بسم الله الرحمن الرحيم

Republic Of Sudan

University Of Shendi
Faculty of Postgraduate Studies and Scientific Research

Detection of Antibody against Cytomegalovirus in Rejected Renal Transplant Recipients and Hemodialysis Patients in Khartoum State, 2018

Dissertation Submitted in Partial fulfillment of the Requirement for the M.Sc. Degree in Medical Laboratory Science,
(Microbiology)

By:

Hala Ahmad Adbalrahem Saeed

B.Sc. in medical laboratory science (microbiology)

Sharq El Neil collage 2013

Supervisor:

Dr. Ahmed Mohammed Ahmed Ibrahim

August, 2018

الايه

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ (1)

الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ (2) الرَّحْمَنِ الرَّحِيمِ (3) مَالِكِ يَوْمِ الدِّينِ (4) إِيَّاكَ نَعْبُدُ وَإِيَّاكَ نَسْتَعِينُ (5) اهْدِنَا الصِّرَاطَ الْمُسْتَقِيمَ (6) صِرَاطَ الَّذِينَ أَنْعَمْتَ عَلَيْهِمْ غَيْرِ الْمَغْضُوبِ عَلَيْهِمْ وَلَا الضَّالِّينَ (7)

صدق الله العظيم

DEDICATION

To my family

To my dear friends and colleagues

With love and respect

ACKNOWLEDGEMENTS

I would like to express my all sincere gratitude to my supervisor
Dr. Ahmed Mohamed Ahmed Ibrahim, Department of Microbiology,
Faculty of medical laboratory, University of Shendi for his patience, guidance, advice and support to accomplish this work. Also, I wish to extend my special gratitude to the Shendi University. My thanks are also due to the Faculty of Pure and Applied Science, and I would like to acknowledge the Doctor Salma for Kidney Disease, for giving me permeation to take the samples. In addition, I greatly acknowledge the assistance of the staff of Department Microbiology, Faculty of Pure and Applied Science. Last but not least, my appreciation and heartily thanks to my relatives, friends, colleagues and everyone who helped me.

ABBREVIATIONS

CMV	Cytomegalovirus
CPE	Cytopathic effect
HCMV	Human Cytomegalovirus
IR	Inverted repeat
IRL	Inverted repeat sequence
TR	Terminal repeat
TRS	Terminal repeat sequence
U_L	Unique long region at CMV genome
U_S	Unique short region of CMV genome

الملخص

اجريت هذه الدراسه بغرض تحديد مدي انتشار فريوس مضخم الخلايا في مرضي عمليات زراعه الكلي المرفوضه من الجسم ومرضي غسيل الكلي في الفتره من مارس الي يونيو 2018.

تم جمع عينات الدم من مجموعات مختلفه 9 عينات من زارعي الكلي المرفوضه ويخضعون للغسيل الدموي ,91عينه من مرضي الفشل الكلوي المزمن ويعالجون بالغسيل الدموي , وكان عدد المرضي المنضوين في الدراسه (56%) من الذكور و (44%) من الاناث الذين يتعالجون بالغسيل الدموي وتم تقسيم هذه الفئات علي حسب العمر الي اربع مجموعات , المجموعه الاولي من عمر 20_30 عام, ومن 31_40 عام, ومن 41_50 عام والمجموعه الاخيره من عمر اكثر من 50 عاما , متوسط الاعمار 46 سنه ووجدت الاغلبيه من المتزوجون 80% و 20%من غير المتزوجين .

تم تشخيص العينات باستخدام فحص الانزيم المرتبط المناعي (الاليزا) وذلك لقياس الاجسام المضاده للفيروس من النوع (IgM) للاصابات الحاده والاجسام المضاده من النوع (IgG) للاصابات المزمنه.

اوجدت الدراسه ان العدد الكلي للمصابين بفيروس مضخم الخلايا (74/100)%74 وكانت الاصابات الحاده (18/74)%24.3 والاصابات المزمنه(56/74)%75.7 و (26/100)%26 لم يصيبو بفيروس مضخم الخلايا.

وكان العدد الكلي للاصابه لدي مرضي زارعي الكلي (5/100)%55, (2/100)%22 للاصابات المزمنه و (3/100)%33 للاصابات الحاده.

معدل انتشار فيروس مضخم الخلايا في مرضي الفشل الكلوي المزمن المعالجون بالغسيل الدموي معدل عالي جدا خصوصا في المرضي اللذين اجريت لهم عمليه زراعه الكلي التي رفضت بواسطه الاجسام المضاده لديهم .

