بسم الله الرحمن الرحيم

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Sero Frequency of Cytomegalovirus Among Type I Diabetic Children Attending Different Hospitals in Khartoum State

نسبة تكرار الاجسام المضادة للفيروس المضخم للخلايا وسط الاطفال المصابين بداء السكر من النوع الاول بمختلف مستشفيات ولاية الخرطوم

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الآية

بِسْمِ اللّهِ الرّحْمَنِ الرّحِيمِ

قال الله تعالى :

قُلْ لَوْ كَانَ الْبَحْرُ مِدَاداً لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَداً

صدق الله العظيم

سورة الكهف- الآية (109)

DEDICATION

This Research is lovingly dedicated

To my respective parents who have been my constant source of inspiration. They have given me the drive and discipline to tackle any task with enthusiasm and determination.

To my husband without his love and support this project would not have been made possible.

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My thankful and praise to ALMIGHTY ALLAH for helping me to fulfill my desire and accomplish this research aiming to get master degree in medical microbiology. The acknowledgement and thanks also to outstanding scholar **Dr. Wafa Ibrahim** for her supporting ,facilitating and supervising me to easy achieve this research , great efforts has a profound impact during the past time.

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ABSTRACT

Cytomegalovirus is one of the most common microorganisms that cause opportunistic infections; the virus can cause severe diseases with multiple complications.

This was descriptive, cross sectional study, aimed to detect cytomegalovirus among type I diabetic children in Sudan and to determine the risk factors (gender,age,and family history) associated with cytomegalovirus infection.

Sera of 90 children were collected 45 (50%) were diabetic patients (case group) and 45(50%) are apparently healthy children (control group) who attending different Hospitals in Khartoum State, during the period from March to May 2015.

The samples were tested for IgG anti cytomegalovirus by using enzyme-linked immunosorbent assay (ELISA) technique. Demographic and clinical data were collected via direct interviewing questionnaire.

The results showed that 43/45 (95.6%) of the case group and 40/45 (88.9%) of the control group were positive for CMV IgG (P value=0.242).

40(44.4%) of study population were males, whereas 50(55.6%) were female .All participants fall in age ranged from four to fifteen years old, 54(60%) categorized in age group of 4-9 years old, while the rest 36(40%) children were in group of 10-15 years old.

The results revealed higher prevalence rate of CMV in males 37/40(92.5%).Only12 (13.3%) of all participants had family history of diabetes mellitus.

The study showed that there was no relation between sero-positive IgG anti-cytomegalovirus among type I diabetes mellitus and gender (P value=.931) and also with past history of family diabetes (P value=.939)

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There was an insignificant association between cytomegalovirus and type I diabetes mellitus in Sudanese children.

الملخص

يعتبر الفيروس المضخم للخلايا احد الكائنات الحية المجهرية التي تسبب العدوى الانتهازية,كما انه يمكن ان يسبب الفيروس امراض حادة ومتعددة المضاعفات0

هذه كانت دراسة وصفية مستعرضة تهدف لدراسة نسبة تكرار الفيروس المضخم للخلايا في الاطفال المرضى بالنوع الاول من داء السكري بمستشفيات مختلفة في الخرطوم وعوامل الخطر المرتبطة بالفيروس خلال الفترة من مارس الى مايو 2015.

هدفت هذه الدراسة إلى الكشف عن تواتر الفيروس المضخم للخلايا (CMV) بين مرضى داء السكر من النوع الأول وتحديد العوامل (الجنس والعمروالتاريخ الاسري لمرض السكري) المرتبطة بالعدوى الفيروس المضخم للخلايا.

تم جمع صل من 90 طفلا 45 (50٪) من المشاركين هم مرضى السكري (المجموعة الحالة) و 45 (50٪) من المشاركين هم من الأطفال اصحاء (المجموعة الضابطة). تم اختبار العينات ل IgGمضاد الفيروس المضخم للخلايا باستخدام الفحص المناعي (ELISA). تم جمع المعلومات عن العمر والجنس والتاريخ العائلي لمرض السكري النوع الأول عن طريق الاستبيان.

النتائج أظهرت 45/43 (95.6٪) من مجموعة الدراسة و45/40 (88.9٪) من المجموعة الضابطة)

(*P*value.242%).

40 (44.4٪) من جميع الفئات هم من الذكور، في حين أن 50 (55.6٪) كانت من الإناث في سن تتراوح 4-15 سنة، 54 (60٪) تصنيفها في الفئة العمرية من 4-9 سنوات من العمر، في حين تم فرز بقية 36 (40٪) الأطفال في مجموعة من 10-15 سنة.

أظهرت النتائج ارتفاع نسبة انتشار CMV في الذكور 40/37 (92.5٪). فقط 12 (13.3%) من جميع المشاركين لديهم تاريخ عائلي لداء السكر من النوع الاول .دلت الدراسة على انه لا توجد علاقة بين المصلية الايجابية IgG وعامل العمر (*P* value .931). ايضا لاتوجد علاقة مع التاريخ العائلي لداء السكر من النوع الاول (*P* value 939)

دلت الدراسة على عدم وجود علاقة معنوية بين الفيروس المضخم للخلايا والنوع الاول من داء السكري في الاطفال السودانين.

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List of Abbreviation

AIDS	Acquired immunodeficiency syndrome
CMV	Cytomegalovirus
CPE	Cytopathic effect
CFT	Complement Fixation Test
CD4	Cell of differentiation 4
CD8	Cell of differentiation 8
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent assay
EBV	Epstein Bar Virus
GAD 65	Glutemic Acid Decarbolase 65
HCMV-1	Human cytomegalovirus-1
HCMV-5	Human cytomegalovirus-5
HCMV-8	Human cytomegalovirus-8
HIV	Human Immunodiffeciency Virus
HLA	Human Leukocyte Antigen
HLA-A8	Human Leukocyte Antigen-A8
HAART	Highly Active Antiertroviral Therapy
HPC	Hematopoietic Cell
IgG	Immunoglobuline class G
IgM	Immunoglobuline class M

ICT	Immunochromatography Test
ICA	Islet Cell Antibodies
IFIHI	Interferon Induced Helicase 1
NK	Natural Killer cell
MHC	Major Histocomatipility Complex
PCR	Polymerase chain reaction
PU 157	Power Unit 157
SPSS	Statistical package service solution
TMB	Tetra Methyle Benzidine

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Diabetes mellitus is one of the most important public health problems in the world, and characterized by group of disorders with hyperglycemia as a main feature of the diseases (Brickell *et al*., 2007).

