بسم الله الرحمن الرحيم

Sudan University of science and Technology

College of Graduate Studies

Seroprevalence of Cytomegalovirus Infection among Neonates with Congenital Anomalies

الإنتشار المصلي للإصابة بفيروس مضخم الخلايا بين حديثي الولادة المصابين بعيوب خلقية

A thesis submitted to the college of Graduate Studies in partial fulfillment for the requirement of MSc. Degree in Medical Laboratory Science

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

قال تعالى:

﴿ وَقُلِ اعْمَلُوا فَسَيَرَى اللّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ ﴾

صدق الله العظيم

سورة التوبة

الآية (105)

DEDICATION

To my honorable parents and my beloved brother and sisters.

To my dear friends.

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First of all, my thanks to AlMighty ALLAH for giving me help and patience to finish this study.

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ABSTRACT

This is a qualitative, descriptive, cross-sectional, hospital-based study , It was conducted among neonates with congenital anomalies attending Omdurman Maternity Hospital, Alshaab Teaching Hospital, Ibrahim Malek Teaching Hospital in Khartoum State, During the period from September to December 2013.

A total of 92 neonates with congenital anomalies (n=92) were included in this study,39 (42.4%) of them were males, and 53 (57.6%)were females. There age ranged from 1 day to 4 months (mean2.6 month).

Serum samples were tested for Cytomegalovirus IgM by a capture enzyme- linked immunosorbent assay (ELISA).

Data were analyzed by SPSS version 16.0 computer software. The results obtained showed that while 2 (2.2%) out of 92 neonates with congenital anomalies were Cytomegalovirus IgM positive, 90(97.8%) were Cytomegalovirus IgM negative.

One (1.1%) of 92 neonates with congenital anomalies has neural tube defect (NTD) and the other one (1.1.%) has hydrocephaly(HC).

From the above findings it is concluded that, there was 2.2 % of neonates with congenital anomalies are Cytomegalovirus IgM positive in Khartoum state. There is no differences in the proportion between congenital anomalies associated with Cytomegalovirus.

ملخص الأطروحة

أجريت هذه الدراسة الكمية،الوصفية، المستعرضة، على حديثي الولادة المصابين بتشوهات خلقية في مستشفى أم درمان للولادة، مستشفى الشعب التعليمي، مستشفى إبراهيم مالك التعليمي، خلال الفترة من سبتمبر إلى ديسمبر2013.

شملت هذه الدراسة 92 من حديثي الولادة المصابين بتشوهات خلقية، 39 (42.4%) منهم من الذكور و53 (57%) منهم من الإناث، تراوحت أعمارهم بين (يوم واحد- أربعة شهور) ومتوسط الأعمار (2.6 شهر).

تم جمع عينات مصل الدم، واختبار القلوبيولين المناعي من النوع **M** لفيروس مضخم الخلايا بواسطة إختبار الروز المناعي الإنزيمي.

وقد تم تحليل البيانات إحصائيا بواسطة برنامج الكمبيوتر (**SPSS**) الإصدار 16.0. أظهرت نتائج هذه الدرسة 2 من أصل 90 من هؤلاء المرضى أعطوا نتيجة إيجابية لفيروس مضخم الخلايا ب (2.2%)، و90 أعطوا نتيجة سلبية (97.8%).

في الدراسة الحالية واحد(1.1%) من أصل92 من حديثي الولادة المصابين بعيوب خلقية كان مصابا بعيب خلقي في الأنبوب العصبي. و الاخر(1.1%) كان مصابا بزيادة ماء الرأس.