Abstract

The aim of this study was to find out the frequency of human cytomegalovirus (HCMV) infection in rejected renal transplant and haemodialysis patients in Khartoum state, from March to June 2018. Blood samples were selected randomly, 100 haemodialysis patients included in the study 9 of them had past history of rejected renal transplant. 56 were males and 44 females, the patient's age range from 20 to 60 years, the mean age was 46 years(SD±13), the study showed 80% of patients were married and 20% were not.

The blood samples were investigated with the Enzyme Linked Immunosorbant Assay (ELISA) to detect HCMV IgG and IgM antibodies.

The study showed total HCMV sero-positivity were 74% (74\100) and sero-negativity were 26% (26\100), 24.3% (18\74) were acute infection, and 75.7% (56\74) revealed chronic sero-positivity for HCMV on haemodialysis patients, and total sero-positivity on rejected renal transplant were 55%(5\100), 22%(2\100) were chronic infection, and 33%(3\100) revealed acute sero-positivity for HCMV.

The frequency of HCMV in haemodialysis patients was high especially in rejected renal transplant patients.

LIST OF TABLES

LIST OF FIGURES

LIST OF CONTENTS

Page

CHAPTER ONE

INTRODUCTION AND OBJECTIVE

1. Introduction

Human cytomegalovirus (HCMV) is a member of the genus Herpes virus and belongs to the family Herpesviridae. As with all members of the family, the virus has the ability to persist in the host in a latent state after primary infection (Emery**,** 2001). It is a ubiquitous virus, the seroprevalence of which varies between 30 to 100 % in different countries. Acquisition of the virus in the general population mainly occurs early in life. Transmission of the virus can occur vertically or horizontally via direct contact with infectious body fluids or blood. The virus can also be transmitted by blood products or transplanted organs. After the primary infection, the virus will remain in a latent state in the host life-long but may reactivate later. Although rarely pathogenic in immunocomptent individuals, the virus posses a significant health threat to immunocompromised individuals and is a significant cause of morbidity and mortality especially in organ allograft and bone marrow transplant patients (Partel *et al*., 1992; Benz, 2000).

In the immunocompetent individual, the virus and host exist in a symbiotic equilibrium, such that disease manifestations are rarely encountered. However, when the host immune system is compromised, either through infection—for example, by human immunodeficiency virus (HIV), immaturity (neonate), or through iatrogenic means following organ transplantation, the virus is able to exert its full pathogenic potential (Emery**,** 2001). In immunocompromised patients, severe HCMV infections may occur. HCMV is a significant pathogen in organ transplanted patient causing symptomatic infections and end-organ disease. Many risk factors for the development of symptomatic infection have been suggested. Viral load has been shown to be a major factor in the development of HCMV disease. In most developed countries Human cytomegalovirus (HCMV), seroprevalence steadily increases after infancy, and

10-20% of children are usually infected before puberty. In adults the prevalence of antibodies ranges from 40-100%.

Infection with HCMV being more common in developing countries and in areas with low socioeconomic condition which is predominantly related to closeness of contacts within these population (De Jong *et al*., 1998). HCMV causes 8% of mononucleosis syndromes (Britt and Alford, 1996) atherosclerotic coronagy artery, congenital infections (Boppana *et al*., 1992), and clinical syndromes associated with HCMV infections in AIDS patients (Britt and Alford, 1996). Prepheral blood mononuclear cells expressing CD13+ antigen as monocytes and certain CD8+ lymphocytes are permissive to HCMV (Soderberg *et al*.,1993). Monocytes have been identified as the major site of latency of HCMV in peripheral blood of healthy carriers and are more likely to transform latent infection. Allogeneic stimulation of peripheral blood mononuclear cells by T cells provides an immunologic stimulus that facilitates reactivation of latent HCMV (Soderbergy *et al*., 1997).

Thus HCMV may be reactivated from latently infected cells after blood transfusion. In general; transfusion of unscreened cellular components leads to TT- HCMV incidence of approximately 30% in seronegative recipients (Hillyer *et al*., 1994). The first surveillance of HCMV infection in Sudan was done in 2004, in blood donors the second surveillance was done in 2006, in candidate recipients, kidney candidate donors and blood donors (Bushera, 2006). Diagnosis of HCMV was made by clinical symptoms, laboratory conformation was achieved by serological methods Enzyme linked Immunosorbent assay (ELISA).

Rationale

Investigate the prevalence of HCMV lgG and IgM antibodies among rejected renal transplant recipient and hemodialysis patients', HCMV is significant cause of increased morbidity and mortality in population, CMV can predispose to rejection of kidney graft.

Objective

General Objective

To detect the antibodies against cytomegalovirus infection in rejected renal transplant and hemodialysis patients.

