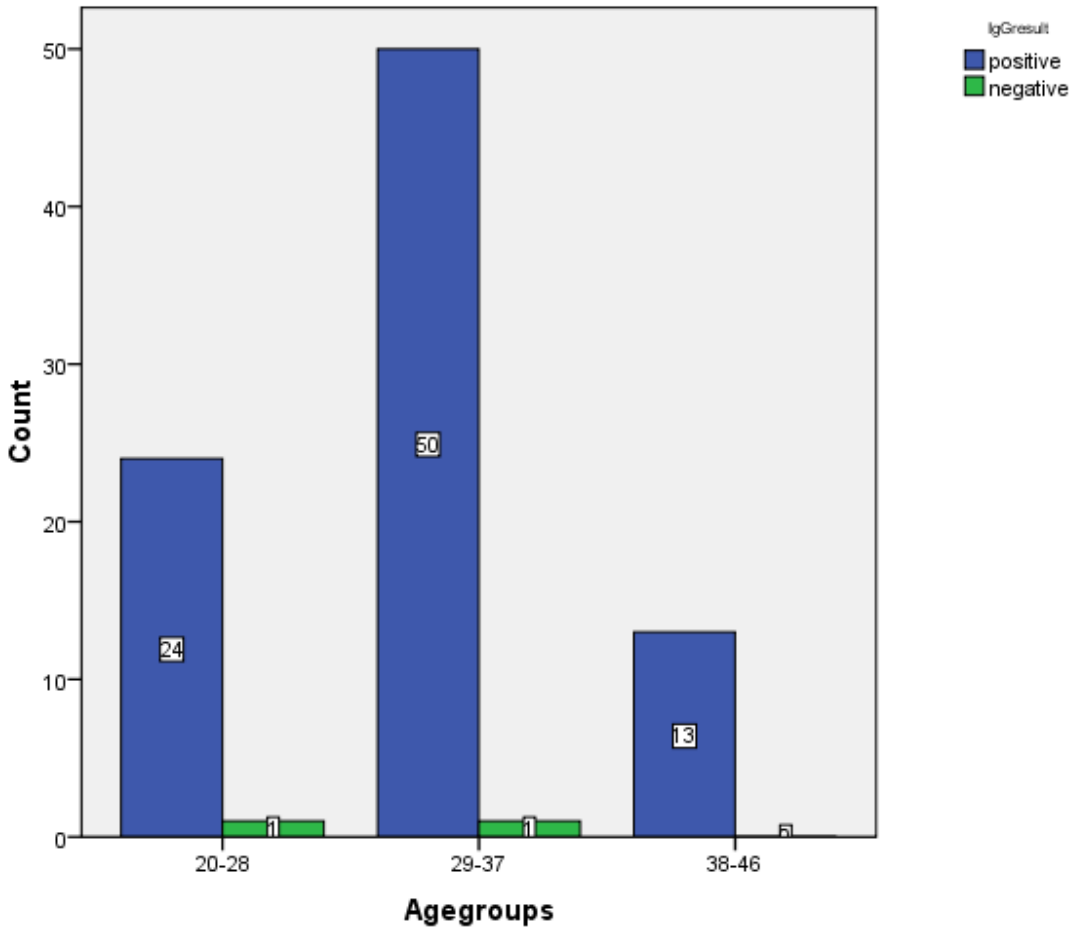

Fig 4.2 Relationship between age groups of aborted women and IgG results *P.* value (0.716)