من النتائج أعلاه خلصت الدراسة إلى أن (2.2%) من حديثي الولادة المصابين بتشوهات خلقية مصابين بفيروس مضخم الخلايا. وأنه ليس هناك فرق في النسب بين التشوهات الخلقية المتعلقة بفيروس مضخم الخلايا.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
CMV	Cytomegalovirus
CNS	Central nervous system
CPE	Cytopathic effect
DNA	Deoxyribonuleic acid
ELISA	Enzyme-linked immunosorbent assay
HC	Hydrocephaly
HCMV	Human cytomegalovirus
HCMV-5	Human cytomegalovirus-5
HCMV-6	Human cytomegalovirus-6
HCMV-7	Human cytomegalovirus-7
HSV	Herpes simplex virus
IgG	Immunoglobulin class G
IgM	Immunoglobulin class M
MC	Microcephaly
NTD	Neural tube defect
PCR	Polymerase Chain Reaction
RCS	Rubella congenital syndrome

RNA	Ribonuleic acid
SPSS	Statistical Product and Social Solution

CHAPTER ONE

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 of double-stranded linear DNA, the virus capsid is of icosahedral symmetry, and a viral envelope. They also share the biological properties of latency and reactivation. CMV grows slowly in cell culture and strict species specifically (Murray *et al*; 2007).

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).

It is called Cytomegalovirus because of swollen state of infected cells in culture and tissues. Nuclei of productively infected cells contain a large inclusion bodies, giving a typical " owl's " eye appearance. The virus can be transmitted by different modes such as across the placenta, within birth canal and in breast milk that in early life. In young children, it is most common mode of transmission is via saliva (Levinson; 2010).

Cytomegalovirus can also be transmitted sexually and during blood transfusion and organ transplants. The infection of CMV is worldwide, and more than 2% of adults have antibodies against the virus (Levinson; 2010).

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

In immunocompetent adults, CMV can cause heterophil negative mononucleosis and in immunosupressed patients there are systemic CMV infection, especially pneumonitis and hepatitis, e.g., those with renal and bone marrow transplants (Levinson; 2010).

1.2. Rationale

Cytomegalovirus is found throughout the world among all socio-economic groups and infects between 50% and 85% of adults by the age of 40 years. CMV infection is more widespread in developing countries and areas of low socio-economic conditions (Hodinka; 2007).

The majority of CMV infections are asymptomatic but CMV infections can cause serious disease in the newborn infants and the immunocompromised individuals. About 2% of pregnant women have either a primary or a reactivated CMV infection during pregnancy, and it is estimated that 10% -20% of congenitally infected newborns will show evidence of disease (Gaytant *et al*; 2003).

The infection among neonates can be presented as:

Generalized infection which may occur in the infant, and cause complications such as low birth weight (Intra Uterine Retardation), microcephaly, hydrocephaly Nueral tube defects, petechial rash similar to the "blueberry muffin" rash of congenital rubella syndrome, and moderate hepatospleenomegaly (with jaundice).

Though severe cases can be fatal, with supportive treatment most infants with CMV disease will survive. However, from 80% to 90% will have complications within the first few years of life that may include hearing loss, vision impairment, and varying degrees of mental retardation.

Another 5% to 10% of infants who are infected but without symptoms at birth will subsequently have varying degrees of hearing and mental or coordination problems (Yoshikawa et al; 2005).

1.3. Objectives

1.3.1. General Objective

To detect the frequency of CMV virus IgM among neonates with congenital anomalies.

1.3.2. Specific Objectives

1.3.2.1 To detect specific CMV IgM in sera of neonates with congenital anomalies.

1.3.2.2 To determine the frequency of congenital anomalies associated with CMV among neonates with hydrocephaly and neural tube defects.

CHAPTER TWO

2. Literature Review

2.1. Herpesviruses

The name of the herpes viruses came from 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 it's host and especially the immune status of the host (Murray *et al* ; 2007).

2.1.1. History

Cytomegalovirus was first noticed by Ribbert in 1881, when he saw large "protozoan-like" cells in the kidney of still-birth infant (Barry *et al* ; 2004).

The first report of the visualization of CMV by electron microscopy appeared in 1953, when cytomegalic inclusion cells from an infant's pancreas were viewed and particles were observed in both the cytoplasm and the clear halo around the inclusions (Ho; 2008).

