بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

College of Graduate Studies

Seroprevalence of Cytomegalovirus Infection among Leukemic Patients in Khartoum State

الانتشار المصلي للإصابة بفيروس مضخم الخلايا بين مرضى سرطان الدم معدل بولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirement of M.S c in Medical Laboratory Science (Microbiology)

By:
ALaa Babiker Yousif Dafalla

B.S.c in Medical Laboratory Science,(Microbiology)
Khartoum University (2013)

Supervisor:

Prof. Yousif Fadlalla Hamed Elnil

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بسم الله الرحمن الرحيم

قال تعالى:

(وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا)

113 (سورة النساء الايه)

الإهداء

الهي لايطيب الليل إلابشكرك ولايطيب النهار إلابطاعتك ولاتطيب اللحظات إلابذكرك ولاتطيب الآخرة إلابعفوك ولاتطيب الجنة إلابرؤية وجهك الكريم .

إلى رسولي الكريم سيدي محمد صلى ا لله عليه وسلم

إلى الذي علمني معاني البذل و العطاء .... والتماسك والصمود ..... والأمل والنجاح..... والتطلع إلى أفق بعيد.

إليك والدي العزيز

إلى التي علمتني أن أحب الناس .... وأحب النجاح .... وأن أسير نحو غد بإقدام أكثر التصاقا بقيمها النبيلة .

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إلى أخواني وأخواتي الذين يحملون في أعينهم ضياء لكل الرائعين حولي ... الى الذين يحترقون لكي يضيئوا للأخرين نورا ... إليكم رفقاء الدرب... زملاء المسير .

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ABSTRACT

This study was amid to determine the seroprevalence of human cytomegalovirus (HCMV) among leukemic patients attending at Radiation Isotope-center-Khartoum (RICK)from April toAugust 2015

A total of 90 Subjects were included 70 Leukemic Patients and 20 of them were hematological healthy (Control group)

Personal and clinical data were collected by questionnaire after verbal consent, serum samples were collected, tested for CMV IgG&IgM using enzyme-linked immune sorbent assay (ELISA).

Out of the 70 leukemic patients 68/70(75.6%) were positive for CMV IgG while 11/70 (12.2%) of them were positive for CMV IgM. All the 20 control group (100%) were negative for CMV IgM and 13/20 (14.4%) were IgG positive.

This study had also tried to find a relationship between the possible risk factors of age, gender and type of leukemia on CMV IgM/IgG antibodies

The results showed that the prevalence of cytomegalovirus (CMV) according to the type of leukemia was high in case of chronic myeloid leukemia CML. the IgG positive patients was 42 (60%) and the IgM was 6 (8.5%), with no significant difference between types of leukemia.

The results furtherly showed that 57.8% of male had IgG & 10% IgM while the 32% of female had IgG & 2.2% had IgM . This was no significant effect of age. The highest prevalence of CMV was in children less than 20 year.

This Study concluded that there was significant correlation between leukemia and human cytomegalovirus (HCMV).

ملخص الأطروحة

اجريت هذه الدراسة لتحديد الانتشار المصلي لفيروس مضخم الخلايا بين مرضى سرطان الدم (اللوكيميا)بالمركز القومي للعلاج بالإشعاع والطب النووي بولاية الخرطوم في الفترةما بين ابريل الى اغسطس من العام2015

وقد شملت هذه الدراسه تسعون شخصا (90) منهم (70)من مرض سرطان الدم وباقي العدد (20) كانواا صحاءظاهريا يمثلون العينة الضابطة للاختبار.

تم جمع المعلومات الشخصية والطبية عن طريق الاستبيان بعد موافقتهن الشفوية ومن ثم اخذت العينات وفحصت لمعرفه احتوائها علي اجسام مضادة من النمط IgG والنمط IgM لفيروس مضخم الخلايا باستخدام اختبار الاليزا.

من مجموع 70 من مرضي سرطان الدم 70/68 (75.6%)كانت لديهم اجساممضادة من النوع IgG بينما 70/11 (12.2%) كانت لديهم اجسام مضادة من النمط IgM. بينما لم تظهر العينة الضابطة أي اجسام مضادة من النمط IgM ،بينما لنمط IgG كانت 20/13(14.4%).

وقدبحثت هذه الدراسة أيضا العوامل التي تزيد من معدل الإصابة بفيروس المضخم الخلايا مثل العمر،النوع،الجنس ونوع سرطان الدم على الأجسام المضادة.

وأظهرت النتائج زياده في معدل انتشار فيروس مضخم الخلايالأجسام المضادة من النوع IgG 42(60٪) و6 (8.5٪) لنوع IgM.لسرطان الدم النخاعي المزمن(CML)

كما أظهرت النتائج نسبة عالية منا لأجسام المضادة بين الذكور أكثر من الإناث ،الذكو ر بنسبه 57.8% IgG.ولنوع IgM 10% . اما الاناث فكانت لنوع IgG 32% , و 2.2%لنوع IgM. في حين ليس هناك تأثيركبيربالنسبة للتفاوت في العمر .وكان اكثرهم من صغار السن .

وخلصت هذه الدراسة في العثور على وجود علاقة ذات دلالة إحصائية بين سرطان الدم وفيروس مضخم الخلايا.

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Abbreviation

AIDS: Acquired Immunodeficiency syndrome

ALL: Acute lymphoblastic leukemia

AML: Acute myeloid leukemia

CID :Cytomegalic Inclusion Disease.

CMI: Cell mediated immunity.

CML: chronic myeloid leukemia

CMV: Cytomegalovirus.

CLL: Chronic lymphocytic leukemia

CPE: cytopathic effect

DNA: Deoxyribo nucleic acid

Ds DNA: double strand DNA

ELISA: Enzyme- linked immunosorbent assay

HCMV:Human cytomegalovirus

HH-6:Human Herpes-6

HH-7:Human Herpes-7

HIV: Human Immunodeficiency Virus

IgM: Immuno globulin M

IgG: Immunoglobuin G

NK cell: Natural Killer Cell

PCR: Polymerse chain reaction

PP 65: Phosphoprotein 65

SOT:solid-organ transplantation

SCT: stem cell transplantation

SPSS: Statistical Package of Social Science.