The incidence of diabetes mellitus is continuing rising all over the world, and this may be a result of many reasons that can be collectively or separately lead to the disease . These factors include genetic factors, obesity, autoimmunity disorders and infections (Brickell *et al*., 2007).

Certain viruses can infect human and may cause diabetes mellitus through different mechanisms such as pancreatitis or hepatitis and their subsequent complications .These viruses include Mumps virus , Rubella virus , Cytomegalovirus ,Coxsakie B virus , Cytomegalovirus is a wide distributed virus that belongs to betaherpesvirinea subfamily of (DNA) with icosahedra coat (Haaheim *et al*.,2002,Elhawary *et al*.,2011) .

Cytomegalovirus (CMV) does appear to be somehow associated with autoimmune type I diabetes since there is a strong correlation between the presence of islet cell auto-antibodies and persistent infections. Regarding genetic factors, there are distinct markers related to the susceptibility to CMV-associated type I diabetes (Yoon *et al*., 1989).

Virus could potentially act in initiation, promotion of the viral infection and the timing of infection may be of key importance. A causal role would thus imply

exposure early in life (before or shortly after birth); alternatively, viral infection could promote an existing immune process or compromise beta cell function sufficiently to precipitate the onset of hyperglycaemia (Pak *et al.*, 1988).

There are many potential mechanisms by which viral infection might promote autoimmunity. These include cell rupture with exposure of intracellular antigens to the immune system; alternatively persistent or latent infection might lead to altered recognition of the cell by the immune system, followed in turn by chronic low grade damage (Pak *et al.*, 1988).

Genetic and environmental factors are implicated in the beta cell destruction. As environmental factor affecting the induction of type I diabetes, diabetogenic viruses, chemicals, toxins and diet are likely candidates as either primary injurious agents of beta cells or triggering agents for the induction of autoimmunity (Pak *et al.*, 1988). Regarding viruses as a triggering factor of type I diabetes, there are at least two different pathogenic mechanisms in virus-induced diabetes :cytolytic infection of beta cells , leading to their destruction ,and triggering of autoimmunity ,leading to the autoimmune-mediated destruction of beta cells (Pak *et al.*,1988).

In Khartoum State , the immunoglobulin class G (IgG) antibodies against cytomegalovirus were found in 77% ,73%,97.3%and 84% of donated blood(Eldoma,2004, Bushra,2006, Ibrahim,2012 and Mohamed,2014) , 95% of pregnant women ,96% of kidney pretransplanted patients ,98% of renal

transplanted patients and 17% of healthy candidate donors of renal transplantation(Bushra,2006,Awad,2007). Besides that,the anti-cytomegalovirus antibodies were found in 72.2% in pregnant women in western Sudan (Hamdan *et al.*,2011) , and in 87.8% of Sudanese living in Jeddah city in Saudi Arabia (Redwan *et al*.,2011).

Locally, there is only a case control study conducted on the pathogenesis of CMV on type 1 diabetes mellitus (Mudathir *et al*.,2014), who used sera of 81 children, 27(33.3%) with diabetes which represent the study group and 54 (66.7%) without diabetes which represent the control group. The samples were tested for IgG anti-cytomegalovirus using (ELISA) technique. 18 (22.2%) of the total population of study were positive, 55.6% were diabetic patients, the results indicated significant association of CMV IgG antibodies with type 1 diabetes mellitus in children (Mudathir *et al.*,2014).

1.2 Rationale

Cytomegalovirus is a causative agent of many form of diseases includes atypical infectious mononucleosis and congenital cytomegalovirus infection with cytomegalic inclusion disease, also the virus can cause extensive organ damage in immunocopromised patients. The complication of the viral infection can be interstitial pneumonia, hepatitis, retinitis, gastrointestinal infection and diabetes mellitus (Uyar *et al*.,2008).

Several authors indicate that the cytomegalovirus is widely distributed in Sudan, Eldom.,(2004), Bushra,(2006), Ibrahim,(2012)and Mohamed,(2014) had reported high prevalence of IgG anti-cytomegalovirus among blood donors 77% , 84% ,97.3%and 73% respectively .

Awad, (2007) and Hamdan *et al.*, (2011) were found sero-prevalence of cytomegalovirus in pregnant ladies 95% and 72.2% respectively.

To the best of our knowledge there were few researches regarding cytomegalovirus in diabetic children in Sudan, done by Mudathir *et al* (2014). They found IgG antibodies of cytomegalovirus in 11.9% of test group (diabetic) and 4.8% in the control group.

1.3objectives

General objective:

- To determine sero-frequency of cytomegalovirus among type I diabetic children attending different hospitals in Khartoum State during March to May 2015 using ELISA technique.

Specific objectives:

- To detect the presence of cytomegalovirus IgG antibodies among diabetic patients (case group) and healthy individuals (control group).
- To determine the relation between sero-frequency of CMV and Diabetes Type I and different factors (age, gender and family history of type I diabetic children).

CHAPTER TWO
LITERATURE REVIEW

2 Literature review

2.1History

The name of herpesviridae was derived from Greek word herpein which means to creep. This family contains more than one hundred viruses that infected wide range of hosts including human, animals, birds, plants and fish. Only eight viruses cause human disease and named in numbers from human herpes virus 1 to human herpes virus 8 (HHV1-HHV8). Cytomegalovirus is the common name of human herpes virus 5 (HHV5), and classified within betaherpesvirinae subfamily of herpesvirinae family (Sone *et al.*,2010).

The owl's eye cytopathic effects associated with cytomegalic inclusion disease was recognized at early 1930's, and related to viral infection at early 1950's after detection of virus structure in cytomegalic cells by electron microscope and by isolation of the virus(Knipe and Howley,2007).