The virus was cultured for the first time in 1956 by Rome, Smith and Weller, all of whom worked independently (Bhatia and Ichhupujani; 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 Betaherpesvirinae with cytomegalovirus of other animal species based on it's tropism for salivary glands, slow growth on cell culture and strict species specificity (Brooks *et al*; 2010) .

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

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

2.1.3. Structure of the virus

The general structure of the virus seen in (Figure 2.1). Cytomegalovirus characterized by slow growing, cytomegalic (Cytopathic effect, CPE). Which from its name, and enlargement of cell with acidophilic inclusion bodies in the nuclei that resemble owl's eye, (Figure 2.2). It is the genus megalo that its official name HHV-5 (Brooks *et al*; 2010).

Compared to other human herpesviruses, HCMV is largest, with genome of 235 kb encoding 165 genes (Davinson et al; 2003).

Cytomegalovirus is composed of large DNA genome (240kpb), which is double strand, linear and in form of atoroid. In spite of its genetically different strain 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. 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 phosphoprotien 65(pp65) (Varnum et al; 2004).

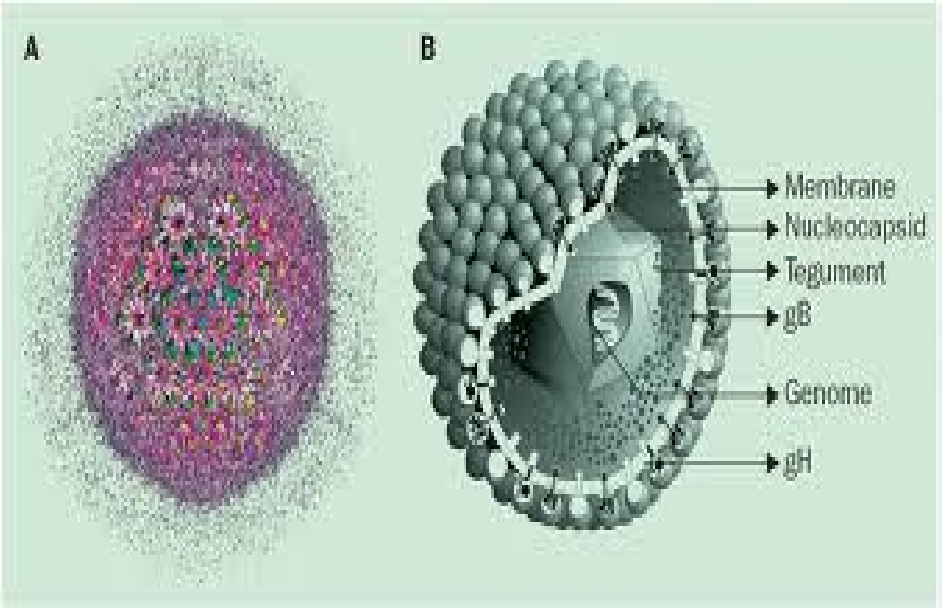

Figure 2.1: General structure of the CMV (Brooks *et al*; 2010).