TMB: Tetramethylbenzidine.

Chapter One

INTRODUCTION

1-1: Introduction

Cytomegalovirus (CMV) is a member of human herpesviridae family. It is known as the human herpes virus 5 (HHV-5) which belongs to *Betaherpesvirinae* subfamily. *Betaherpesviruses*, also include human herpes virus 6 (HHV-6) and human herpes virus 7 (HHV-7), viruses that are more closely related to each other than to other herpes viruses (Ryan and Ray.,2004).

CMVis an ubiquitous virus infection with worldwide distribution. The seroprevalence of CMV in adults ranges from 55%in developed countries to as high as over 90% in developing countries.(Mocarski *et al* ., 2007).

It is one of the most successful human virus that survive in its human host by infecting man both vertically and horizontally. The virus can be transmitted by either route during primary infection, reinfection or reactivation. At all times the virus causes minimal disability, allowing infected individuals to remain active and so maintain the maximum opportunity of encountering susceptible contacts (Zhang *et al* .,2010)

CMV infections remain inapparent or harmless in the immunologically healthy, but can cause generalized, fatal infections in immunocompromised individuals. It is characterized by a narrow spectrum of hosts, slow replication, frequently involving formation of giant cells and late, slow development of cytopathology. The virus apparently persists in the latent state in mononuclear cells. Reactivation can also run an asymptomatic course, but symptoms may also develop that are generally relatively mild, such a mononucleosis like clinical pictures and mild forms of hepatitis or other febrile illnesses. The situation is different in AIDS, transplantation or malignancy patients, in whom a fresh CMV infection or reactivation can result in severe generalized infections with lethal outcome (Kayser *et al*., 2005).

CMV disease manifestations include pneumonia, enteritis, encephalitis, retinitis, hepatitis, cholangitis, cystitis, nephritis, sinusitis and marrow suppression. T-cell function is paramount in the control of CMV, and T-cell depleting agents (e.g., alemtuzumab) and aggressive chemotherapy (e.g., hyper-CVAD (Cyclophosphamide, Vincristine, Adriamycin, Dexamethasome) and acute leukemia induction) appear to increase the risk of CMV infection and disease.(Wade ,2006).

The laboratory diagnosis of CMV infection depends on the growth of the virus from urine or other body fluids or on the demonstration of virion components such as viral antigens or viral DNA, using tissue culture or serologically by Enzyme Linked Immunosorbent Assay (ELISA) or polymerase chain reaction (PCR) subsequentially. Diagnosis of CMV disease cannot be made on clinical grounds alone and need laboratory confirmation,

(Enan *et al*.,2011).

This study aims to identify the seroprvalance of CMV among leukemic patient in khartoum state.

1-2: Rationale

CMV infection and its worldwide distribution depend on many factors such as age, clinical manifestation, route of infection ,personal immunity, chronic diseases, and chemotherapy. The virus is characterized by latent infection or reactivation with long-life persistest infection (Akhter and Tood , 2011).

The incidence of CMV antigenemia among patients who have severe underlying diseases, such as leukemia, lymphoma, or solid tumors. In this population, CMV antigenemia has been reported only in small series or case studies. As the incidence of cancer increases, and as patients with cancer survive longer, various infectious complications have become more pronounced. Therefore, in-depth knowledge of the epidemiology of CMV antigenemia in cancer patients is important not only clinically for risk assessment and the timely diagnosis and treatment of the infection to allow

better management of underlying cancers but also scientifically for better understanding of the virus-host interaction (Xiang, 2007).

The seroprevalence of the CMV in Khartoum State was reported by Kafi (2003) in 77% of blood donors using ELISA test (Kafi *et al.,* 2009). Also Suliman in 2006 detected the presence of CMV antibodies in healthy blood donors which were 84% for 1gG and 22% for 1gM using ELISA. He observed that the presence of 1gM antibodies varied in the various age groups.

(Suliman ,2006).

A previous study done among 68 children with acute lymphoblastic leukemia at Cairo University, Egypt (2001-2003). They used ELISA in and the seroprevalence of CMV IgG antibody in both leukemic children and their control was observed to be 100% in each. All patients were negative for CMV IgM antibody. No difference in seroprevalence was found among both genders (Loutfy *et al:* 2006)

1-3: Objectives

1-3-1 General Objective

To determine the seroprevalence of CMV among leukemic patients at Radiation and Isotopes Centre, Khartoum, Sudan.

1-3-2 Specific Objectives

1-To detect CMV anti IgG and IgM in serum of leukemic patients using ELISA

2-to determine the possible major risk factor predisposing to CMV infection among leukemic patient.

3-To compare finding between leukemia patients and control.

4-To compare finding among Lymphoblastic leukemia (ALL and CLL) and myeloid leukemia (AML and CML)

Chapter Two

2. LITERATURE REVIEV

2-1: History

CMV infections were first described in the early years of the twentieth century when the typical ‘owl’s eye’ intranuclear inclusions were found by histopathologists in tissues from fetuses stillborn following cytomegalic inclusion disease. These strange inclusions were thought to result from a protozoan infection. In 1956, three laboratories simultaneously isolated CMV, having successfully developed cell culture technology, so CMV is named after its cytopathic effect (Griffiths, 2004).

2-2: Classification

CMV formally designated human herpesvirus 5(HHV-5) by the International committee on taxonomy of virus,is a member of family Herpesviridae , and it is classified in subfamily Beta herpesvirinae with cytomegalovirus's of other animal species based on its tropism for salivary gland ,slow growth in cell culture, and strict species specificity. Human CMV(HCMV) is the type species of the genus cytomegalovirus, and it name is derived from the enlargement of cell (cyto=cell,mega=large)infected by the virus Herpesvirus 6(HHV-6) and Herpesvirus7(HHV-7) are now classified with CMV among the beta herpesviruses (Hodinka,2007)

2-3: Structure

CMV is the largest member of the herpesvirus family, with a genome of approximately 230 kbp encoding 160 genes (Figure.1). CMV virions range from 150 nm to 200 nm in diameter and may be distinguished from other herpesviruses by having a more pleomorphic envelope (Burbelo *et al*., 2009)

