1. Introduction

1.1. Background:

Human cytomegalovirus (HCMV) is a member of herpesviridae family, subfamily Betaherpesvirinae, with human CMV and other animal species. The genome is of double-stranded linear DNA, the virus capsid is icosahedra symmetry and has envelope (Murray *et al;* 2007).

The virus can be transmitted by different modes such as across placenta, within birth canal and in breast milk that in early life, via saliva most common in children, sexually, during blood transfusion and organ transplants (Levinson;2010).

Latency following a primary infection may be punctuated by periodic reactivation that gives rise to recurrent infection, and *in utero* transmission may occur during either primary or recurrent infections (Boppana *et al;*2000).

Now CMV is believed to be the most common pathogens of man. Most of CMV infections remain asymptomatic. The outcome is usually fatal in rarely cases (Bhatia and Ichhpujani; 2008).

There is significant relation between CMV infection and spontaneous abortion (Enders *et al*; 2001). There are also evidences which suggest that CMV will lead to complicated pregnancies (Hammouda *et al;* 1993). It has been reported that the risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy (Stagno *et al;* 1986).

1.2. Rationale:

Human cytomegalovirus (CMV) infection, which usually has a benign course in immunocompetent individuals, can have catastrophic consequences during pregnancy (Britt; 1999). Primary CMV infection during gestation poses a 30 to 40% risk of intrauterine transmission and clinical disease (Stagno *et al*, 1982; Stango *et al;* 1986). Reactivated infection is associated with at least a 10-fold-lower rate of transmission. Congenital CMV infection is a relatively common occurrence, as approximately 1 to 4% of newborns in the United States and Europe are infected with CMV (Britt; 1999), and transmission could be higher in developing countries (De Jong; 1998).

Despite numerous reports between association of CMV and pregnancy loss, the role of CMV in recurrent spontaneous abortion (RSA) remains to be elucidated (Pandey *et al*; 2005).

The role of altered immune response, and therefore, recurrence or reactivation of latent CMV infection may relate to RSA is unclear, whereas few data are available in this regard.

In Sudan 6% of pregnant women IgM positive and 97.5% positive IgG to CMV and 10.2% of them had a history of miscarriage (Khairi *et al;* 2013). Kafi *et al* (2013) reported that there was significant association between CMV infection and frequency of abortion and malinformation in children among Sudanese women with spontaneous abortion.

1.3. Objectives:

1.3.1. General objective:

To detect the frequency of cytomegalovirus IgM among aborted women.

1.3.2. Specific objectives:

1. To detect specific CMV IgM among aborted women.

2. To determine the frequency of abortion associated with CMV among recurrent spontaneous abortion history women.

2-Literature Review

2.1. Herpesviruses

The name of the herpes viruses came from the Greek word *herpein*, meaning to creep. More than 100 herpes viruses had been isolated from a range of hosts that includes mammals, birds, fishes, reptiles, amphibians and mollusks (Carter and Saunders; 2007).

A notable characterization of herpes viruses is that, once they have infected a host, they often remain as persistent infections for the life time of the host. These infections are often latent infections, which can be reactivated from time to time, especially if the host becomes immunocompromised. Both primary and reactivated herpes virus infections can either be asymptomatic or can result in disease of varying severity. The outcome depends on the interplay between the particular virus and its host and especially the immune status of the host (Murray *et al*; 2007).

2.1.1. History

CMV was first noticed in 1881 by German scientist Ribbert, as large cells in section of kidney of a luetic stillborn and in the parotid gland of children. These cells were described and considered as "protozoan-like" by Jesionek and Kiolemenoglou (Ho; 2008). The new disease was called "generalized cytomegalic inclusion disease (CID)" (Ho; 2008).

The virus was cultured for the first time in 1956 by Rome, Smith and Weller, all of whom worked independently (Bhatia and Ichhpujani; 2008).

2.1.2. Classification

Cytomegalovirus formally designated human herpes virus 5 (HHV-5) by the International Committee on Taxonomy of Viruses, is a member of the family Herpesviridae, and it's classified in the subfamily Beta herpesrvirinae with cytomegalovirus of other animal species based on its specificity (Brooks *et al*; 2010).

Human CMV (HCNV) is the type of species of the genus cytomegalovirus, and its name is derived from the enlargement of the cells (cyto=cell, mega=large) infected by the virus (Brooks *et al;* 2010).

Herpes virus 6 (HV-6) and herpes virus 7 (HV-7) are now classified with CMV among the betaherpes viruses (Brooks *et al*; 2010).

2.1.3. Structure of the Virus

Cytomegalovirus is composed of large DNA genome (240kpb), which is double strand, linear and in form of atoroid. In spite of its genetically different strains, it is being in human population. The DNA genome is surrounded by a protein coat that gives icosahedral symmetry with 160 capsomeres. Its nucleocapsid is surrounded by an envelope that derived from nuclear membrane of the infected cell, measured 150-200 nm and contain glycoprotein spikes 8 nm act as Fc receptor for non specific binding (Brooks *et al*; 2010).

Between the envelope and capsid, found an amorphous layer called the tegument. The naked virion measures 100 nm contains one of the immediate early stronger enhancers due to concentration of binding sites for cellular transcription factors (figure 2.1). Herpesviruses encode an array of virus-specific enzymes involved in nucleic acid metabolism,

DNA synthesis and protein regulation (DNA polymerase- thymidine kinase- protein kinase; Brooks *et al*; 2010).