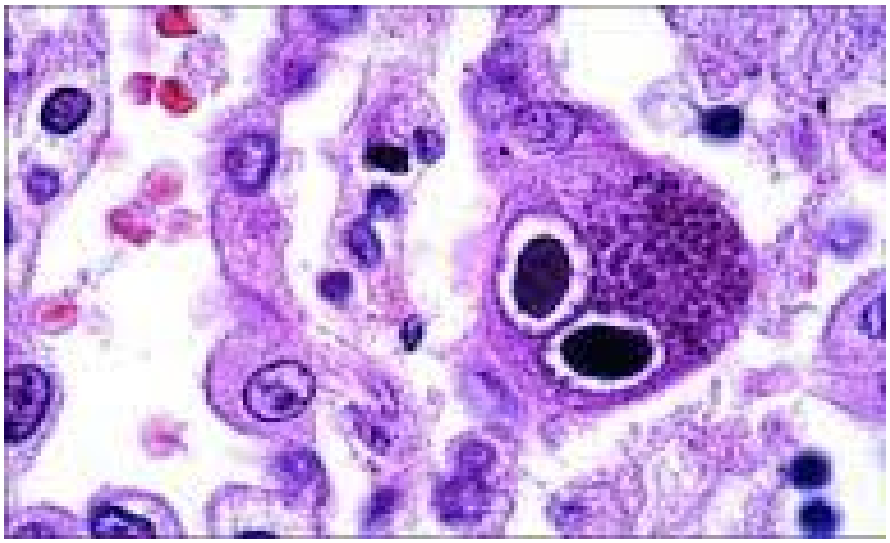

Figure 2.2: CPE of the virus (Owl's eye) (Brooks *et al*; 2010).

2.1.4. Properties of the virus

Cytomegalovirus has the largest genetic content of the human herpesviruses, its DNA genome (240 kpb) 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 surface glycoprotein, act 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 fibroblast, 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 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 to give its structural component after viral DNA replication and translation, 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 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 beast milk of seropositivity women. The risk of CMV transmission in infants breast-fed by 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, male homosexuais, diabetes, chronic disease, chemotherapy, newborns) (Akhter and Tood; 2011).

Human cytomegalovirus is highly species-specific, with humans 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).

2.1.8. Pathogenesis

Cytomegalovirus causes no symptom in children and most mild disease in adult. The virus first infects 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 humoral and cell mediated immunity from the possibility of spread from cell to cell. If suppressed, the virus later reactivate, 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 immunosuppressed patients like retinitis in up to 15% of all AIDS patients, interstitial pneumonia, colitis, esophagitis, hepatitis, myelitis, colitis, uveitis and encephalitis are seen in some patients (Greenwoon *et al*; 2007).

2.1.9. Lab diagnosis

The virus can be diagnosed by electron microscope, cell culture, biopsy staining , serology and molecular method (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. 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 encluding 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 (PCR)

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 sell 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 antibody.

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 the 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 valyl prodrug vanciclovir (Nichols and Boeckh; 2000).

Intravenous gancyclovir is used as a first- line treatment of CMV disease in bone marrow and solid organ transplant recipients.

Reversible bone marrow suppression is the most common adverse effect of ganciclovir. Other adverse effect of the drug are rash, pruritus, diarrhea, nausea, vomiting and increased levels of serum creatinine and lver enzymes. Neurotoxicity may occur occasionally (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, especially those unable to tolerate ganciclovir and those infected with ganciclovir-resistant virus (Razonable; 2011).

Cidofovir is a nucleoside analogue used for the 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 (Razonbale; 2011).

2.1.10.1. Prenatal management and treatment

The management of the pregnancy in cases of primary CMV infection is a matter of debate (Demmler and Nance; 2005). Suspected foetal 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 foetus needs to be taken into consideration when planning strategies for prenatal diagnosis (Collinet *et al*; 2004).

2.1.10.2. Postnatal treatment

Ganciclovir treatment of symptomatic newborns has been evaluated in several studies. Ganciclovir therapy begun in the neonatal period in children with symptomatic CMV infection

involving the CNS prevents hearing deterioration in the first six months of life and may prevent hearing deterioration in the first year of life. Ganciclovir is toxic to the bone marrow, and two thirds of the treated infants suffered from side effects such as significant neutropenia. Recent studies in neonates with symptomatic congenital CMV infection reported that comparable plasma concentrations can be reached by oral administration of valganciclovir and intravenous administration of ganciclovir . Currently it is recommended to use of 6mg/Kg intravenous ganciclovir twice daily for six weeks in babies born with CNS involvement and proven congenital CMV infection (Kimberlin *et al*; 2003).

2.1.11. Prevention

Prevention strategies are classified as primary, secondary and tertiary prevention. Primary prevention strategies try to avoid an infection and are mostly accomplished by precautions against exposition to the virus, i.e. hygiene measures and change of behaviour. Secondary prevention strategies allow identifying infected patients at an early stage, with the aim of stopping progression of infection and disease. In the case of symptomatic disease, tertiary prevention strategies try to prevent the development of severe sequelae after infection(Nigro and Jeon; 2009).