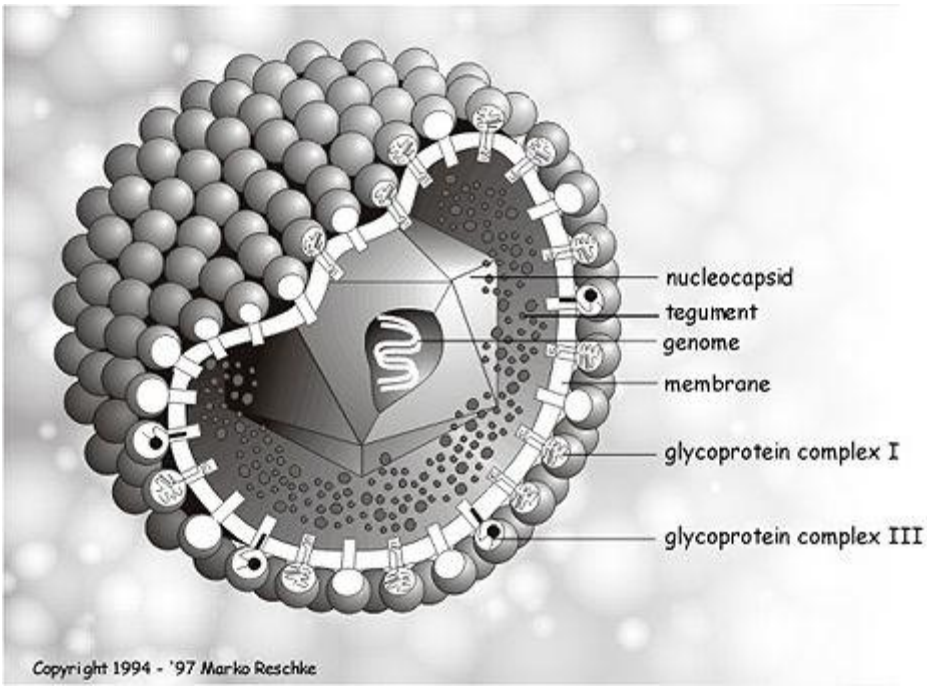

Fig 1: Structure of Cytomegalovirus

The genome of characterized CMV is all linear DNA molecule ranging in size from 200 to 240 kbp encoded more than 200 proteins. This is significantly larger than those of other herpesviruses. Human CMV genome also exhibits a pattern of terminal and inverted repeats that vary in size depending on the virus strain and passage history. The human CMV genome has two unique components designated unique larger region (UL) and unique short region (Us).(Mohammed , 2007).

2-4: Human Cytomegalovirus replication

In the replication cycle of HCMV, the virion initially fuses with the cell membrane of the target cell, and the (unenveloped) virus migrates towards the nucleus. Viral DNA is replicated in the nucleus, and virus-encoded proteins are synthesized in the cytoplasm, the nucleocapsid is assembled in the nucleus. HCMV genes are transcribed in strict temporal sequence which are classified into three distinct phases, termed alpha (immediate early), beta (delayed early), and gamma (late). The 'immediate–early' genes (expressed within 2–4 hours of infection) encode regulatory proteins that are needed for synthesis of 'early' and

'late' genes. The 'early' gene products include a DNA polymerase which is required for DNA replication. 'Late' gene products are dependent upon prior DNA replication and include capsid proteins (Hudnall and Stanberryl, 2005). Culture of human CMV is carried out in human fibroblasts. In vivo, replication appears to occur primarily in epithelial cells (e.g. in salivary glands, kidneys); HCMV is also known to infect peripheral blood leukocytes and haematopoietic progenitor cells (Singleton and Sainsbury, 2006)

2-5: Epidemiology

Seroepidemiologic studies showed that CMV is universally distributed among human populations. It is endemic in all parts of the world; epidemics are unknown. It is present throughout the year, with no seasonal variation seen in infection rates .In general, prevalence of infection is greater and varies with socioeconomic status, living conditions, and hygienic practices which is acquired earlier in life in developing countries (Brooks *et al*., 2007).

CMV is more widespread in developing countries and in areas of low socioeconomic conditions. After primary infection CMV is not eradicated but establishes life-long infection in its host. CMV dispersed and become dormant or latent in multiple end organs, and can later be reactivated by a number of different stimuli, including immunosuppresion and inflammation (Redwan *et al* .,2011)

The rate of infection with the virus varies widely from 40 -80 % in high socioeconomic groups in developed countries to 90 – 100% among childrenand adults in developing counties respectively. (Kafi *et al.*,2009*).*

2-6: Transmission

HCMV infection is associated with a variety of pathological conditions including retinitis, pneumonia, hepatitis and encephalitis that may be transmitted congenitally, horizontally and parentally and occurs both as a primary infection and as reactivation in immunocompromised individuals **(**Burbelo *et al*.,2009**)**

HCMV can be transmitted via saliva ,sexual contact ,placental transfers, breast feeding, blood transfusion solid-organ transplantation (SOT), or hematopoietic stem cell transplantation (SCT).(Sia and Patel,2000).

Day care center are significant source of CMV infection. Children less than three years of age with postnatally acquired CMV infection have been demonstrated to excrete CMV in their urine and saliva for 6 to 42 months (Nyholm and Schleiss,2010).

CMV is excreted in the breast milk of seropositive women . the risk of CMV transmission infants breast fed by seropositive women have been reported to be 58% to 69% (Nyholm and Schleiss,2010).