Cytomegaloviruses are ubiquitous herpesviruses that are common causes of human disease. The name for the classic cytomegalic inclusion disease derives from the propensity for massive enlargement of cytomegalovirus-infected cells (Uyar *et al.*,2008).

Cytomegalic inclusion disease is a generalized infection of infants caused by intrauterine or early postnatal infection with the cytomegaloviruses (Jawetz *et al.*,2007). Cytomegalovirus poses an important public health problem because of its high frequency of congenital infections, which may lead to severe congenital anomalies. Inapparent infection is common during childhood and

adolescence. Severe cytomegalovirus infections are frequently found in adults who are immunosuppressed (Jawetz *et al.*, 2007).

2.2 **Structure of the virus**

Cytomegalovirus has the largest genetic content of the human herpesviruses. The virus particles consist of core, capsid, tegument and envelope. The core is DNA genome (240 kbp) and it is double stranded which encodes viral protein. Only a few of the many proteins encoded by the virus (over 200) have been characterized (Jawetz *et al.*, 2007). The protein coat or capsid of the virus is icosahedra symmetry composed of 162 capsomers. Both core and capsid form nucleocapsid that surrounded by envelope from which an eight nanometer (nm) surface glycoprotein projections called spikes are raised. The cell surface glycoprotein, acts as an Fc receptor that can nonspecifically bind the Fc portion of immunoglobulins. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins(Jawetz *et al*,2007). The tegument is amorphous matrix or space lies between the capsid and envelope. It considers about 40% of the whole mass of the virion. (Knipe and Howley, 2007)

2.3 Pathogenesis

2.3.1Transmission of the virus

Cytomegalovirus can be transmitted and cause infection by both ways: vertical transmission (cross placenta from infected pregnant woman to her fetus or during birth) and horizontal transmission (from person to other usual

transmission)(Knipe and Howley,2007)in several different ways, all requiring close contact with virus-bearing material. There is a 4 to 8weeks incubation period in normal older children and adults after viral exposure (Jawetz *et al.*, 2007).

Touching the eyes or inside of nose or mouth after coming into contact with the body fluids blood, saliva, tears, urine, vaginal fluids, cervical secretion and semen of an infected person could spread the disease. This is the most common way CMV is spread because it's absorbed through the mucous membranes. In addition to that, infected breast milk can infect the child through lactation from infected women (Knipe and Howley, 2007)

2.3.2 **Replication of the virus**

Although the virus is often isolated from epithelial cells of the host, human cytomegalovirus replicates in vitro only in human fibroblasts, and it replicates very slowly in cultured cells, and it may take several weeks for an entire monolayer of cultured cells to become involved with growth proceeding more slowly than that of Herpes simplex viruses or varicella-zoster virusa. Infection is spread primarily cell-to-cell but very little virus becomes cell-free(Jawetz *et al.*,2007).

The Perinuclear cytoplasmic inclusions form of cytopathic effect is the characteristic of cytomegalovirus in addition to the intranuclear inclusions typical of herpesviruses. Multinucleated cells and many affected cells become greatly enlarged (Jawetz *et al.*, 2007).

2.3.3 **Immune response**

Innate and adaptive immune responses are cooperating to control primary cytomegalovirus. During primary infection, natural killer cells (NK) and Interferon (INF) are playing an important role in defense and clearance of cytomegalovirus. The adaptive response is mainly T-cell mediated with secondary role by the antibodies (Knipe and Howley, 2007)

The virus possesses several proteins which play roles in NK cells down modulation and also encodes genes that down regulate cell surface expression of major histocompatibility antigens class I(MHC class I) and class II(MHC class II) (Knipe and Howley,2007,Tomasec *et al.*,2005).

Cell-mediated immunity is depressed with primary infections, and this may contribute to the persistence of viral infection. It may take several months for cellular responses to recover (Jawetz *et al.*, 2007).

An immune response to viral antigens in the host cells or to host-cell-specific antigens that are exposed as a result of infection might cause a disease. If the CMV infection persists in the beta cells and either expresses viral antigens or induces an aberrant expression of class II major histocompatibility complex (MHC) antigen on the cells in certain circumstances, such as particular genetic background and environmental effects (eg, drug, diet, toxins, infections), persistent CMV infection might trigger beta-cell-specific autoimmune. Lately it was reported that chronic viral infection of beta cells could result in synthesis

and release of alpha-interferon, which in turn induced class I MHC hyper expression on adjacent endocrine cells. The aberrant expression of class II MHC antigen on the beta cells was also reported in a type1 diabetic patient (Pak *et al.*, 1988).

2.3.4 **Persistence and latency**

Once person infected with CMV, the virus remains with him for life, but it's not always active. CMV may cycles through periods during which it lies dormant and then reactivates. If in healthy person, it mainly stays dormant. The patient can pass the virus to others during reactivation. The tendency of reactivation following immune suppression or immunodeficiency is an important factor leading to cytomegalovirus associated diseases (Jarvis and Nelson, 2006, Mocarski *et al.*, 2006).

The infection can be inactive (historical), latent, or still active. Most virus infections are asymptomatic or trivial, yet these might nonetheless trigger an immunological chain reaction which results in diabetes long after all evidence of the original infection has vanished. Persistent replication occurs in epithelial cells of respiratory tract and kidneys of the host cell and persists in lymphocytes with irregular shedding in body fluids through life without seeking to replicate, while hematopoietic cells (HPC) either myeloid progenitor cell types or more differentiated cells lineage are harbor latent virus and it may be reactivated to pursue the pathway of replication and cell lysis (Reeves *et al.*, 2005).

2.3.5**Indirect Evidence: The "Viral Signature"**

Foulis and coworkers were the first to systematically document the hyper expression of HLA Class I and interferon-α within islets of recently diagnosed diabetic children .Normal islets are completely devoid of these markers. The presence of these markers is commonly referred to as a "viral signature," as the up-regulated expression of HLA/MHC class I molecules is typically driven by type I IFNs following viral infection. The islet-specific MHC class I expression would render the cells suitable targets to CD8 T cells that are known to be an integral part of insulitic lesions. Indeed, in a mouse model, only β cells that have been unmasked by MHC class I expression were attacked by activated, auto-reactive T cells, demonstrating the necessity of MHC class I up-regulation for immune-mediated β-cell destruction the defined polymorphisms in the IFIH1 gene may result in lower levels of type I interferons (IFNs) in response to viral infections and may confer protection from autoimmune diabetes (Jeffrey *et al.*,2012)

2.3.6 Cytomegalovirus and type I diabetes mellitus

The classical classification of diabetes mellitus divides it into two types: type I which also called insulin dependent diabetes mellitus, and type II which is insulin independent (Brickell *et al*., 2007).