The tegument compartment contains the majority of the virion protein, with the most abundant tegument protein being the lower matrix phophoprotein 65 (pp 65), also termed unique long 83, UL83 (Varnum *et al*; 2004).

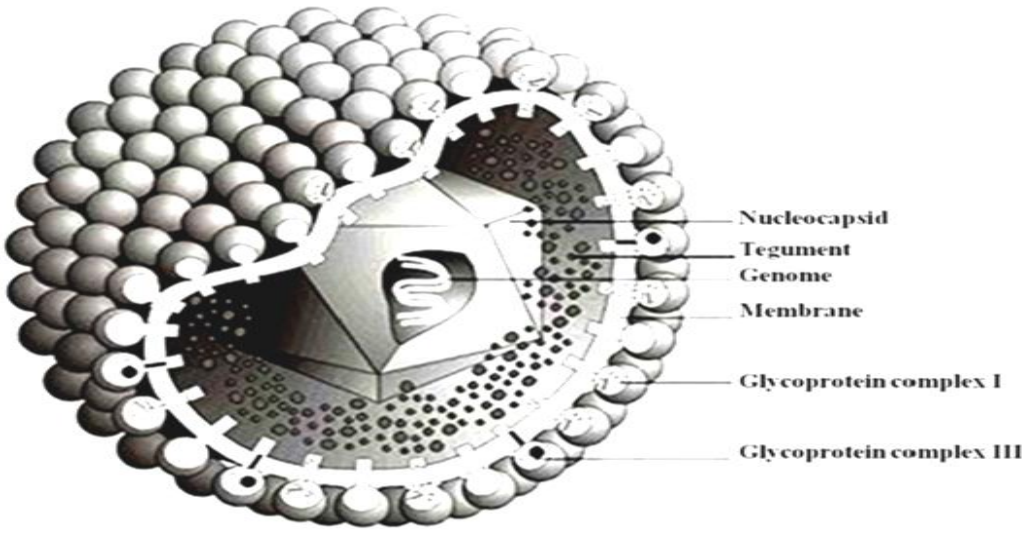

Figure 2.1: General structure of CMV (Brooks *et al;* 2010).

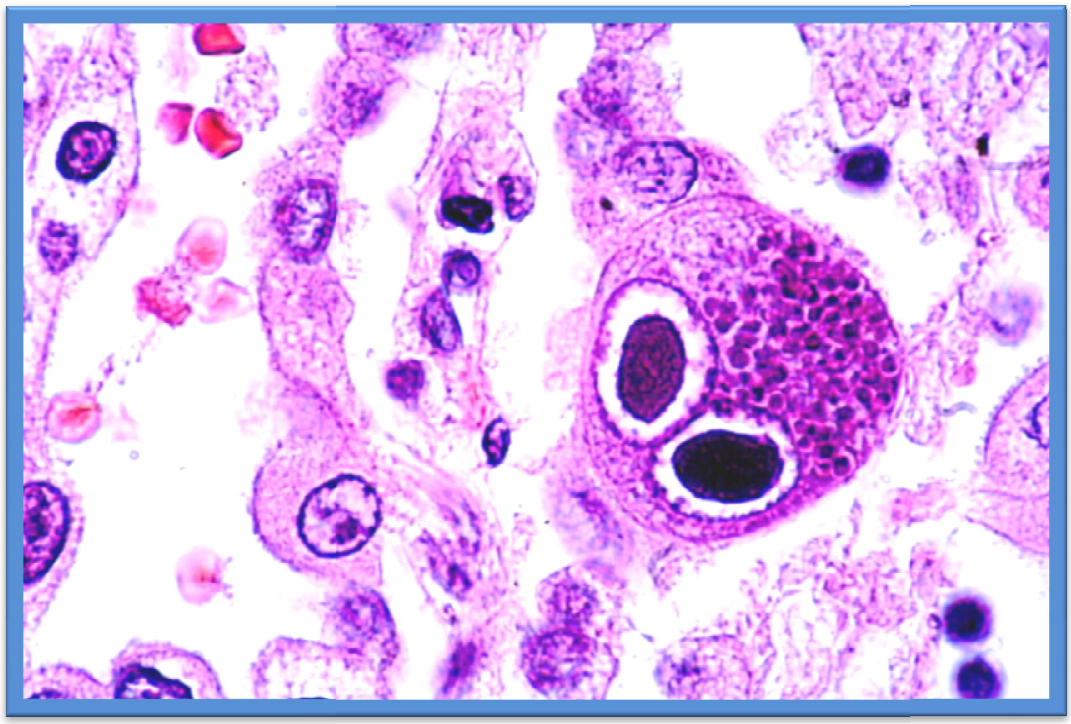

Figure 2.2: CPE of the virus (Brooks *et al;* 2010)

2.1.4. Properties of the virus

Cytomegalovirus has the largest genetic content of the human herpesviruses, its DNA genome (240kpb) is significantly larger than of herpes simplex virus. Only a few of the many proteins encoded by the virus (over 200) have been characterized. One, a cell surfaceglycoprotein, acts as Fc receptors that can non-specifically bind the Fc portion of the immunoglobulins.This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins. The major immediate early promoter- enhancer of CMV is one of the strongest known enhancers, due to concentration of binding sites for cellular transcription factors. It is used experimentally to support high-level expression of foreign genes. Many genetically different strains of CMV are circulating in the human population. The strains are sufficiently

related antigenically, however, so that strains differences are probably not important determinants in human disease (Brooks *et al*; 2010).