2.2. CMV among neonates with congenital anomalies

Congenital HCMV infection occurs when the mother suffers a primary infection (or reactivation) during pregnancy. Due to the lower seroprevalence of HCMV in industrialized countries and higher socioeconomic groups, congenital infections are actually less common in poorer communities, where more women of child-bearing age are already seropositive. In industrialized countries up to 8% of HCMV seronegative mothers contract primary HCMV infection during pregnancy, of which roughly 50% will transmit to the fetus. Between 22-38% of infected fetuses are then born with symptoms, which may include pneumonia, gastrointestinal, retinal and neurological disease. HCMV infection occurs in roughly 1% of all neonates with those who are not congenitally infected contracting the infection possibly through breast milk. Other sources of neonatal infection are bodily fluids which are known to contain high titres in shedding individuals: saliva ($<10^7$copies/ml) and urine ($<10^5$copies/ml) seem common routes of transmission. (Barry *et al*; 2004). CMV remains the most important cause of congenital viral infection in the United States. HCMV is the most common cause of congenital infection in humans and intrauterine primary infections are more common than other well-known infections and syndromes, including Down Syndrome, Fetal Alcohol Syndrome, Spina Bifida, and Pediatric HIV/AIDS. (Ryan and Ray; 2004).

2.2.1. Presentation of the infection

For infants who are infected by their mothers before birth, two potential adverse scenarios exist:

- Generalized infection may occur in the infant, and can cause complications such as low birth weight (Intra Uterine Retardation), microcephaly, hydrocephaly Nueral tube defects, petechial rash similar to the "blueberry muffin" rash of congenital rubella syndrome, and moderate hepatospleenomegaly (with jaundice). Though severe cases can be fatal, with supportive treatment most infants with CMV disease will survive. However, from 80% to 90% will have complications within the first few years of life that may include hearing loss, vision impairment, and varying degrees of mental retardation.
- Another 5% to 10% of infants who are infected but without symptoms at birth will subsequently have varying degrees of hearing and mental or coordination problems.

These risks appear to be almost exclusively associated with women who previously have not been infected with CMV and who are having their first infection with the virus during pregnancy (Yoshikawa *et al*; 2005).

CHAPTER THREE

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

Neonates with congenital anomalies admitted to hospital suspected to be infected with CMV.

3.4. Study area

The blood samples were collected from neonates born with congenital anomalies who are hospitalized in Omdurman Maternity Hospital, Alshaab Teaching Hospital, Ibrahim Malek Teaching Hospital. The experimental work was carried out in the Central Laboratory, Khartoum, Sudan.

3.5. Study duration

The study was carried out during the period, from September to December, 2013.

3.6. Sample size

Ninety two blood samples (n=92) from neonates with congenital anomalies.

3.7. Sample collection

Venous blood samples were collected from each subject. Disinfection by 70% alcohol .Syringe was used to collect the sample in plain tubes. Blood samples were allowed to clot at room temperature and serum was separated by centrifugation at 3000 RPM/ 3 minutes.

3.8. Sampling technique

A 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 interviewing questionnaire (Appendix).

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 parents of participant neonates were informed before collection of blood for the purpose of the study, and verbal consent was taken from all parents.

3.13. Laboratory diagnosis of CMV

3.13.1. Detection of anti-CMV IgM antibodies using ELISA

Determination of IgM class antibodies to CMV virus in serum was performed using IgM ELISA kits(G.E.N.E.S.I.S Diagnostic,Omega Diagnostic Group PLC, Cambridge Shine,Uk).