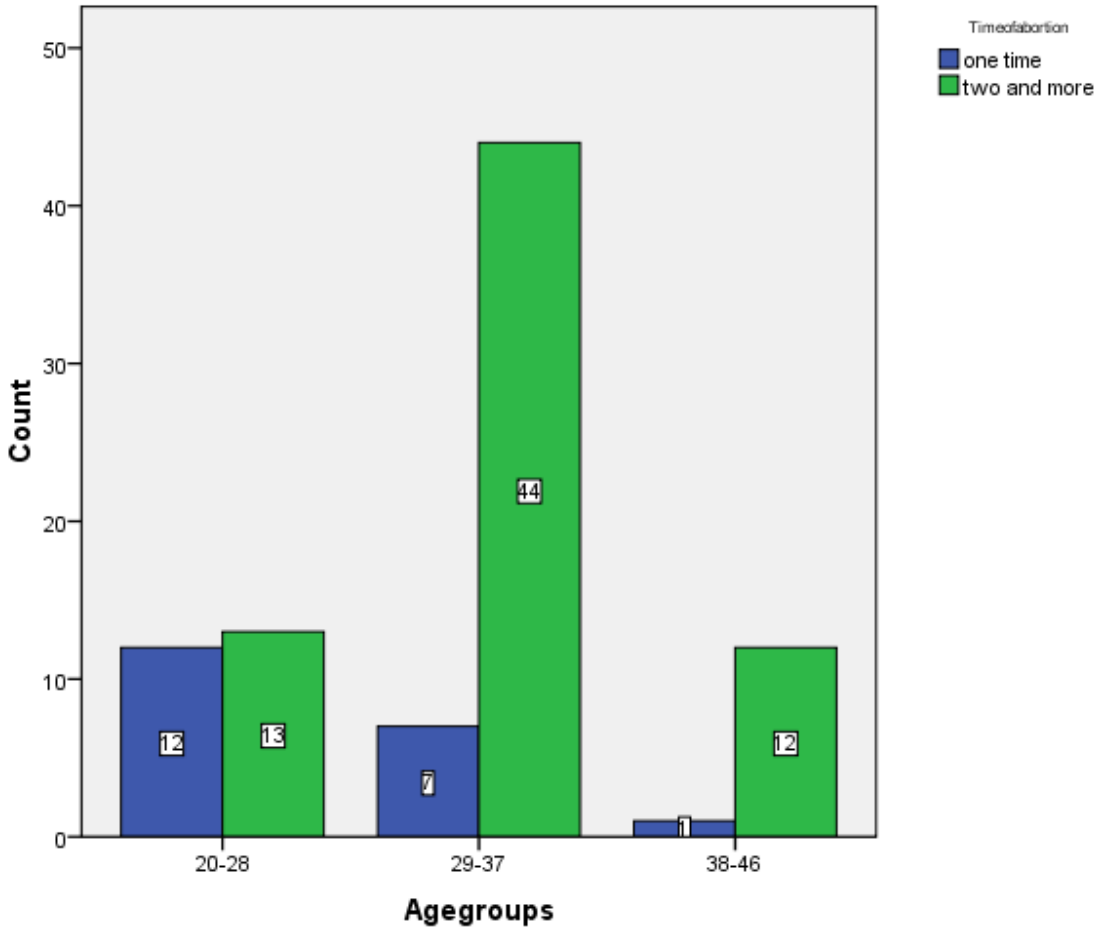

Fig 4.3 Relationship between the number of abortions and age groups of aborted women *P*. value (0.001)

CHAPTER FIVE
5. DISCUSSION

The aim of this study was to find the distribution of CMV infection in women with history of abortion by detection of CMV IgG and IgM antibodies using ELISA technique.

The results explained that among 89 women with history of abortion 87 (97.8%) of them showed the presence of CMV IgG whereas the CMV IgM wasn't detected. The detection of CMV IgG indicates that the women were previously infected with CMV, while the presence of IgM indicates recent infection or re infection and the IgM was formed immediately after infection and disappeared of short period.

The results obtained in this study were similar to the results reported in Sudan by Kafi *et al* (2013) and Khairi *et al* (2013) in which 97.8% and 97.5% CMV IgG positive respectively .

In this study there was insignificant association between CMV infection and the frequency of abortion and age. In a study conducted by Khairi and his colleagues the age was significantly associated with CMV IgM and the history of miscarriage was significantly associated with IgG positive CMV (Khairi *et al*., 2013). In a study by Kafi and his colleagues there was significant association between CMV infection and the frequency of abortion, age and congenital malformation in children (Kafi *et al*.,2013)and CMV IgM was 38.3%. The difference between the results of this study and those reported by Khairi and Kafi could be attributed to the large sample size used in their studies and criteria of samples collection.

In a similar study reported by (Sherkat *et al*., 2014) (90.6%) of CMV IgG were positive, while only one case (2.3%) of CMV IgM was positive, and (Hama and Abdurahman, 2013) reported (90.2%) of CMV IgG and (9.1%) of CMV IgM .

In a study in Iran CMV IgGwas positive in (94%) cases and (75%) in control groups while the CMV IgM was (5.2%) in cases and negative in control groups (Jahromi *et al*., 2010). These reports agrees with the results of this study with few exceptions of positive CMV IgM cases that could be due to the variations in the sample size.

The results of this study were higher than the results reported by Hassan *et al*., 2014 which were (80.4%) of CMV IgG, Bagheri *et al*., 2012 (72.1%) of CMV IgG and IgM (2.5%) respectively and Paschale *et al*.,2009 who reported the presence of (68.3%) anti CMV IgG antibodies in urban areas of northern Italy. The results were also higher than the presence of CMV IgG (55.3%) and IgM (3.2%) among Sudanese women with recurrent pregnancy loss reported with Elamin and Omer (2015).This may be attributed to the different in socioeconomic status and hygienic practices.

The CMV IgM was not detected in this study and low percentage in the other studies which above. Higher percentage of CMV IgM antibodies were reported in studies by Khalf *et al* (2012) where (15.7%) were detected and Lone *etal* (2004) who reported the presence of CMV specific IgM antibody in (15.98%) and even more higher results were reported by Hassan *et al* (2014) in which (32.6%) anti CMV (IgM) positive were found. The reasons for the presence of CMV IgM antibodies in these studies and the absence of them in this study could be justified by the variation in sample size and duration of the study. Although the presence of CMV IgM indicate active recent infection or re infection there was cross reactivity of about 3.3% for IgM positivity with other viral infections including EBV, measles, herpes simplex varicella- zoster influenza vaccine as reported in Hama and Abdurahman (2013)

The results in this study disagree with the results obtained by Falahi*etal* (2010) IgG was found in 6 (14.28%) and IgM in 12 (28.58%) and the results showed that a high seroprevalance of HCMV IgM than IgG and that may be due to the long study period from 2007 to 2008.

According to age groups the percentage of CMV IgG positive were (27.6%), (57.5%), (14.9%) in age 20-28, 29-37, 38-46 respectively, these results were higher than the results reported by Abdul Wahab(2012) in which the percentage of immunoglobulin at age 20 was (16.7%) while at age 40 to 45 was(21.7%) and women at age more than 30 years showedhigher percentage of abortion.

4.2 Conclusions

From this study we conclude that the high anti CMV IgG positive results in aborted women indicates the exposure of those women to the virus and this high percentage confirms the importance of screening pregnant women for CMV IgG antibodies as this infection might play an important role in abortion. Prevention of CMV infection in pregnant women will prevent congenital infections in their infants.

4.3 Recommendations

Routine screening of CMV infection in pregnant women should be carried out in maternity wards. Any women with a history of abortion must be investigated before and after pregnancy. Further research needed to be carried out to detect the source of transmission of this virus and further research more advanced molecular approaches should be done to confirm the results of this research. Future research should involve larger samples size with well structured questionnaire to identify the source of transmission as well as women who are at risk of getting the infection with this virus.

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