Specific Objectives

1. To screen hemodialysis patients for acute and chronic CMV infections.
2. To determine the frequency of CMV infections among rejected renal transplant patients.
3. To find out the risk factors which increase the incidence of CMV infection such as diabetes mellitus and HBV and HCV infections.

CHAPTER TWO

LITERATURE REVIEW

2. LITERATURE REVIEW

Definition

Cytomegalovirus (CMV) infections are common and usually asymptomatic infection; however, the incidence and spectrum of disease in newborns and in immunocompromised hosts establish this virus as an important human pathogen. CMV infection can be classified as those acquired before birth (congenital), at the time of delivery (prenatal), or later in life (postnatal) (Hodinka *et al.,* 1991).

History of Cytomegalovirus

Cytomegalovirus (CMV) was first isolated from the salivary gland and kidney of two dying infant with cytomegalic inclusion bodies and reported in 1956 (Smith, 1956). Two other laboratories isolated CMV at approximately the same time. Thus CMV was initially called "Salivary gland virus" or" “Salivary gland inclusion disease virus”.

In 1960, Weller *et al* (1957) proposed the use of the term cytomegalovirus. Klemola and Kaarianinen first described CMV mononucleosis CMV was first isolated in renal transplant recipients in 1956.

The Genus Cytomegalovirus

Human cytomegalovirus (HCMV) is member of the Betaherpesvirinae subfamily, of the family Herpesviridae. Its replication cycle is significantly longer and infected cells typically are greatly enlarged and multinucleated thus the name cytomegalo (William *et al*., 2001).

Structure and biology of human cytomegalovirus

Human cytomegalovirus is a herpesvirus, with enveloped virion, about 150 nm in diameter, and consists of an icosahedral nucleocapsid about 100 nm in diameter, composed of 162 hollow capsomers -150 hexamers and 12 pentamers. The viral genome is wrapped around a fibrous spool –like core, which has the shape of a torus and appears to be suspended by fibrils that are anchored to the

inner side of the surrounding capsid and passs through the hol of the torus (Murphy *et al*., 1999). The genome of CMV is also characterized by linear DNA molecules ranging in size from 200- 248 kbp. This is significantly larger than those of the other a herpesvirus.

Human cytomegalovirus genome also exhibits pattern of terminal and inverted repeats that vary in size depending on the virus strain and passage history (knipe *et al* ., 2001). Surrounding the capsid is a layer of globular material, known as the tegument which is enclosed by atypical lipoprotein envelope with numerous small glycoprotein peplomers at least 25 proteins of which are phosphorylated and also several transcripational transactivator protein have also been localized in the tegument layer between the virion capsid and envelope (knipe *et al* ., 2001) .

Classification of human cytomegalovirus

The family herpesviridae is divided into four subfamilies: Alphaherpesvirinae, Betaherpesvirinae, Gammaherpesvirinae, and Unnamed subfamily. This division was originally based on biological properties, but in general it has accorded well with subsequent molecular characterization, including nucleotide sequence and phylogenic analyses (Murphy *et al*., 1999). The same study group classified a number of herpes virus into genera based on DNA sequence homology. The similarities in genome sequence arrangement and relatedness of important viral protein demonstrable by immunological methods (Knipe et al., 2001).Subfamily Alphaherpesvirinae, these viruses have a relatively rapid, cytocidal growth cycle the prototypic viruses of the genra of this subfamily is human herpesvirus I (herpes Simplex virus I: genus simplexvirus) and human herpesvirus III (Varicella-zostervirus; genus Varicellavirus). Gallid herpesvirus I (infectious laryngotracheitis virus) and marek's disease virus.

Subfamily Betaherpesvirinae these viruses have a relatively slow replication cycle. The protype of the subfamily is human herpesvirus

VI (human cytomegalovirus). Subfamily Gammaherpesvirinae, these viruses replicate in mucosal epithelium where they also establish latent infection and this subfamily comprises the herpesvirses that are lymphotropic. The prototype is human herpesvirus - V (Epstein- Barrvirus; genus lympocrytovirus). Unnamed subfamily, at present this subfamily comprises only channel catfish herpes viruses (William *et al*., 2001; Murphy et al., 1999).

Genome structure

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 cytomegalovirus 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). Flanked by inverted repeats b (TRL / IRL) and c (IRS / TRS) (Figure. 2). A directly repeated sequence is found at the genome termini and also present in inverted orientation at the L-S junction. The arrangement of a sequence promotes genome inversion. The sequence carries Cissignals called pac-1 and pac-2 for cleavage and Packaging of viral genome.

The human CMV genome is G +C rich and contains a significant number of direct and inverted repeats (Murphy et al.,1999). The regions show an extremely high density closely spliced inverted and direct repeats. Human CMV generates defective viruses carrying DNA molecules that are shorter than the genome length. The novel restriction fragments are found in the defective CMV DNA, suggesting that specific deletions or rearrangement occur relative to standard viral (Knipe *et al*., 200l).