3.13.1.1. Principle

ELISA test system is designed to detect IgM antibody to CMV in human sera. CMV IgM Antigens are adsorpted in solid phase to the polysterene reaction strop. If there is CMV IgM antibody in test sample, it will binds to CMV IgM antigen forms antigen-antibody complex, and then binds to the enzyme labled anti-antibody and forms antigen-antibody-antibody complex, and 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, all samples were diluted 1:100 with sample

diluents. 100 µl of the negative control, 100 µl of 10 IU/ml standard, 100µl of positive control, and 100µl of diluted samples were incubated in microplate wells coated with CMV antigen at room temperature for 15 minutes, the wells were washed 3 times by BPS/Tween washing buffer (which diluted firstly by distilled water 1:9) to remove residual plasma. After another washing step to eliminate unbound material, an enzyme conjugate (Horseradish peroxidase) was added (100µl/ well) and the plate was incubated for 15 minutes. The wells were washed 3 times by BPS/Tween washing buffer. Substrate solution (TMB substrate) was added (100µl/ well) and plate was incubated for 10 minutes. The blue color changed to yellow after adding of the (0.2 M H2 SO4) stop solution (100µl).The cut-off value was calculated by optical density (O.D) in a microplate ELISA reader was read within 10 minutes at 450 nm wavelength .

3.13.1.3. Measurement

ELISA reader microplate 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 Negative Control (MNC) and mean absorbance values of Positive Control (MPC) were calculated. The cut-off value was then calculated following this equation:

Cut-off value COV= MNC + (0.2 × MPC)

The result run was validated according to the manufacture's criteria for validity as below:

1- Ab substrate blank < 0.150

2- MNC < 0.250

3- MPC > 0.500

3.13.1.5. Interpretation of the results

To determine the presence or absence of CMV-IgM, the measured O.D is compared to O.D mean of IU/ ml as follows:

Negative samples : O.D < O.D of 10 IU/ml standard

Positive samples: O.D > /= O.D of 10 IU/ml standard.

CHAPTER FOUR

4. RESULTS

A total of ninety two subjects (n=92) were enrolled in this study, 39(43%) of them were males and 53(57%) were females, age range was from 1 day to 4 months (Mean: 2.6 month).

4.1. Detection of CMV IgM among neonates with congenital anomalies

Table (4.1) demonstrates that 2 (2.2 %) out of 92 neonates with congenital anomalies were found IgM to CMV positive, 1of them was male and 1 was female, and 90 (97.8%) were found IgM to CMV negative (97.8%), 38 (42.4%) of them were males and 52 (57.6%) females.

Table (4.1): Detection of Cytomegalovirus IgM antibodies in 92 of congenitally born neonates.

			Gender		
			Male	Female	Total
CMV	Positive	Count	1	1	2
		of Total	1.1%	1.1%	2.2%
	Negative	Count	38	52	90
		of Total	41.3%	56.5%	97.8%
Total		Count	39	53	92
		of Total	42.4%	57.6%	100.0%

4.2. Detection of CMV IgM among neonates with congenital NTD

Table (4.2) shows that 1(1.1%) out of 92(45.7%) neonates with congenital NTD was shown CMV IgM positive.

Table (4.2): The frequency of IgM to CMV antibodies among neonates with congenital NTD.

			NTD		
			Yes	No	Total
CMV	Positive	Count	1	0	2
		of Total	1.1%	1.1%	1.1%
	Negative	Count	41	49	90
		of Total	44.6%	53.3%	98.9%
Total		Count	42	50	92
		of Total	45.7%	54.3%	100.0%

4.3. Detection of CMV IgM among neonates with congenital HC

Table (4.3) out of 92 (71.7%) neonates with congenital HC there was 1 positive CMV IgM (1.1%).

Table (4.3): The frequency of IgM to CMV antibodies among neonates with congenital HC.