Type I diabetes mellitus is usually a deficiency in insulin, and consider as one of an organ specific autoimmune diseases that caused by selective destruction of insulin producing pancreatic beta cells by the action of cytotoxic T

lymphocytes and autoantibodies (Goldsby *et al.,* 2003).Where type II diabetes mellitus is insulin resistance with or without relative insulin deficiency, and usually occur as result of obesity and unhealthy lifestyle (Sone *et al*., 2010).

Diabetes in children is usually type I, and happened with severe manifestations with very high elevation in blood glucose, noticeable glucosuria and ketonuria (WHO, 1999).

The autoimmune paradigm of the 1960s proposed that the link between organ-specific autoimmunity and human leukocyte antigen (HLA) susceptibility could be bridged by environmental triggers. Viral infection was an obvious candidate (Pak *et al*., 1988).

Early investigators assumed that juvenile diabetes resulted from acute viral infection, but prospective family studies made it clear that immune-mediated or type 1A diabetes has a long prodrome, indicating that any encounter with a causative virus must have occurred months or years before the onset of beta cell failure (Pak *et al*., 1988).

Type I diabetes mellitus can be result of many factors, one of the most important factors is cytomegalovirus owing to its ability to induce immunological beta cells damage (Aarnisalo *et al*., 2008).

Molecular mimicry is one of the most important immunological mechanisms that lead to destruction of the pancreatic beta-cells. This mimicry could be involved in cytomegalovirus-induced diabetes by inducing islet cell antibodies. Besides that, the auto-reactive CD4 T cell clone reactive to self antigen called

GAD65 which contains cross-reacting sequence that cytomegalovirus DNA-binding protein pUl57 (Hielmesaeth *et al.*,2005). Hiemstra *et al* .,(2001) found the loss of T-cell tolerance to GAD65 self-antigen may be due to processing and presentation of molecularly mimic cytomegalovirus protein pUl57 by denderitic cells Hiemstra *et al* .,(2001).

The cytomegalovirus specific viral genome was found in22% of diabetic patients, correlating with present of islet cell antibodies in their serum, suggesting the persistence cytomegalovirus infections may relevant to pathogenesis in some cases of type I diabetes mellitus (Pak *et al.*,1988).

Several studies were conducted to correlate cytomegalovirus with type I diabetes mellitus, one of these studies is that of Nicoletti *et al.*, (1990) who reported a significant association between high titer of anti-cytomegalovirus and anti-islet cell antibodies. Also other new studies have shown that asymptomatic cytomegalovirus infection is associated with increased risk of new onset type I diabetes and impaired insulin release after renal transplantation (Hjelmesaeth *et al*.,2004). In contrast, Hiltunen *et al.*,(1995) did not found any correlation between the presence of anti-cytomegalovirus IgG antibodies and anti-islet cell antibodies in children with newly diagnosed type I diabetes mellitus.

2.4 Clinical features of cytomegalovirus infections :

2.4.1 Mononucleosis

Most cytomegalovirus infections are silent or asymptomatic, but it also can present as acute febrile illness with feature of mononucleosis that very similar to mononucleosis of Epstein Bar Virus (EBV) when doing Paul-Bunnell test it has characterstic of heterophile antibodies (Haaheim *et al*., 2002). Cytomegalovirus is responsible for approximately 20-50 % of heterophile negative mononucleosis and that is consider about 8% of all cases of mononucleosis (Klemola *et al.*,1970). The main picture of the illness is characterized by fever for more than 10 days, myalgias, malaise, headache and fatigue. hepatomegaly, splenomegaly, adenopathy and rash are present in some patients (Knipe and Howley,2007,Klemola *et al.*,1970).

The laboratory findings of mononucleosis-like illness of CMV is specific anti-cytomegalovirus immunoglobulin class M (IgM), lymphocytosis with atypical lymphocytes and abnormal liver function, especially elevation of bilirubin and transaminases (Knipe and Howley.,2007)The virus causes a systemic infection; it has been isolated from lung, liver, colon, kidneys, esophagus, monocytes , T and B lymphocytes. Virus can be shed in the urine for months to years after primary infection and intermittently from the pharynx. Prolonged cytomegalovirus infection of the kidney does not seem to be deleterious in normal persons. Salivary gland involvement is common and is probably chronic (Jawetz *et al*., 2007).

2.4.2 **Congenital features of cytomegalovirus infection**

Occurs by vertical transmission which includes three ways: transplacental, intrapartum and by way of breast milk which is the most common mean of infection (Knipe and Howley, 2007).

Congenital cytomegalovirus infection is an important medical and public health problem due to its damage to the central nervous system and organs of perception (Boppana *et al.*,1992).

Fetal and newborn infections with cytomegalovirus may be severe. A high percentage of babies with this disease will exhibit developmental defects and mental retardation (Jawetz *et al*., 2007).

The signs and symptoms of this infection are manifested as non neurologic abnormalities include petechiae or purpura, small gestational age, jaundice, hepatosplenomegaly, hemolytic anemia and pneumonia. Neurologic abnormalities include microcephaly, intracranial calcification, hearing impairment, chorioretinitis, seizers and finally neonatal death (Knipe and Howley, 2007, Stratton *et al.*,2001).

2.4.3 **Cytomegalovirus in immunosuppressed host**

Cytomegalovirus is one of the most common microorganisms that cause opportunistic infections and complicate the clinical care and progress of immunocompromised patients. The disease can be result of primary infection, reinfection or activation of latent virus from previous infection. Primary

cytomegalovirus infections in immunosuppressed hosts are much more severe than in normal hosts. And the most problems for those patients it is the way of infection consider same way of hospitalization and caring procedures such as blood transfusion and organ transplantation (Knipe and Howley, 2007).