2-7: Pathogenesis

CMV is an enveloped double stranded DNA virus. On this envelop ,there are molecules called glycoproteins. These molecules, especially glycoproteins B are determinants of humoral immunity, that the can cause a humoral immune response. Proteins in the viral tegument, the proteinaceous layer between the capsid and the envelope, elicit cellular responses to CMV. Specifically the abundant molecule phosphoprotein 65 is the molecule targeted by cytotoxic T cells in the cellular response. Although the host has the ability to defend itself against a CMV invasion, CMV has the ability to evade the host immunity ,and in this way it can cause infection in the host . CMV has genes that interfere with the antigen presentation of infected cells, thus limiting the immune response to it .there fore, it can remain alive for a long time in a host,(Riley,1997)

The virus causes a systemic infection; it has been isolated from lung, liver, esophagus, colon, kidneys, monocytes, ىand T and B lymphocytes. The disease is an infectious mononucleosis-like syndrome, although most CMV infections are subclinical. Like all herpesviruses, CMV establishes lifelong latent infections. Virus can be shed intermittently from the pharynx and in the urine for months to years after primary infection. Prolonged CMV infection of the

kidney does not seem to be deleterious in normal persons. Salivary gland involvement is common and is probably chronic (Brooks *et al*., 2007)

The majority of infected individuals do not show clinical symptoms and signs; few may be associated with serious manifestation such as mononucleosis, abortion, stillbirth and congenital cytomegalic disease. However reactivation of the virus in immunocompromised patients, may be associated with serious complicationssuch as chorio-retinitis (leading to blindness) and colitis. (Kafi *et al*., 2009)

When the host immune system is compromised, the virus is then able to exert its full pathogenic potential (Enan *et al*., 2009)

2-8: Immunity to human Cytomegalovirus

The virus activates humeral and cell mediated immunity. the virus has ability to evade the host immunity like removal of class 1 fromendoplasmic reticulum with the production of cytokine receptor homology, pp28,pp150 and pp65 virion tegument protein (Abbas *et al*;2007)

The innate immune system plays important role in the defense against HCMV and also in priming the adaptive immune respons. It is becoming increasingly apparent that HCMV is subject to innet sening by Toll-Like Resptors (TLRs). The stimulation of TLRs by pathogens such as HCMV activates singal transduction pathways, which induce the secration of inflammatory cytokines that recruint cells of the innate immune system,and the up regulation of co stimulatory molecules such as CD80 and CD86, Which are important for the activation of adaptive immunity(Brooks *et al*;2007).

2.8.1.Cell-mediated immunity (CMI)

Resistance to CMV infection depends on successful collaboration between the innate and adaptive immune responses. Both natural killer (NK) cells and cytotoxic T lymphocyte (CTL) whose presence correlates with protection from CMV disease, play prominent role in immune clearance.

In human with naturally acquired CMV-infection, CD4 + helper T lymphocyte

responses to multiple structural and nonstructural CMV protein have been demonstrated. Cytotoxic T lymphocytes (CTL) responses to CMV were associated with clinical recovery from CMV disease. CD8+ CTL responses to a number of CMV proteins (Suliman, 2006)

2.8.2. Humoral immunity

A crucial part of the immune system defense against CMV is the development of CMV specific antibodies. CMV-specific antibodies of the IgM, IgA, and IgG classes have all been detected. The first type of antibody to develop in response to CMV is IgM, which develops within a few days following primary infection. While CMV IgM remains detectable for six to nine months.IgM can also be detected during some secondary infection (Brooks *et al*;2007).

The second antibody type to respond to CMV is IgG. This antibody develops within 1 to 2 weeks after infection and, once developed, can be detected throughout life. Reactivation of latent infection occurs in the presence of humoral immunity (Redwan *et al*.,2011).

2-9: Human Cytomegalovirus infection and disease

2.9.1: infection in immunocompetent hosts

Immunocompetent individuals with primary infection are frequently asymptomatic (Zanghllini *et al*., 1999). But CMV occasionally effects clinical illness i.e. a self- limited mononucleosis-like syndrome. fever, fatigue , and muscle pain and malaise are hollmarks of CMV mononucleosis and can persist for weeks .Other clinical abnormalities have been associated with CMV infection in normal hosts ,I ncluding Guillain-Barre syndrome, meningoencephalitis, hemolytic anemia, and thrombocytopenia .

(Mocarski *et al*., 2007)

2-9-2: Human Cytomegalovirus and immunocompromised patients

CMV can cause severe disease in immunocompromised patients; either via reactivation of latent CMV infection or via acquisition of primary CMV infection.Both morbidity and mortality rate increase with primary and recurrent

CMV infection in immunocompramised individual.The host immune response presumably maintains CMV in a latent state in seropositive individuals. Reactivated infections are associated with disease much more often in immunocompromised patients than in normal hosts (Alipourfard *et al*; 2009).

Clinical syndromes that may be observed in this setting include encephalitis, pneumonitis, hepatitis, uveitis, retinitis, colitis, and graft rejection. Furthermore, CMV infection affecting the human embryo, a host with immature immunologic responses, is often associated with serious complications, such as microcephaly, mental retardation, spastic paralysis, hepatosplenomegaly, anaemia, thrombocytopenia, deafness, and optic nerve atrophy leading to blindness (Rafailidis *et al*., 2008)

2-9-3: Congenital human Cytomegalovirus

Congenital CMV infection most often follows primary maternal infection. Early congenital infection often leads to fetal death or permanent neurologic damage. The most severe form of congenital infection, cytomegalic inclusion disease (CID), is characterized by disseminated infection leading to thrombocytopenic purpura, and hemolytic anemia (Hudnall and Stanberry, 2005).

2-9-4: Human *Cytomegalovirus* and leukaemic patients

The patient with malignant disease such as lymphoma or leukemia or hodgkins disease may have sever or protracted illness with CMV infection , and they may suffer from more episode of pneumonitis or fever with rash but not more episode of hepatitis , and may other possible syndrome such as encephalitis or ulcerative colitis (Baron *et al*.,1989)

2-10: Diagnosis of human Cytomegalovirus

Diagnosis of HCMV infection and disease is based on clinical symptoms, however, the symptoms of HCMV are confused with EBV, and this may lead to difficulties in diagnosis. But, it has been enhanced with the development of techniques for rapid culture, CMV antigen assays and PCR-based molecular tests (Enan *et al*., 2009).