			HC		
			Yes	No	Total
CMV	Positive	Count	1	0	1
		of Total	1.1%	0%	1.1%
	Negative	Count	64	26	90
		of Total	69.6%	28.3%	98.9%
Total		Count	66	26	92
		of Total	71.7%	28.3%	100.0%

CHAPTER FIVE

5. DISCUSSION

Despite the recognized importance of congenital CMV infection in the world, only limited information is available about the incidence and in the natural history of this infection in Sudan. In this hospital-based newborn screening study, we found a 2.2% birth prevalence of congenital CMV infection, and 1.1% of infected infants exhibited at least one clinical finding suggestive of congenital infection in the newborn period. In this result, CMV seroreactivity may underestimate the actual prevalence of infection. Because of small sample size, it is conceivable that, the rate of CMV nonprimary maternal infection accounts for > 95% of infants with congenital CMV infection (Yamamoto *et al*; 2001).

The rate of congenital CMV infection observed in this study in infants

born to Sudanese women from a predominantly low socioeconomic level is similar to that seen in other highly immune populations in Africa (Van der Sande *et al*; 2007) , Chile (Miura *et al*; 2007) , Korea (Sohn *et al*; 2000), Mexico (Noyola *et al*; 3003), China (Tsai *et al*; 1998), and India (Dar *et al*; 2008) and among low-income North American women (Boppana *et al*; 2000). However, these results differed from the others that observed in populations with European (Barbi *et al*; 2006) or North American (Murph *et al*; 2000) populations in which the prevalence of congenital CMV infection ranges from 0.18% to 0.48%. The findings of this study are consistent with those of previous studies and a recent review of the literature documenting that the incidence of congenital CMV infection increases with increasing maternal CMV seroprevalence and low socioeconomic status of the mother (Kenneson and

Cannon; 2007). The discrepancy in the reported results of congenital CMV infection between several studies, including our study, could be due to several factors. One could be attributed to differences in the various techniques used for the detection of CMV . Differences in the sensitivity of the methods used for detection of CMV, different prevalence of CMV in a geographical area, and differences in the socioeconomic status and nutrition and prenatal infection.

Conclusion

This study provides frequency of CMV infection in neonates with congenital anomalies. CMV IgM antibodies was detected in NTD and HC affected neonates with 1.1% in each.

Recommendations

1. Routine screening of CMV antibodies for the women during pregnancy with a highly sensitive and specific approach.
2. Routine screening of CMV antibodies for the neonates with a highly sensitive and specific approach.
3. A closer follow- up is recommended in patients with positive results of CMV test.
4. Routine screening of Rubella virus for women during pregnancy and for neonates because some symptoms of congenital CMV infections are similar to symptoms of congenital rubella infections (CRS).
5. More investigation is needed to explain the results obtained by various authors in different geographical locations using larger

samples size, and further to study the role of CMV in the congenital infection.

6. Further confirmation is needed for positive results by using PCR.

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Appendix

Appendix (1)

Sudan University of science and Technology

College of Graduate Studies

Questionnaire

Seroprevalence of Cytomegalovirus among Neonates with Congenital Anomalies.

Name: ……..... Age: ……..

Gender: …… Gestational age: …..…

Nervous system:

Neural Tube Defect: Yes( ) No( )

Hydrocephaly: Yes( ) No( )

Microcephay: Yes( ) No( )

Eye:

Anophthalmos: Yes( ) No( )

Cataract: Yes( ) No( )

Ear:

Ear defect: Yes( ) No( )

Congenital heart disease:

Anomalies of cardiac champers and connections: Yes No)

Malformations of cardiac :

1. Ventricular septal defect: Yes( ) No( )

2. Arterial septal defect: Yes( ) No( )

3. Artioventricular septal defect: Yes( ) No( )

4. Malformation of valves: Yes( ) No( )

5. Malformations of the great arteries and veins: (Yes) (No)

Cleft lip with or without palate: Yes( ) No( )

Cleft palate: Yes( ) No( )

Digestive system anomalies: Yes( ) No( )

Hepatospleenomegaly: Yes( ) No( )

Jaundice: Yes( ) No( )

Limb defect: Yes( ) No( )

Appendix (2)

ELISA microplate (*Virol. J*)

Appendix (3)

ELISA reader (*Virol. J*)