Cytomegalovirus is very species-specific and cell type-specific. All attempts to infect animals with HCMV have failed. A number of animal CMV exists, all of them species-specific. HCMV replicates in vitro only in human fibroblasts, although the virus is often isolated from epithelial cells of the host. CMV replicates very slowly in cultured cells, with growth proceeding more slowly than that of HSV or Varicella-zoster virus. Very little virus becomes cell-free; infection spreads primarily cell-to-cell. It may take several weeks for an entire monolayer of cultured cells to become involved (Brooks *et al*; 2010).

Cytomegalovirus produces a characteristic cytopathic effect. Perinuclear cytoplasmic inclusion form in addition to the intranuclear inclusion is typical of herpesviruses. Multinucleated cells are seen. Many affected cells become greatly enlarged. Inclusion-bearing cytomegalic cells can be found in samples from infected individuals (Brooks *et al*; 2010).

2.1.5. Replication of the virus

The virus replicates in the cell after binding to cell receptor via envelope glycoprotein. The capsid is transported to the nuclear pore through cytoplasmic membrane, un-coating occurs, and then the virus genome changes to circular shape in order to introduce in the DNA and to express "alpha" proteins. After activation of gene expression by VP16, a tegument protein then forms complex with several cellular proteins to be translated to "beta" protein enzymes (Brooks *et al*; 2010).

Starting the virus transcription by cellular RNA polymerase II with virus factors called rolling-circle mechanism, after viral DNA replication and translation, to give "gamma" proteins its structural component with

production of virus component. This component assembled and after that the virus is released by budding through nuclear membrane, envelope virus particle transported by vesicular movement to cell surface (Brooks *et al*; 2010).

2.1.6. Transmission

Human cytomegalovirus (HCMV) can be transmitted via saliva, sexual contact, placental transfer, breast feeding , blood transfusion, organ transplantation, or hematopoietic stem cell transplantation (Sia and Patel; 2000).

CMV is excreted in the breast milk of seropositivity women shedding virus in their breast milk has been reported to be 58% to 69% (Nyholm and Schleiss; 2010).

2.1.7. Epidemiology

At least 60% of the US population has been exposed to CMV, with a prevalence of more than 90% in the high risk groups (e.g. homosexual males, diabetes, chronic disease, chemotherapy, newborns) (Akhter and Tood; 2011).

HCMV is highly species-specific, with human being the only host. Furthermore, CMV has been found in every human population tested. The prevalence of infection is greater in developing countries and among lower socioeconomic groups of developing countries. Overall, the seroprevalence of infection varies between 65% to 90% among middle age adults in the USA (Nassetta *et al;* 2009). The prevalence of congenital infection caused by CMV ranges from 0.2%-2.2% in different populations (Demmler; 1991). In Sudan seroprevalence was found to be 97.8% and 38.3% for IgG and IgM respectively among spontane[[ous

aborted women (Kafi *et al*; 2013). 12.9% of pregnant with complications in India were positive to CMV specific IgM (Kapil and Broor; 1992). CMV specific IgM were positive in 25% of aborted women in Jordan (Nuha *et al*; 2011).

2.1.8. Pathogenesis

Cytomegalovirus causes no symptoms in children and most mild disease in adult. The virus first infect the upper respiratory tract then local lymphocyte, circulating lymphocytes then spread the virus to other lymphocytes and monocytes in the spleen and lymph nodes. The virus finally spread to a variety of epithelial cells including those of the salivary glands, kidney tubules, testes and cervix. Infections are usually asymptomatic (sub-clinical) but glandular fever is sometimes seen in young adults. The virus can inhibit T cell responses. The virus elicits both humeral and cell mediated immunity from the possibility of spread from cell to cell. If suppressed, the virus later reactivates, particular in cases of immunosuppressants. Indeed, infection by the virus can, itself, be immunosuppressive (Hunt; 2010).

The virus causes congenital diseases including: microcephaly, rash, brain calcification, CNS defects and hepatospleenomegaly, also causes diseases in immunesuppressed patients like retinitis in up to 15% of all AIDS patients, interstitial pneumonia, colitis, esophagitis, hepatitis, myelitis,uveitis and encephalitis are seen in some patients (Greenwood *et al;* 2007).

Primary CMV infection in an individual can be detected by demonstration of CMV specific IgM antibody (Griffiths *et al;* 1982).

Also primary infection in pregnancy has a higher incidence of symptomatic congenital infections and fetal loss (Stagno *et al;* 1986). The

proposed mechanisms for infectious causes of pregnancy loss include: (1) direct infection of the uterus, fetus, or placenta,(figure 2.3) (2) placental insufficiency, (3) chronic endometritis or endocervicitis, (4) amnionitis, or (5) infected intrauterine device (Summers; 1994).