2-10-1: Histopathology

Histologic technique may be applied in an attempt to find characteristics intranuclear inclusions in specimens. Inclusion-bearing cells may be found in saliva, milk, cervical and tracheal secretions, and in touch preparations from biopsy or necropsy tissues. The microscopic hallmark of CMV infection is the large (cytoplasmic) 25-35 μm cell containing a large, central, basophilic intranuclear inclusion is referred to as "owl's eye" because it is separated from the nuclear membrane by a halo. These inclusions are seen well withPapanicolaou or hematoxylin-eosin stains (Jahan,2010)

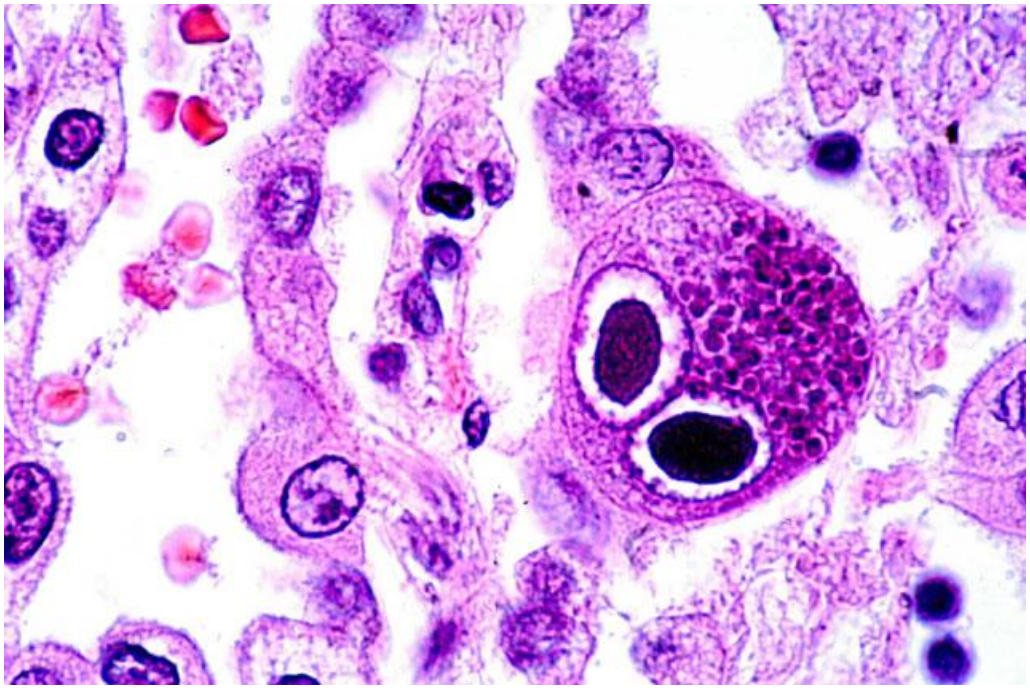

Fig 2. Characteristic interanuclear owl's eye appearance within kidney bean shape like nucleus (Olyaee *et al*., 2003)

2-10-2: Culture methods

2-10-2-a: Conventional cell culture

Viral culture of urine and saliva obtained within first two weeks of life continues to be the gold stander for diagnosis of congenitally infected infants (Ross *et al*., 2011)

For conventional cell cultures, human fibroblasts are used routinely. Foreskins or embryo lungs may be employed as a source of fibroblasts and must be used only at low passage (<25). The characteristic CMV cytopathic effect (CPE) consists of small round or elongated foci of rounded, enlarged retractile cells(Pillay and Griffiths ,1992).

All cultures should be observed at least twice weekly for the typical focal cytopathic effect (CPE) of CMV. Occasionally, urine samples from cases of congenital infection produce widespread CPE within 24–28 h which resembles that of HSV. Usually, however, the CPE evolves only slowly, so the cultures must be maintained for a minimum of 21 days before being reported as negative(Griffiths ,2004)

2-10-2-b: Shell virus culture

The shell vial culture is rapid modification of conventional cell culture. In this technique the virus is detected more quickly because infected cell monolayer is stained for viral antigens found soon after infection before the development of CPE (Mohammed, 2007)

2-10-3: Serology

a-Enzyme linked immunosorbent assay (ELISA) is the most commonly available serologic test for measuring antibody to CMV which is available from several manufacturers. The result can be used to determine whether it is due to acute infection, previous infection, or passively acquired maternal antibody in an infant. Results are typically available in a few hours (Jahan ,2010)

ELISA-based methods can be easily automated; they are fast and convenient to perform. They usually have good sensitivity and they are also commercially available (Piiparinen, 2004).

b- Immunofluorescence test (IFT)

c-Immunoblot

d-Immunochromatography technique (ICT):

The CMV ICT or one step test device (serum/plasma) is a rapid immunoassay for the qualitative detection of antibodies to CMV(Suliman 2006)

2-10-4: Molecular techniques:

The detection of CMV has become increasingly important in the care of immunocompromised patients. Over time, molecular methods have been implemented as a primary means of quantitatively detecting this and other systemic viruses, particularly in peripheral blood specimens. , molecular techniques have also evolved, with end-point PCR methods now being supplanted by real-time technologies. Real-time PCR can offer decreased turnaround time, improved ease of use and result interpretation, and an improved ability to quantify viral targets (Vincent *et al*.,2009)

Polymerase chain reaction (PCR)CMV DNA can be detected with the PCR technology, which is used in detecting the viral nucleic acids (DNA or RNA).The PCR assay is more versatile and can be used either qualitatively (diagnostic PCR) or quantitatively to measure the viral load, which is proportional to the level of CMV DNA(KalKan and Dagli ,2010)

2-11: Treatment

The drugs ganciclovir, foscarnet, cidofovir and fomivirsen (intravitreal only) have been licensed for serious or life-threatening CMV infections in immunocompromised patients, while valaciclovir is licensed only for prophylaxis(Griffiths,2004).

Immunosuppressed patients can be treated with hyperimmunoglobulin to provide passive immunity against infection or recidivation. Ganciclovir and foscarnet are therapeutically useful in transplantation, and particularly in AIDS patients,to combat CMV-induced pneumonia, encephalitis, and retinitis (Kayser *et al*; 2005).

2-12: prevention and control:

Specific control measures are not available to prevent CMV spread. Isolation of newborns with generalized cytomegalic inclusion disease from other newborns is advisable.

Screening of transplant donors and recipients for CMV prevent some transmissions of primary CMV. The CMV-seronegative transplant recipient population represents a high-risk group for CMV infections. Administration of human IgG prepared from plasma pools obtained from healthy persons with high titers of CMV antibodies (*Cytomegalovirus* immune globulin) has given discordant results in tests to decrease the incidence of viral infections in transplant recipients. *Cytomegalovirus* immune globulin is in limited supply.