Viraemia is notable feature of the disease in immunocompromised patients, and the severity of the infection is nearly corresponding to the degree of immune compression The host immune response presumably maintains cytomegalovirus in a latent state in sero-positive individuals, so that Individuals at greatest risk for cytomegalovirus disease are those receiving organ transplants, recipient of stem cell transplants, those with malignant tumors who are receiving chemotherapy, and those with acquired immunodeficiency syndrome AIDS patients those with very low CD4 count(Knipe and Howley,2007). The severity of the disease ranged from short and self limiting febrile illness to multisystem disease with extensive organ damage that can be life threatening or debilitating. Reactivated infections are associated with disease much more often in immunocompromised patients than in normal hosts. Although usually less severe, reactivated infections may be as virulent as primary infections. Pneumonia, vision and hearing problems are the most common complications (Jawetz *et al*., 2007).

Approximately 20% to 40% of adults with AIDS develop cytomegalovirus disease, but this percent and severity of the disease is diminished by using of antiviral drugs such as highly active antiretroviral therapy (HAART) that

results in improving the degree of immunocompression and rising CD4 count (Knipe and Howley, 2007).Approximately 90% of human immunodeficiency virus (HIV) infected women of childbearing age are co-infected with cytomegalovirus. It has been thought that HIV infected pregnant women would result in increase rate of congenital cytomegalovirus infection in their offspring and those women are more likely to shed cytomegalovirus than HIV negative women (Quinn *et al.*, 1987, Clarke *et al.*, 1996, Doyle *et al.*, 1996).

2.5 **Epidemiology**

Cytomegalovirus is broadly distributed over all human population. The prevalence is increased with age and more common in people with low economic status and with female is slightly higher than male (Knipe and Howley, 2007).

The sero-prevalence of IgG anti-cytomegalovirus was studied and reported in different nations. Ghebrekidan *et al.,* (1999) found prevalence more than 90% in Eritrea, Jama *et al.*, (1987) report 90% in Mogadishu-Somalia, and Zhao *et al.,* (2009) found the frequency of 48.07% in eastern China. Redwan *et al.*, (2011) studied the prevalence of cytomegalovirus among foreign manpower in Jeddah city-Saudi Arabia, and found the frequency of 88.9% in Ethiopian, 87.8% in Sudanese, 83.6% in Bangladeshi,82.7% in Pakistani, 78.7% in Yemen and 66.7% in Indian.

Because cytomegalovirus is vertically transmitted and can cause congenital infection with multiple complications, many authors focusing their studies on

pregnant women and women in childbearing age (Knipe and Howley, 2007). In pregnant women, ,El-Nawawy *et al.*,(1996*)* found the prevalence of 96% in Egypt ,Uyar *et al.*,(2008) report prevalence 97.3% in northern Turkey , Ghazi *et al.*,(2002) report 92.1% in Saudi Arabian pregnant ladies, Wong *et al.*,(2000) found prevalence 87% in Singaporean pregnant women . Other authors report 99% in Ankara-Turkey (Hizel *et al.*, 1999), 97% in Cotonou-Benin (Rodier *et al.*, 1995) 84% and 67% in Sao Paulo-Brazil in low and middle socio-economic status pregnant women respectively (Pannuti *et al.*,1985). In Khartoum state-Sudan, the sero-prevalence of IgG anti-cytomegalovirus among pregnant women was reported to be 72.2% by Hamdan *et al.*, (2011) while Awad *et al.*, (2007) found 95% in western Sudan.

De mattia *et al.*, (1991) study the prevalence of cytomegalovirus antibodies among three to eighteen years old Italian children and reported that 64.2% was positive for IgG anti-cytomegalovirus. White *et al.*, (1989) reported prevalence of 43% in American ageing from 6-22 years old, and Ali, (2013) reported 77.8% highest frequency 86% of IgG detected among the age group 26-31 years old, lowest 72% detected among the age group 20-25.Whereas liu *et al.*, 1990 reported prevalence of 60% in age 4-6 years and 52% in age less than one year old in Chengdu-China.

2.6 **Prevention**

Although cytomegalovirus is highly contagious and rapidly spread in households and closed societies due to shedding of the virus in all biological

secretions such as saliva ,urine ,tears ,vaginal secretion and semen , but simple ways of protection like avoidance of contact with possibly contaminated body fluids , good hand washing, avoid sharing food or drinking out of the same glass as others ,be careful with disposable items ,practice safe sex, clean toys and countertops, increasing awareness and personal hygiene can control the virus spread and infection (CDC,2012) .

2.7 **Treatment and vaccination of cytomegalovirus**

There's no cure for CMV, and treatment for the virus generally isn't necessary or recommended for healthy children and adults.

Newborns and people with compromised immune systems, however, need treatment when they're having symptoms of CMV infection. The kind of treatment depends on the symptoms and their severity such as pneumonia (Jenkins *et al.*, 2004).

Nucleoside analogue with a modified pentose such as foscarnet, a pyrophosphate analogue and ganciclovir have anti-cytomegalovirus activity and are use in clinical care for both : prophylaxis in transplantations and in suppressive treatment in active infections (Haaheim *et al*.,2002) . Experimental vaccines are being tested for women of childbearing age. These vaccines may be useful in preventing CMV infection in mothers and infants, and reducing the chance that babies born to women who are infected while pregnant will develop disabilities by ways of attenuated live virus ,a recombinant protein in a fowl pox vector , a DNA vaccine ,and a recombinant protein given with a new

adjuvant , but there is no approved available vaccine that can be used routinely (Knipe and Howley,2007).while passive immunization is available ,and high levels of antibody to cytomegalovirus have been evaluated for prevention of cytomegalovirus disease in organ transplant recipients ,premature newborns at risk for postnatal infection and pregnant women (Knipe and Howley,2007).