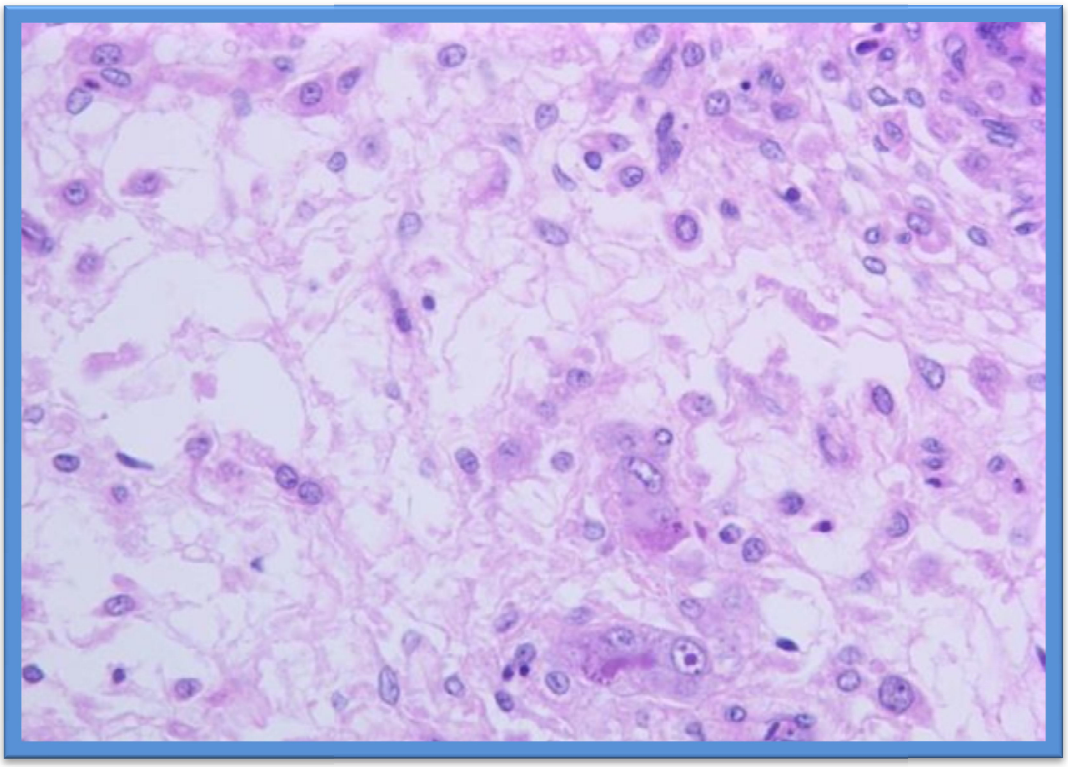

Figure 2.3: CMV placentitis (Roger *et al;* 2012)

2.1.9. Laboratory diagnosis

The virus can be diagnosed by electron microscopy, cell culture, biopsy staining, serology and molecular methods (Steve and Daniel; 2002).

2.1.9.1. Collection and Transportation of samples

Most useful specimens for isolation are throat washings, urine and blood. CMV can also be isolated from saliva, breast milk, cervical secretions and semen as well as various biopsy materials (Bhatia and Ichhpujani; 2008).

All specimens should be sent to the laboratory without delay. If delay of more than a few hours is anticipated, then should be sent refrigerated, or preserve on wet ice, but under no circumstances should any specimen be frozen at any temperature (Griffiths; 2004).

2.1.9.2. Direct detection

2.1.9.2.1. Microscopy

Microscopical examination of tissues and culture is useful in diagnoses of CMV infection, although it has limited utility especially in immunocompromised patients (Steve and Daniel; 2002).

2.1.9.2.2. Cell culture technique

Cultural techniques are complicated, need sterile conditions with safety cabinet, special procedures to prepare the sample before inoculation, take long time, need identification method and also need electron microscopy to detect the presence of the virus by production of CPE (Timbury; 1997).

However, viral culture of the urine and saliva obtained within two weeks of life continues to be the gold standard for diagnosis of congenitally-infected infants (Ross *et al;* 2011). The virus can be recovered most readily from throat washings and urine (Brooks *et al;* 2010).

2.1.9.2.3. Histopathology technique

Autopsy and biopsy need histopathology techniques to prepare the sections, staining and immunoflourescent techniques to detect the virus (figure 2.2). Sometimes CMV does not produce CPE in tissues so other confirmatory methods are needed (Timbury; 1997) (Brooks *et al;* 2010).

2.1.9.3. Antigen detection

Currently the gold standard quantitative assay is the antigenemia assay for the detection of the HCMV pp65 antigen in leukocytes. Flow cytometry has been applied to the antigenemia assay to make it automated and less subjective (Steve and Daniel; 2002).

2.1.9.4. Serological tests

Serological methods for the diagnosis of human cytomegalovirus (HCMV or CMV) infections are including detection of CMV IgM and IgG. IgG avidity testing is now a useful serodiagnostic test to differentiate a new infection from a reactivation (Steve and Daniel; 2002).

2.1.9.5. Polymerase chain reaction

This is designed to detect replicating virus, not latent genomes (Brooks *et al;* 2010).

2.1.9.6. Shell vial assay

The shell vial assay is performed by adding the clinical specimen to a vial that contains a permissive cell line for CMV. The shell vial are centrifuged at a low speed and placed in an incubator. After 24-48 hours, the tissue culture medium is removed and the cells are stained using a fluorescein- labeled anti-CMV. The cells are read using a fluorescent microscope. Alternatively, the cells are stained with an antibody against CMV, followed by a fluorescein- labeled anti-immune globulin. This test has been found to be as sensitive as traditional tissue culture, probably because of thesz enhancement of infectivity provided by centrifugation (Jahan; 2010).