The use of blood from seronegative donors has been recommended when infants will require multiple transfusions. (Brooks *et al.*, 2007).

2.13. leukemia

Leukaemia are cancers of the white bloodcells, which begin in the bone marrow. This information refers to four types of leukaemia; acute lymphocytic leukaemia, chronic lymphocytic leukaemia, acute myeloid leukaemia and chronic myeloid leukaemia.

Leukaemia are grouped in two ways: the type of white blood cell affected – lymphoid or myeloid; and how quickly the disease develops and gets worse – acute leukaemia appears suddenly and grows quickly while chronic leukaemia appears gradually and develops slowly over months to years.((Hoffbrand *et al*.,2006)

2.14. Types of leukemia

There are several types of Leukaemia. Some types appear suddenly and progress rapidly over days to weeks..

Leukemia's are named according to the type of blood cells involved. The myeloid leukemia are those which involve the granulocytes red blood cells platelets and monocytes; the lymphocytic leukemia are those which involve the lymphocytes.

2.14.1Acute lymphocytic leukemia (All)

Acute lymphocytic leukemia is most common in children .its incidence is highest at 3-7 year, falling off by 10 years with a secondary rise after the of 40 (Hoffbrand *et al*.,2006) .

2.14.2Chronic lymphocytic leukemia (CLL)

This leukemia also affects the lymphocytes, but usually develops much more slowly than acute lymphocytic leukemia. It has peak incidence between 60and 80 year (Hoffbrand *et al*,.2006)

2.14.3Acute myeloid leukemia (AML)

This leukemia mainly affects adults, but can occur in children and adolescents. It is common form of acute leukemia in adults and increasing with age (Hoffbrand *et al*,.2006)

2.14.4Chronic myeloid leukemia (CML)

Chronic myeloid leukemia is a clonal disorder of pluripotent stem cell. can occur at any age, but is uncommon below the age of 20 years. (Hoffbrand *et al*, 2006)

Chapter Three

3.MATERAL AND METHODS

3.1. Study design

This is was Descripitive cross –sectional study.

3.2. Study area

The study was conductedat Radiation and Isotopes Centre(RICK),

Khartoum, Sudan.

3.3. Study period:

The study was carried out during the period from April to August 2015.

3.4. Study population

A-Seventy known leukemic patients (70 patients after receiving chemotherapeutic drugs) at Radiation and Isotopes Centre, Khartoum, Sudan.

b- Twenty control group (20 apparently healthy individuals).

3.5. Sampling:

Non-probability sampling-Quota population was selected randomly.

3.5.1-Sample size:

Ninty patients were supposed to be collected .

3.6. Data collection

Personal data were collected from the medical records of the patients based on a constructed questionnaire that included name, age, gender, etc. (See appendix 1).

3.7. Sample processing

Seventy70 blood samples from leukemic patients and twenty 20 control group were taken. These blood samples were collected in plain container by laboratory technologists. Allowed to clot and then separated by centrifugation 3000 rpm for 5 min and stored at -20°C until used.

3.8. Ethical consideration

Permission to carry out the study was obtained from the College of Graduate Studies, Sudan University for Scince and Technology .

All patients examined were informed for the purpose of the study before collection of the sample , and verbal consent was taken from them.

3.9. Laboratory work:

The Enzme-linked Immuno Sorbent Assay (ELISA) was used to detect the specific HCMV IgG and IgM.

3.9.1. ELISA for the detection of CMV IgG antibodies

3.9.1.1. Principle

The HUMAN CMV IgG ELISA is based on the classical ELISA technique**.** (EUROIMMUM,Germany) the test kit contain micro titer strips with 8 break-off reagent wells coated with CMV antigen .In the first reaction step,diluted samples are incubated in the wells . In the case of positive samples, specific IgG antibodies will bind to antigens .To detect the bound antibodies, a second incubation is carried out using an enzyme labeled anti-human IgG (enzyme conjugate) catalysing a colour reaction .A third incubation is carried out using chromogen/substrate solution .

The reaction is then stopped by adding acid. The results of colors are read by micro ELISA reader and then the reading of samples is compared with the controls and calibrators

3.9.1.2. Storage and precautions of ELISA kits

All kits components were stored at 2 - 8°C and used before expiry date on the label. Once opened the kit is stable for three months (or until its expiry date if less than three months).

The assay standards and controls are manufactured from diluted non-infectious human serum. Normal clinical laboratory safety procedures were maintained at all times. Gloves and protective clothing were used when handling any patient sera or serum based products. The stop solution is non-corrosive.

3.9.1.3. procedure

According to manufacturer guidelines (EUROIMMUM,Germany) the following steps were followed(see appendix-2)

All Reagents and sample were brought to room temperature before beginning the procedure. The sample diluted (1:100) with sample buffer and washing solution diluted (1:10) with fresh deionized water, other reagents were ready for use.

Briefly, 100 µ1 of calibrater and the negative controL(NC), and positive control(PC) , were transferred in duplicate form, and 100µ1 of diluted samples were incubated in microtiter strip wells coated with CMV antigen at room temperature for 30 minutes. The wells were washed 3 times by washing solution using automatic washer to remove unbound components. Then 100 µ1 of conjugate(peroxide-labled anti-human IgG) were added to each well and incubated at room temperature for 30 minutes. After another (3times) washing step to remove excess conjugate ,an enzyme substrate reagent (TMB Substrate) was added (100µ1/well)and the plate was incubated for 15 minutes. the blue color was changed to yellow after adding of the100 µ1 stop solution (sulphuric acid) in each well. The optical density (OD)in microplate reader was read within 30 mintes at 450nm.

3.9.1.4. Calculation of result

Result can be evaluated semi quantitatively by calculating a ratio of extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate of the ratio according to the following formula:

$$\frac{\text{Extinction value of the control patients sample}}{\text{Exctintion value of the calibrator 2}} = \text{Ratio}$$

EURO IMMUN recommends interpreting results as follows:

Ratio <0.8: negative

Ratio ≥0.8to ≤1.1: borderline

Ratio >1.1: positive

3.9.2. ELISA for the detection of CMV IgM antibodies

3.9.2.1. Principle

(EUROIMMUM,Germany) the test kit contain micro titer strips with 8 break-off reagent wells coated with CMV antigen .In the first reaction step,diluted samples were incubated in the wells . In the case of positive samples, specific IgM antibodies will bind to antigens .To detect the bound antibodies, a second incubation is carried out using an enzyme labeled anti-human IgM (enzyme conjugate) Catalysing a colour reaction .A third incubation is carried out using chromogen/substrate solution.