2.8 **Laboratory diagnosis of cytomegalovirus infection**

Cytomegalovirus infections clinically characterized by malaise and prolonged fever which often more than ten days. That need to be differentiated from other acute infectious illness of viral, bacterial and parasitic infections this can be done by serological means, viral isolation and molecular techniques (Knipe and Howley.,2007).Several serological techniques were used for identification of anti-cytomegalovirus IgM and IgG such as enzyme-linked immunosorbant assay (ELISA), immunochromatography test (ICT), immune-fluorescent tests and complement fixation test (CFT). The benefit of IgM is the detection of recent infection, where the detection of IgG is indicating last exposure to infection, and give great benefit for organ transplantation and blood transfusion (Knipe and Howley, 2007). Tests for IgM antibody to cytomegalovirus often lack specificity for primary infection due to false positive results from other similar antigen, or because IgM antibody from past cytomegalovirus infection. The avidity of IgG antibody increases with time after initial infection and demonstration of low cytomegalovirus-IgG avidity can improve the accuracy of identification of recent primary infection (Grangeot *et al*., 1997, lazzarotto *et al.,* 1999, Guo *et al.*, 1998).

Virus isolation from urine, throat, genital secretions, milk and blood is the favorite method for diagnosing active cytomegalovirus infections. The sample should be mixed with a suitable transport medium and sent to the laboratory as soon as possible and never be frozen (Haaheim *et al.*, 2002). The suitable type of tissue culture is human embryonic lung or foreskin fibroblast, then identification of the slowly developing, focal cytopathic effect (CPE) that is characteristic of cytomegalovirus, or alternatively identification of the virus by specific monoclonal antibodies (Knipe and Howley,2007).Polymerase Chain Reaction (PCR) is the most common molecular technique that used in laboratory. It extremely sensitive and specific, and replace viral isolation in diagnosis of infection in newborn and immunocompromised (Haaheim *et al.*, 2002, Knipe and Howley, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3 Materials and Methods

3.1 Study type and design

This study was Descriptive cross secional study.

3.2 Study area

The study area was carried out at different hospitals in Khartoum State hospitals including (Gaber abo elez, Gafar ebn aof and Police Hospitals).

3.3 Study population

Children diagnosed with type I diabetes mellitus (as case group) and healthy children (as control group). Participants are of both genders males and females with age ranged from 4-15 years old.

3.4 Sample size

The sample size was 90 participants 45diabetic (case group) and 45 healthy (control group).

3.5 Inclusion criteria

For study group: known diagnosed type I diabetic children of one year history, among age from four to fifteen years old, with or without family history of diabetes mellitus.

For control group: apparently health and normal children with or without family history of diabetes mellitus.

3.6 Method of data collection

Demographic and clinical data were collected through personal interviewing questionnaire.

3.7 Ethical consideration

Permission to perform the study was taken from the College of Graduate Studies, Sudan University of Science and Technology. All subjects participated were informed before blood collection.

3.8 Experimental work:

3.8.1 Specimen collection

3 ml venous blood was collected from all participants under aseptic condition and left to clt for 30 minutes. The blood was centrifuged at 5000 rpm for 10 minutes at room temperature to obtain serum; serum was preserved at -20^{o}C till used.

3.8.2 Detection of IgG anti-cytomegalovirus using ELISA (EURO IMMUN,Germany)

- The serum samples were diluted 1 in 100 in sample buffer.
- One hundred microliter of diluted serum, undiluted calibrator, positive and negative controls were applied into micro-titer strips (each in separated well), and the micro-titer plate was incubated for 30 minutes at 18-25^{o}C.
- Three hundred microliter per well of washing buffer were added 3 times.

- One hundred microliter of enzyme conjugated anti human IgG was pipetted to each well, and then the micro-titer plate was incubated for 30 minutes at 18-25^{o}C.
- Three hundred microliter per well of washing buffer were added 3 times.
- One hundred microliter of chromogenic substrate (TMB/H_2O_2) was pipetted to each well, and then the micro-titer plate was incubated for 15 minutes at 18-25^{o}C.Then One hundred microliter of stop solution (Sulphuric Acid) was added to each well.
- Then the density of color was read photo-metrically at 450 nm.

3.8.3 Interpretation of the cut-off point:

Formula: $$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

1-Negative: < 0.8 Ratio

2-borderline: ≥0.8 to <1.1 Ratio

3-positive: ≥1.1 Ratio

3.9 Data analysis

Correlations and frequencies were computerizing calculated by statistical package for social science (SPSS) program version 19.

CHAPTER FOUR

RESULTS

4. RESULTS

Ninety children were participated in this study, 45(50%) were type I diabetic patients represent case group and 45(50%) were apparently healthy children represent control group. The IgG antibodies against cytomegalovirus were found in 43(95.6%) of study group, 40(88.9%) of control group The participants age ranged from 4-15 years old, 54(60%) categorized in age group of 4-9 years old, while the rest 36(40%) children were sorted in group of 10-15 years old.

Statistical analysis showed insignificant association between IgG antibodies to cytomegalovirus and type I diabetes mellitus in children (*P* value= .242) (table 1), and also between seropositivity and gender (*P* value=.931) 40(44.4%) of all participants were males, whereas 50(55.6%) were females higher percentage of CMV IgG was observed among males 37/40(92.5%) (table 2).

Regarding family history of diabetes, statistical analysis showed insignificant association with CMV seropositivity (pvalue 0.341) (Table4).

Table (1) Association between serofrequency of CMV and diabetes type I among study population

group	CMV IgG		Total
	positive	Negative	
case group(diabetic)	43 (95.6%)	2 (4.4%)	45 (50%)
control group(non diabetic)	40 (88.9%)	5 (11.1%)	45 (50%)
Total	83 (92.2%)	7 (7.8%)	90 (100%)

- *P* value= .242
- *P* value <0.05 consider significant

Table (2) Sero-frequency of CMV among study population n=90 according to their gender

Gender	population	CMV IgG		Total
		positive	Negative	
Males	Case	18 94.7%	1 5.3%	19 21.1%
	Control	19 90.5%	2 9.5%	21 23.3%
Females	Case	25 96.2%	1 3.8%	26 28.9%
	Control	21 87.5%	3 12.5%	24 26.7%
Total		83 92.2%	7 7.8%	90 100%

- *P* value= .931
- *P* value <0.05 consider significant

Table (3) Serofrequency of CMV among study population according to the Family history of diabetes mellitus type I