2.1.10. Treatment

Antiviral agents for CMV infections are available but serious side effects limit their use to life- or slight- threatening complications (Ogilvie; 2007).

Ganciclovir is an acyclic 2-deoxyguanosine analogue for the management of CMV. It is available in oral and parenteral formulations. Oral ganciclovir is poorly absorbed, with a bioavailability of only 5%. Management of active CMV disease is therefore with intravenous ganciclovir or its oral valylprodrugvanciclovir (Nichols and Boeckh; 2000).

Intravenous gancyclovir is used as a first- line treatment of CMV disease in bone marrow andsolid organ transplant recipients. Reversible bone marrow suppression is the most common adverse effect of ganciclovir (Eid *et al;* 2008).

Foscarnet is approved for the treatment of CMV retinitis in patients with AIDS. It has been used to treat other CMV disease in immunocompromised patients, espatialy those unable to tolerate ganciclovir and those infected with ganciclovir-resistant virus (Razonable; 2011).

Cidofovir is a nucleoside analogue used for treatment of CMV other herpesviruses and other DNA viral infection. The major clinical indication for cidofovir is the treatment of CMV retinitis in patients with AIDS (Razonable; 2011).

2.1.10.1. Prenatal management and treatment

The management of the pregnancy in case of primary CMV infection is a matter of debate (Demmler and Nance; 2005). Suspected fetal CMV

infection most often results in amniocentesis, an invasive test that causes spontaneous miscarriages in about 1% of the cases. The danger of amniocentesis for the fetus needs to be taken into consideration when planning strategies for prenatal diagnosis (Collinet *et al;* 2004).

2.1.11. Prevention

Prevention of Cytomegalovirus transmission in women of childbearing age is of utmost importance in order to reduce the rate of congenital infection. Hygienic strategies are important in preventing CMV transmission given that the saliva and urine of infected children are significant sources of CMV infection among women who are pregnant .prevention strategies include washing hands whenever there is contact with a child's saliva or urine, not sharing food, utensils, or cups, and not kissing a child on the mouth or cheek (Nyholm and Schleiss*;* 2010).

The development of an effective prophylactic vaccine for HCMV-associated diseases remains a significant challenge (Crough and Khanna*;* 2009).

More recently a number of attempts have been made to design prophylactic HCMV vaccines that are based predominantly on subunit vaccine technologies. These vaccine formulations have been delivered as recombinant proteins (Mitchell *et al;* 2002).

2.2. Recurrent abortion

Recurrent miscarriage, habitual abortion, or recurrent pregnancy loss (RPL) is the occurrence of three or more consecutive pregnancies that end in miscarriage of the fetus before viability (for example 24 weeks gestation in the United Kingdom). About 1% of couples trying to have children are affected by recurrent miscarriage.

There are various causes for recurrent miscarriage, and some are treatable. Some couples never have a cause identified, often after extensive investigations. These include anatomical condition, chromosomal disorders, endocrine disorders, thromophilia, immune factors, ovarian factors, life style factors and infections (RCOG*;* 2011).

2.2.1. Role of infection in recurrent abortion

Embryo–fetal infections have been reported to cause recurrent spontaneous abortions (RSAs) at a rate lower than 4%. The possible mechanisms include production of toxic metabolic byproducts, fetal or placental infection, chronic endometrial infection, and chorio-amnionitis. Viruses appear to be the most frequently involved pathogens, since some of them can produce chronic or recurrent maternal infection. In particular, cytomegalovirus during pregnancy can reach the placenta by viremia, following both primary and recurrent infection, or by ascending route from the cervix, mostly following reactivation. Another herpesvirus, herpes simplex virus type 2, less frequently type 1, causes recurrent infections of the genital tract, which can involve the feto-placental unit. Parvoviruses have also been implicated in the development of repeated fetal loss. Among bacterial infections, *Chlamydia trachomatis, Ureaplasma urealyticum,* and *Mycoplasma hominis* have been mostly associated with occurrence of RSA. An increased risk of abortion among women with bacterial vaginosis (BV) during early pregnancy was also shown, but questions arise about the role of chronic BV in its occurrence. Although a definitive relationship between recurrently active infections and RSA is still lacking, mostly due to difficulties in demonstrating the pathogenic role of each individual isolated pathogen, diagnosis and therapy of RSA-related infections should be attempted. The diagnosis of infectious agents as a possible cause of RSA might lead to a therapeutic

approach with antiviral drugs and antibiotics or using immunoglobulins, which can display both anti-infective neutralizing and immunomodulating properties (Giovanni *et al*; 2011).

2.2.2. CMV among women with recurrent abortion

Primary CMV infection has been found to be more prevalent in pregnant women than non-pregnant. This difference may be attributed to the susceptibility of seronegative women, at the onset of pregnancy, to the first CMV infection (Stango *et al;* 1982).The risk for fetal infection is greatest with maternal primary CMV infection and much less likely with recurrent infection as the virus remains latent in the host cell after initial infection (Massimo *et al;*2009). Abortion can result from ascending CMV endometritis and the virus has been isolated from post-abortion uterine discharge (Dehner *et al* 1975).