The reaction is then stopped by adding acid .the result of colors are read by micro ELISA reader and then the reading of samples is compared with the controls and calibrators.

3.9.2.2. Procedure

According to manufacturer guidelines (EUROIMMUM,Germany) the following steps were followed(see appendix-2)

All Reagents and sample were brought to room temperature before beginning the procedure. The sample diluted 1:100with sample buffer and washing solution diluted (1:10) with fresh deionized water, other reagents were ready for use.

Briefly, 100µ1 of calibrater ,negative controL(NC), and of positive control (PC) , were transferred in duplicate form, and 100µ1 of diluted samples were incubated in microtiter strip wells coated with CMV antigen at room temperature for 30 minutes. The wells were washed 3 times by washing solution using automatic washer to remove unbound components. Then 100 µ1 of conjugate(peroxide-labled anti-human IgM) were added to each well and

incubated at room temperature for 30 minutes. After another 3times washing step ,an enzyme substrate reagent (TMB Substrate) was added (100µ1/well)and the plate was incubated for 15 minutes. The blue color changed to yellow after adding of 100µ1 stop solution (sulphuric acid). The optical density (OD) in microplate reader was read within 30 mintes at 450nm.

3.9.2.3. Reading and interpretation of test results

Result can be evaluated semi quantitatively by calculating a ratio of extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate of the ratio according to the following formula:

$$\frac{\text{Extinction value of the control patients sample}}{\text{Exctintion value of the calibrator 2}} = \text{Ratio}$$

EURO IMMUN recommends interpreting results as follows:

Ratio <0.8: negative

Ratio ≥0.8to ≤1.1: borderline

Ratio >1.1: positive

3.10. Data analysis

The statistical analysis of the results was performed using the statistical package for social science (SPSS) for windows version 16.

Chi-square test was used for testing difference significance. *P. value* less than 0.05 was considered statistically significant.

Chapter Four

4-RESULTS

4.1. Detection of CMVamong the leukemic patient and control group

Out of the 70 leukemic patients 68/70(75.6%) were positive for CMV IgG while 11/70 (12.2%) of them were positive for CMV IgM. All the 20 control group (100%) were negative for CMV IgM and 13/20 (14.4%) were positive for CMV IgG.. The results of ELISA for both CMV IgG and CMV IgM table 4-1

Table 4-1: Seroprevalence of CMV among leukemic patients and the control group

Study Group	ELISA IgM		ELISA IgG		Total number investigated No (%)
	Positive	**Negative**	**positive**	**Negative**	
Cases	11 (12.2%)	59 (65.6%)	68 (75.6%)	2 (2.2 %)	70 (77.8%)
Control	0 (0.00%)	20 (22.2%)	13 (14.4%)	7 (7.8%)	20 (22.2%)
Total	11 (12.2%)	79 (87.8%)	81 (90%)	9 (10%)	90 (100%)

4.2 CMV IgG and IgM prevalence according to gender :

The results revealed that there were no significance difference between males and females .The males were found to be more infected with CMV infection More than females. (Table 4.2and table 4.3)

Table 4.2 The Prevalence of IgG antibodeis according to gender

Anti CMV IgG			
Target Group	Number of positive	Number of Negative	Total
Male	52 (57.8%)	4 (4.4%)	56 (62.2%)
Female	29 (32.2%)	5 (5.6%)	34 (37.8%)
Total	81 (90%)	9 (10%)	90 (100%)

Table 4.3The Prevalence of IgM antibodies according to gender

Anti CMV IgM			
Target Group	Number of positive	Number of Negative	Total
Male	9 (10%)	47 (52.2%)	56 (62.2%)
Female	2 (2.2%)	32 (35.6%)	34 (37.8%)
Total	11 (12.2%)	79 (87.8%)	90 (100%)

4.3 Anti CMV IgM and IgG prevalence according to age

The result showed that the age of leukemic patient and had no significance effect ($p>0.05$) on CMV IgM and IgG prevalence. in table(Table 4. 4 and 4.5)

Table4-4: **Anti CMV IgG antibodies prevalence according to age**

Age group	**CMV-IgG status**		**Total number examined**
	Positive frequency (%)	**Negative frequency (%)**	
≤ 20years	26 (28.8%)	5 (5.6%)	31 (34.4%)
21-40 years	25 (27.8%)	3 (3.3%)	28 (31.1%)
41-60 years	25 (27.8%)	1 (1.1%)	26 (28.9%)
≥ 61	5 (5.6%)	0 (0.0%)	5 (5.6%)
Total	81(90%)	9 (10%)	90 (100%)

Table 4-5: Anti CMV IgM antibodies prevalence according to age

Age group	**CMV-IgMstatus**		**Total number examined**
	Positive frequency (%)	**Negative frequency (%)**	
≤ 20years	5 (5.6%)	26 (28.9%)	31 (34.4%)
21-40 years	2 (2.2%)	26 (28.9%)	28 (31.1%)
41-60 years	4 (4.4 %)	22 (24%)	26 (28.9%)
≥ 61	0 (0.00%)	5 (5.6%)	5 (5.6%)
Total	11 (12.2%)	79 (87.8%)	90 (100%)

4.4The effect of type of leukemia on CMV Prevalence

Highest frequency of CMV IgG positive patients was observed among CML patients42 (60 %) and 6 (8.5%) in IgM with no significant difference between type of leukaemia (Table4.6 and table 4.7).