Family History of Diabetes Mellitus	sample		Total
	Case group (diabetic)	control group(non diabetic)	
yes	3 6.7%	9 20%	12 13.3%
No	42 93.3%	36 80%	78 86.7%
Total	45 50%	45 50%	90 100%

- *P* value= .939
- *P* value <0.05 consider significant

Table (4) serofrequency of CMV among study population according to their age groups

Age Groups	population	CMV IgG		Total
		positive	Negative	
4-9	Case	29 100%	0 0%	29 32.3%
	Control	23 92%	2 8%	25 27.8%
10-15	Case	14 87.%	2 12.5%	16 17.8%
	Control	17 85%	3 15%	20 22.2%
Total		83 92.2%	7 7.8%	90 100%

- *P* value= .341
- *P* value <0.05 consider significant

CHAPTER FIVE

DISCUSSION CONCLUSION & RECOMMENDATIONS

5.1 Discussion

Several types of viral infections have been associated with increased risk of diabetes mellitus**;** CMV is considered to be the most common pathogen of man. Most of CMV infections remain asymptomatic. The outcome is fatal in rarely cases (Bhatia and Ichhpujani.,2008).

The present study results revealed that the overall sero-frequency of CMV among diabetic children was 95.6% while it was 88.9% among control group, when compared with other previous studies it found closely similar to results of Sosal, (2015) in Sudan-Khartoum who found it as 96.6% among diabetic adults.

The present study shows that the positive rate of IgG against cytomegalovirus in diabetic 43 (95.6%) was higher than that of normal individuals 40 (88.9%).This result is similar to other study carried by Guo *et al*.,(1998) who studied cytomegalovirus infection in patients with diabetes mellitus and concluded that the positive rate of IgG against cytomegalovirus in diabetic is higher than that of normal individuals.

The insignificant finding of this study was association between IgG of cytomegalovirus and type I diabetes mellitus in children. This result disagreed with work of Hjelmesaeth *et al*.,(2004) and Mudathir,(2014), who found significant association between a symptomatic cytomegalovirus infection and increase risk of new onset diabetes mellitus. Also Nicoletti *et al*.,(1990) found a significant association between high titers of anti-cytomegalovirus IgG

antibodies and anti-islet cell antibodies (ICA). Pak *et al.*, (1988) reported the strong correlation between cytomegalovirus genome and islet cell antibodies.

These results found insignificant relation between cytomegalovirus IgG antibodies and family history to type I diabetes mellitus. However several studies were carried out to find the relation between cytomegalovirus and family history. Which was in agree with Santos *et al.*,(2000) who reported there was no evidence of statistical interaction between cytomegalovirus antibodies and the DQB10201 allele or the DQB1 0302 allele. Nicoletti *et al.*, (1990) also reported insignificant relation between the presence of any HlA-A-B-C and DR antigens and the prevalence of anti-cytomegalovirus IgM and IgG antibodies and/or (ICA).

De Mattia *et al.*, (1991) found significant increase of IgG anti-cytomegalovirus in females, but our results indicated slightly increased IgG anti-cytomegalovirus in males (92.5%) than females (92%) with insignificant association between the gender and CMV IgG sero-positivity ,which was different from finding of Knipe and Howely,(2007) who reported slightly increase of anti-cytomegalovirus antibodies in females.

At the level of this study population (case group and control group) we found increase in sero-positive in 4-9 years group, whereas the observations were reported by Knipe and Howely,(2007) and De Mattia *et al.*,(1991) were higher in 10-15 years old group.

The present result indicated insignificant association between anti-cytomegalovirus IgG and type I diabetes mellitus in younger age group (4-9years). The variation of our findings from published literature may be due to differences in geographical location, socioeconomic status type of study population, sample size and methods used.

5.2 CONCLUSION

IgG anti-cytomegalovirus was detected in 95.6% of diabetic children and in 88.9% of apparently health children, IgG anti-cytomegalovirus was higher in males than females.

Statisticaly there was an insignificant association between IgG anti-cytomegalovirus and type I diabetes mellitus, age ,and gender.

5.3 Recommendations:

1. Establish national screening program for cytomegalovirus.
2. Initiate vaccine program against cytomegalovirus in children.
3. Establish diagnostic polices for cytomegalovirus.
4. Further in-depth studies using RT-PCR is recommended to determine the circulating geno types of CMV.

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Appendixes

Appendix1

Questionnaire

Sudan University for science and technology

College of graduate studies

Sero Frequency of Cytomegalovirus Among Type I Diabetic Children Attending Different Hospitals in Khartoum

---

No……

Age……. Gender: male…… female……

Residence: ……

Ethical agreement:

……..

……

Previous history of family diabetes:

……

……

Result:

……

……

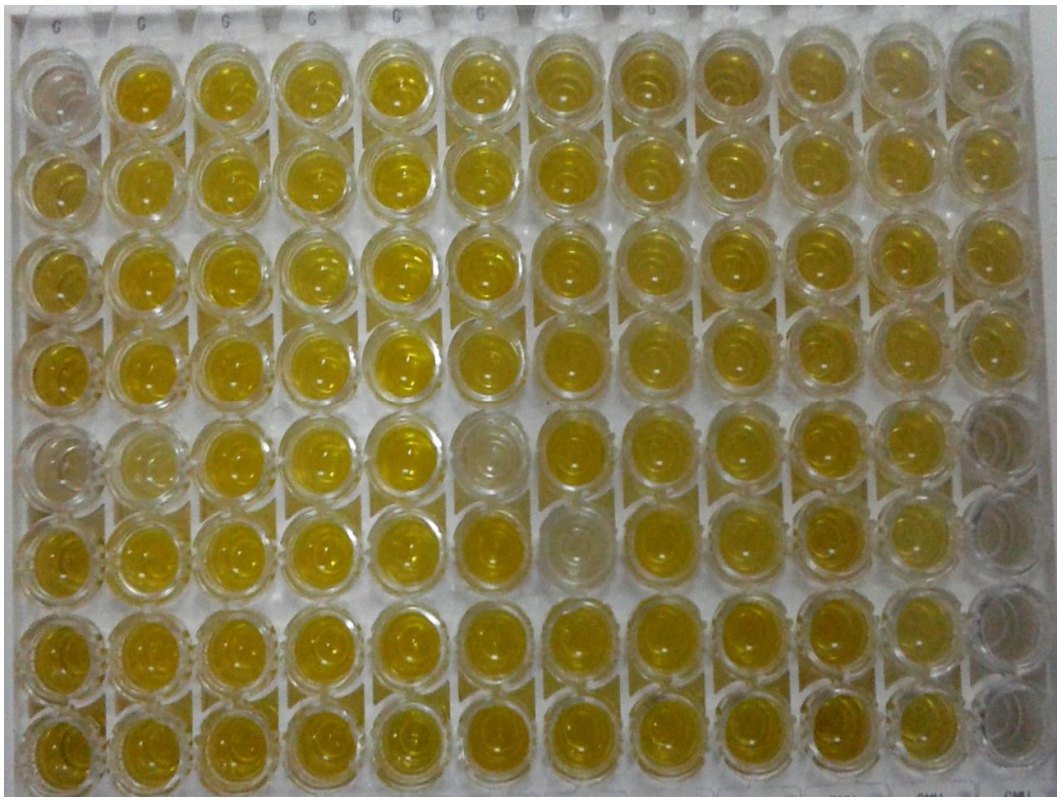


Image1: ELISA microtitre plate of the results

Yellow: positive.

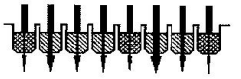
Colorless: negative.

Appendix**2**

EUROIMMUN Medizinische Labordiagnostika AG

(EV_0010_F_DEUK_A04) 13351000-0092_EV_0010_F_DEUK_A04.doc

ELISA-Inkubation
ELISA Incubation

		Antigen-beschichtete Reagenzgefäße *antigen-coated wells*	
	Pipettieren: ***Pipette:***	100 µl je Reagenzgefäß *100 µl per well*	Kalibratoren, Kontrollen, verdünnte Proben *calibrators, controls, samples* 
1.	**Inkubieren:** ***Incubate:***	30 min bei Raumtemperatur (18°C bis 25°C) *30 min at room temperature (18°C to 25°C)*	
	Waschen: ***Wash:***	300 µl (man.)/450 µl (aut.) je Reagenzgefäß Einwirkzeit: 30-60 s je Waschzyklus *300 µl (man.)/450 µl (aut.) per well* *residence time: 30-60 s per washing cycle*	3x 
	Pipettieren: **Pipette:**	100 µl je Reagenzgefäß 100 µl per well	Enzymkonjugat *enzyme conjugate* 
2.	**Inkubieren:** ***Incubate:***	30 min bei Raumtemperatur (18°C bis 25°C) *30 min at room temperature (18°C to 25°C)*	
	Waschen: ***Wash:***	300 µl (man.)/450 µl (aut.) je Reagenzgefäß Einwirkzeit: 30-60 s je Waschzyklus *300 µl (man.)/450 µl (aut.) per well* *residence time: 30-60 s per washing cycle*	3x 
	Pipettieren: **Pipette:**	100 µl je Reagenzgefäß *100 µl per well*	Chromogen/Substrat *chromogen/substrat* 
3.	**Inkubieren:** ***Incubate:***	15 min bei Raumtemperatur (18°C- 25°C) *15 min at room temperature (18°C- 25°C)*	
	Pipettieren: **Pipette:**	100 µl je Reagenzgefäß *100 µl per well*	Stopplösung *stop solution* 
	Auswerten: ***Evaluate:***	Photometrische Messung (450 nm) *photometric measurement (450 nm)*	

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EUROIMMUN Medizinische Labordiagnostika AG

Qualitätskontrollzertifikat
Quality Control Certificate

Produkt: **Anti-CMV ELISA (IgG)** — **Best.-Nr.:** **EI 2570-9601 G**
Product: *Order No:*

Ch.-B.: **E150407AV** — **Verw. bis:** **06-Apr-2016**
Lot: *Exp. Date:*

		Referenzwert *Reference value*		Valider Bereich *Valid range*	
Kalibrator 1 *Calibrator 1*	200 RU/ml	1,408	O.D.	> 0,700	O.D.
Kalibrator 2 *Calibrator 2*	20 RU/ml	0,302	O.D.	> 0,140	O.D.
Kalibrator 3 *Calibrator 3*	2 RU/ml	0,031	O.D.		
Pos. Kontrolle 1 *Pos. Control 1*	quantitativ *quantitative*	134	RU/ml	94 - 174	RU/ml
Pos. Kontrolle 1 *Pos. Control 1*	semiquantitativ *semiquantitative*	3,3	Ratio	1,8 - 4,8	Ratio
Neg. Kontrolle *Neg. Control*	quantitativ *quantitative*	3	RU/ml	0 - 15	RU/ml
Neg. Kontrolle *Neg. Control*	semiquantitativ *semiquantitative*	0,1	Ratio	0 - 0,7	Ratio

O.D. Kalibrator 1 > O.D. Kalibrator 2 > O.D. Kalibrator 3
O.D. Calibrator 1 > O.D. Calibrator 2 > O.D. Calibrator 3

Die Charge wurde von der Qualitätskontrolle getestet und erfüllt alle Spezifikationen.
The lot has been tested by the quality control laboratory and meets the specifications.

Appendix (3)

The principle of the assay

The antiviral IgG antibodies were tested by anti-cytomegalovirus ELIZA (EURO IMMUN). The principle of the test is antigen antibody reaction within ELIZA procedure. The kit was semi-quantitative invitro assay for detection of human antibody class IgG against cytomegalovirus in serum or plasma. The kit contains micro titer stripes with eight break-off reagent wells coated with cytomegalovirus antigens. In the first reaction step, diluted samples were incubated in the wells. In the case of positive sample, specific IgG antibodies could bind to the antigens. To detected bound antibodies, a second incubation was carried using enzyme labeled anti-human IgG (enzyme conjugate) to catalyze a color reaction. The intensity of the formed color was proportional to the concentration of the antibodies against cytomegalovirus antigens.