Rubina *et al* reported that 16.37% of women with recurrent abortion were positive IgM to CMV. 14% of women in Iraq suffering from recurrent abortion showed positive result for IgM to CMV (Maysra *et al;* 2012).

3. Materials and Methods

3.1. Study approach:

Qualitative approach.

3.2. Study design:

This study is a descriptive cross-sectional study.

3.3. Study population:

Pregnant women with vaginal bleeding admitted to hospital with history of recurrent abortion suspected to be infected with CMV.

3.4. Study area:

The blood samples were collected from women with vaginal bleeding which were hospitalized in AL-Saudi Hospital and Ibrahim Malik Hospital. The experimental work was carried out in central laboratory, Khartoum, Sudan.

3.5. Study duration:

The study was carried out during the period, from March to June, 2014.

3.6. Sample size:

A total of eighty nine venous blood samples (n=89) were collected. Fifty eight samples (n=58) from pregnant women with vaginal bleeding and history of recurrent abortion. A thirty one blood samples (n=31) were collected from healthy pregnant women with no history of abortion as control group.

3.7. Sample collection:

Venous blood was collected from each subject after disinfection by 70% alcohol. Blood was collected in plain tubs, allowed to clot at room temperature and serum was separated by centrifugation at 300 rpm/3 minutes. The serum was kept at -20^{o} C until tested.

3.8. Sample technique:

Randomized, non-probability convenience sampling technique during admission to these hospitals was used in the study.

3.9. Sample processing:

All serum samples were tested for the presence of CMV IgM antibodies using commercially 3rd generation available ELISA kits (IgM).

3.10. Data collection:

Demographic data was collected by direct interviewed questionnaire (appendix 1)

3.11. Data analysis:

The data obtained were analyzed and presented using Statistical Package for Social Science (SPSS) version 16.0 computer software version. Significance of differences was determined using Chi-square test.

3.12. Ethical consideration:

Permission to perform the study was taken from the college of Graduate studies, Sudan University for Science and Technology. All women participate were informed before collection of blood for the purpose of the study, and verbal consent was taken from all women.

3.13. Laboratory diagnosis of CMV:

3.13.1. Detection of anti-CMV IgM antibodies using ELISA:

Determination of IgM class antibodies to CMV in serum was performed using IgM ELISLA kits (Foresight, ACON laboratories, Inc.USA).

3.13.1.1. Principle:

ELISA test system is designed to detect IgM antibody to CMV in human sera. CMV IgM antihuman monoclonal antibody is adsorbed in solid phase to the polystyrene reaction strip, if there is CMV IgM antibody in test sample, and then binds to the enzyme labled anti-antibody and forms antigen-antibody –antibody complex, and finally binds to the surface of the micro-well, and display blue color in corresponding well by the action of substrate. Therefore, it can detect specifically the CMV IgM in human serum.

3.13.1.2. Procedure:

All materials were brought to room temperature before beginning the procedure. In brief, Working Wash Buffer was prepared by diluting the concentrated Wash Buffer 1:25 by distilled water .A1 was leaved as Blank well, 100 µl of the Negative Control was added to B1and C1, 100µl of Cut-Off Calibrator was added to D1and E1, 100µl of Positive Control was added to G12 and H12. 100µl of Specimen Diluents' was added to assigned wells starting at H1 (sample wells), 5µl of specimen starting at H1 (sample wells) was added, on a flat bench microwell plate was mixed gently by swirling for 30 seconds. The microwell plate was covered and incubated at $37^{o}C \pm 2^{o}C$ for 30 minutes; the wells were washed 5 times by Working Wash Buffer to remove residual serum, 100µl of Conjugate was added to each well except for the blank well.

Microwell plate was covered and incubated at 37^{o}C for 30 minutes, then was washed by Working Wash Buffer for 5 times. Then substrate (3,3',5,5'-tetramethylbenzidine) which has two parts (Substrate A and Substrate B) 50µl of each part were added to each wells, then was incubated at 37^{o}C for 10 minutes.50µl of Stop Solution was added to each well. The optical density (O.D) in a microwell plate ELISA reader was read within 30 minutes at 450 nm.

3.13.1. 3. Measurement:

ELISA reader micro plate was adjusted to zero using the substrate blank in the first well, and the absorbance of all wells measured by UV light at 450 nm.

3.13.1.4. Calculation of control values and cut-off:

Mean absorbance values of NC (MNC), mean absorbance value of Cut-Off Calibrator (COC), and mean absorbance of positive control (PC) were calculated. The result run was validated according to the manufacture's criteria for validity as below:

1-Ab of substrate blank at 450 nm > 0.100

2- Ab of MNC after subtraction of blank absorbance < 0.150

3-Mean Ab of COC after subtraction of blank absorbance < 0.400

4-Mean Ab of PC after subtraction of blank absorbance > 0.500

The Index Value was calculated to obtain qualitative specimen results as following:

1-Cut-Off Value was obtained by this equation:

Cut-Off Value = Mean Ab of Cut-Off Calibrator – Blank Ab.

2-the Index Value was calculated by dividing the specimen Ab by the Cut-Off Value.