Table4-6:

Frequency of CMV IgG Antibodies according to type of leukemia

Type of leukaemia	**CMV-IgG status**		**Total number examined frequency (%)**
	Positive frequency (%)	**Negative frequency (%)**	
CML	42 (60%)	0 (0.00%)	42 (60%)
AML	1 (1.4 %)	0 (0.00%)	1 (1.4 %)
CLL	9 (12.8%)	0 (0.00%)	9 (12.8%)
ALL	16 (22.8%)	2 (2.8 %)	18 (25.7%)
Total	68(97.1)	2(2.8)	70 (100%)

Table 4-7:

Frequency of CMV IgM antibodies according to type of leukemia

Type of leukemia	CMV-IgM status		Total number examined frequency(%)
	Positive frequency(%)	Negative frequency(%)	
CML	6 (8.5%)	36 (51.4%)	42 (60%)
AML	0 (0.00%)	1(1.4 %)	1 (1.4 %)
CLL	0 (0.00%)	9 (12.8%)	9 (12.8%)
ALL	5 (7.1%)	13 (18.5%)	18 (25.7%)
Total	11 (15.7%)	59 (84.2%)	70(100%)

Chapter Five

5. DISCUSSION

5-1-Discussion

Viral infections are important causes of morbidity and mortality for patients with a hematological malignancy. This study was focused on the serologic diagnosis of human CMV in leukemic patients and control group in Khartoum State, Sudan. A number of 90 serum samples were collected from these two groups (70 leukemic patients and 20 control group) .

The seroprevalence of anti HCMV IgG antibodies was done using ELISA and seroprevalence of HCMV IgG among the two groups was found 81/90 (90 %) .This may be attributed to the surrounding environment as high prevalence of HCMV was previously observed in poor socioeconomic status, poor living conditions, The findings in the present study are in agreement with those of (Suliman,2006) and(Kafi *et al*., 2009) indicating that HCMV is very common in Sudan

In contrast to IgG results, IgM antibodies were detected in 11/70(12.2%) and 0/20 (0.0%) of the investigated leukemic patients and control group respectively. This finding may reflect an active recent infection or reactivation of the virus The seroprevalence of HCMV IgM antibodies might reflect an alarming picture of the disease in such population (Licciardello *et al*., 2011)

The prevalence of HCMV infection in the general population of African countries as well as in leukemic patients reportedly high . The seroprevalence has been reported to be 100% in Egypt (Loutfy,2006) ,90% in Eritrea(Ghebrekidan,1999). and 77.6% in Ghana (Andrew *et al*.,2008).

From the present study it we found that the prevalence of CMV IgM/IgG antibodies was high in children less than 20 years and young adults. However

there was no significant difference. This is in agreement with the finding of (Suliman, 2006) but disagree with that reported in Saudi Arabia (Redwan *et al.*,2011) and USA which were found that seroprevalence of HCMV increased with age (Staras *et al* .,2006).

There was no significance difference between males and females in the prevalence with increase in male to reach 52% for IgG. This in contrast to the finding in Saudi Arabia (Redwan *et al.*, 2011)

In the present study. the prevalence of both IgG &IgM OF CML was high is in contrast to ALL this comes in line with that study of (Hermouet *et al*.,2003).

5.2 Conclusion

Based on the result of this study that indicated high prevalence of CMV among leukemia patient specially thase having Chronic myeloid leukemia (CML) than other types of leukemia.

5.3Recommendations

1. The leukemic patients receiving chemotherapy should be periodically screened for CMV to prevent serious complications of disease.
1. Large sample size is critical for better result.
2. PCR technique should be used beside traditional laboratory methods as a routine technique in the diagnosis of HCMV in Sudan.

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Appendices

Appendices

❖ Appendix 1

Questionnaire

Serologic detection of *Cytomegalovirus* among leukaemic patients at Radiation and Isotopes Centre, Khartoum, Sudan

By:

Alaa Babiker yousif

1. No: …… 2. Date:……..

3. Name:…….4. Place:……

5. Age:……6. Tribe:……

7. Gender:…….

8. Type of leukemia:…….

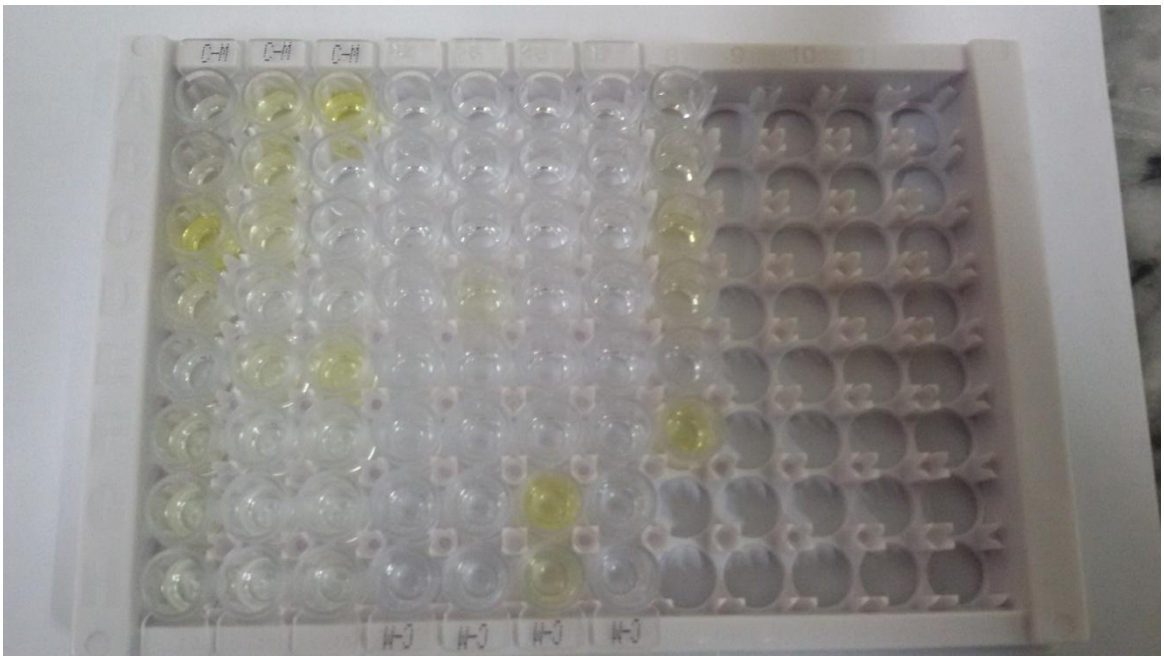

Fig 3: Micro titer ELSA plate