3.13.1.5. Interpretation of the result:

To determine the presence or absence of CMV-IgM, the Index Value was obtained to each specimen then read the result by referring to the interpretation of results below:

1-Negative samples < 0.9

2-Positive samples >1.1

3-Equivocal ≥ 0.9 and ≤ 1.1

4. Results

4.1. Frequency of Cytomegalovirus IgM among the women with recurrent abortion

Out of the 58 women with recurrent abortion tested, one woman (1.7%) was CMV IgM positive, while all healthy pregnant with no history of abortion (n=31) were negative.

Table (4.1): Frequency of Cytomegalovirus IgM antibodies in women with recurrent abortion and control group

group	Anti-CMV IgM positive		
	No. subjects	**No. positive**	**%positive**
Women with recurrent abortion	58	1	1.7%
Control group	31	0	0%
Total	89	1	1.1%

4.2. Relation between Cytomegalovirus infections with recurrent abortion according to the age of women.

The woman suffering from recurrent abortion was (36-40) years were the only positive Cytomegalovirus (1.7%).

Out of the 58 of women with recurrent abortion 22(37.9%) represent the highest numbers of patients belong to age group 20-25 years (Table 4.2).

Table (4.2) Relation between Cytomegalovirus infections with recurrent abortion according to the age of women.

Age/year	Women with recurrent abortion		+ve IgM to CMV	
	No.	%	No.	%
20-25	22	37.9%	0	0%
26-30	10	17.2%	0	0%
31-35	15	25.9%	0	0%
36-40	11	19%	1	9%
Total	58	100%	1	1.7%

4.3. The gestational age among women with recurrent abortion and history of congenital anomalies neonates or abortion complications

The mean gestational age was 14 weeks, and out of 58, 10 women were suffering from abortion complications (fever, infection ...etc.). Only one was positive IgM to CMV, and there were no congenital anomalies baby history to these patients (Table 4.3).

Table (4.3) Gestational age among women with recurrent abortion and number of congenital anomalies neonates or abortion complications

Weeks	No. of patient with recurrent abortion	No. of patient with abortion complications		No. of congenital anomalies baby	+ve IgM CMV	
		No.	%		No.	%
0-10	21	3	14.3%	0	0	0%
11-20	27	2	7.4%	0	1	3.7%
21-24	10	5	50%	0	0	0%
Total	58	10	17.2%	0	1	1.7%

4.4. Relation between chronic disease or genetic disorders and positive IgM to CMV among women with recurrent abortion

Out of 58, four women (6.9%) were had chronic disease or genetic disorder (e.g. sickle cell anemia) and there was no case of positive IgM to CMV at this group, while there one case at this group with no disease (1.9%) (Table 4.4).

Table (4.4) Number of women with chronic or genetic disease among those with recurrent abortion

Disease	women with recurrent abortion		+ve IgM to CMV	
	No.	%	No.	%
Yes	4	6.9%	0	0%
No	54	93.1%	1	1.9%
Total	58	100%	1	1.7%

4.5. Relation between education level among women with recurrent abortion and positive IgM to Cytomegalovirus

Out of 58 women with recurrent abortion 18 (31%) were well educated and no case of positive IgM was found. 14 (24.1%) were with medium education (24.1%) and one case of positive IgM were found. 26 was poor or never educated which were representing the highest percentage (44.8%) and no positive IgM was found (Table 4.5).

Table (4.5) Relation between education level among women with recurrent abortion and positive IgM to Cytomegalovirus

Education level	Women with recurrent abortion		+ve IgM to CMV		Control group		+ve IgM to CMV
	No.	%	No.	%	No.	%	
Well or High*	18	31%	0	0%	10	32.3%	0
Intermediate *	14	24.1%	1	7.1%	5	16.1%	0
Poor or Non *	26	44.8%	0	0%	16	51.6%	0
Total	58	100%	1	1.7%	31	100%	0

Keys:

*Well or High: secondary school or above.

*Intermediate: primary school.

*Poor or Non: only have basics in reading and writing or non.

4.6. The effect of work on women with recurrent abortion and positive IgM to Cytomegalovirus.

Out of 58 women with recurrent abortion, 8 (13.8%) were workers, no one of them was IgM positive, while out of 50 (86.2%) didn't work and one case of positive IgM was found (2%) (Table 4.6).

Table (4.6) The effect of work on women with recurrent abortion and positive IgM to Cytomegalovirus

Occupation	Women with recurrent abortion		+ve IgM to CMV		Control group		No. of +ve IgM to CMV
	No.	%	No.	%	No.	%	
Yes	8	13.8%	٠	0%	4	12.9%	0
No	50	86.2%	1	2%	27	87.1%	0
Total	58	100%	1	1.7%	31	100%	0

5. Discussion

5.1. Discussion

Primary CMV infection has been found to be more prevalent in pregnant women than non-pregnant. This difference may be attributed to the susceptibility of seronegative women, at the onset of pregnancy, to the first CMV infection (Stango *et al;* 1982).The risk for fetal infection is greatest with maternal primary CMV infection and much less likely with recurrent infection as the virus remains latent in the host cell after initial infection (Massimo *et al;*2009). Abortion can result from ascending CMV endometritis and the virus has been isolated from post-abortion uterine discharge (Dehner *et al* 1975).

Rubina *et al* reported that 16.37% of women with recurrent abortion were positive IgM to CMV. 14% of women in Iraq suffering from recurrent abortion showed positive result for IgM to CMV (Maysra *et al;* 2012), and 12.9 % in India reported by Kapil and Broor (1992).

This study revealed that the Cytomegalovirus prevalence among women with recurrent abortion in Khartoum state was 1.7% for Immunoglobulin M (IgM).

This result was close to that reported by Gaytant *et al* (2003) among pregnant women worldwide. And it was within the range 0.2%-2.2% reported by Demmler (1991). Yet, it does not agreed with those result obtained by Rubina *et al* (2004), Nuha *et al* (2012), Kapil an Broor (1992), and Maysra *et al* (2012).

Locally, the prevalence of CMV in this study is lower compared to that obtained by Khairi *et al* in 2013, this might be due to smaller sample size,

shorter period of study, or present of other etiologic causes of abortion among these subjects under study, which need to be confirmed in other researches with larger sample size, longer period of study, and use of other modern, specific and sensitive methods of diagnosis.

Although this study showed high incidence of women under study with poor education and non workers suffering from recurrent abortion which represent 44.8% and 86.2% respectively, there was no case of positivity among them, and no case with history of congenital anomalies neonate birth, which agreed with Khairi *et al* (2013) who reported that no significant association between congenital abnormalities, occupation or education level and CMV infection. However, it does not agree with Kafi *et al* (2013) who reported that there was significant association between CMV infection and congenital malinformation in children.

5.2. Conclusion

This study provided information about Cytomegalovirus infection in women with recurrent abortion, CMV antibodies was detected in 1.7%. And no positive CMV IgM among healthy pregnant women with no history of abortion.

5.3. Recommendations

1. Routine screening of CMV antibodies for the women during pregnancy with high sensitive and specific approach.

2. Routine screening of females of child bearing age for CMV infection is desired in order to reduce the fatal outcome of the pregnancy occurring due to CMV infection.

3. Routine screening of the TORCH for women during pregnancy because some infections cause recurrent abortion as CMV infection.

4. More investigation is needed to explain the results obtained by various authors in different geographical locations using larger sample size, and further to study the role of CMV in the recurrent abortion.

5. Advanced techniques are needed by using PCR to validate these results.

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Appendix (1)

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Questionnaire

Seroprevalance of Cytomegalovirus among women suffering from recurrent abortion.

1. Sample No. ……..

2. Age …….

3. Numbers of abortion ……..

4. Gestation weeks …….

5. Did you have history of birthing baby with congenital anomalies?

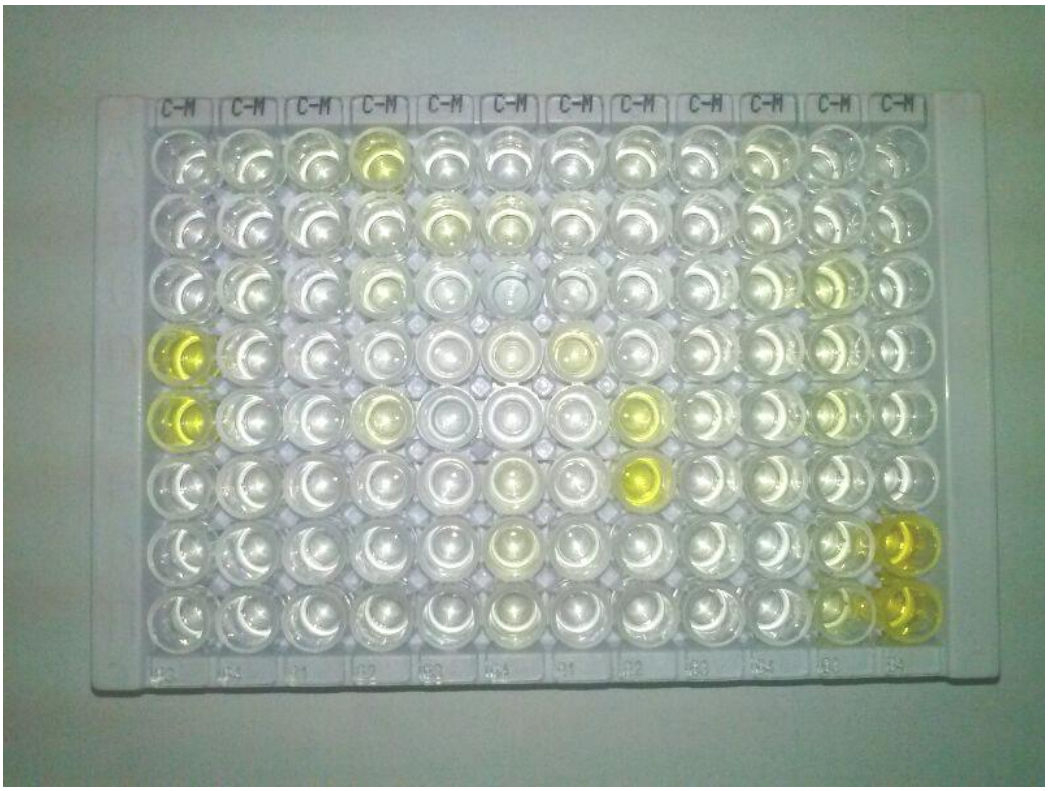
(Yes) (No)

6. Chronic or genetic disease? (Yes) (No)

7. Occupation? (Yes) (No)

8. Education level (low) (intermediate) (high or well)

Appendix (2)



ELISA microplate

Appendix (3)

ELISA reader