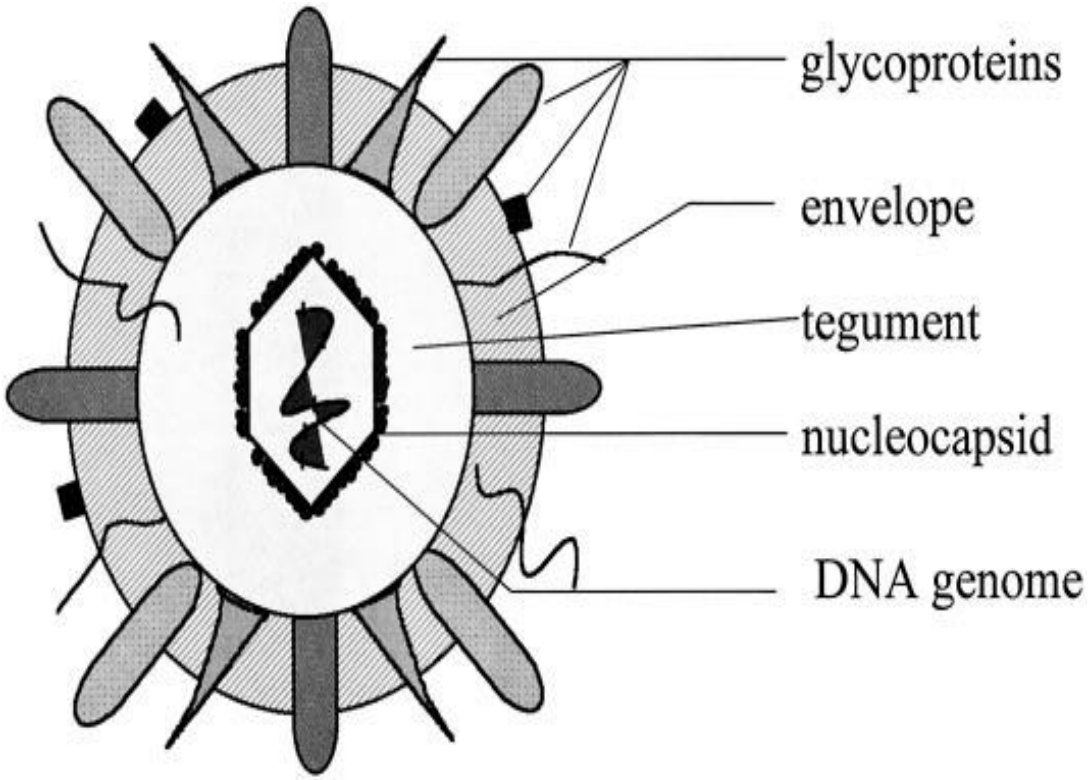

Figure 1. Schematic diagram of cytomegalovirus (CMV). The CMV virus shows a similar composition to all human herpes virus.

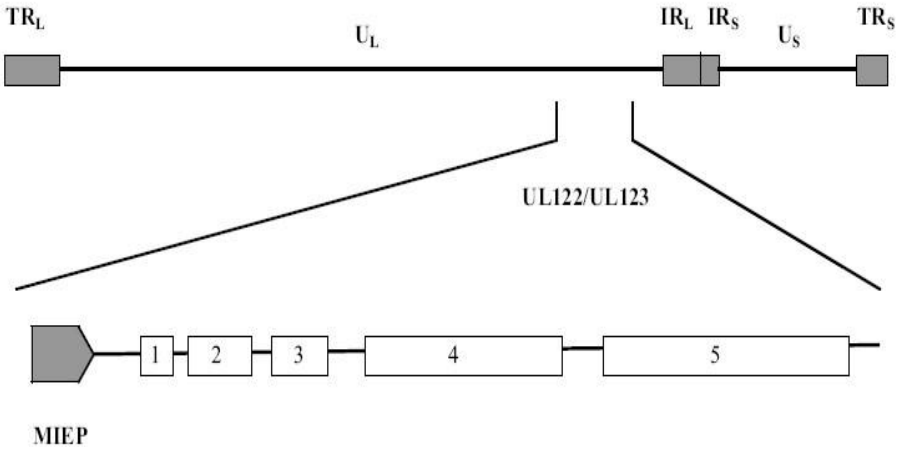

Figure 2. Simplified structure of the CMV genome (UL = unique larger region, US = unique short region, TRL and TRS = terminal repeat sequence, IRL and IRS = inverted repeat sequence).

Epidemiology of HCMV

Cytomegalovirus is endemic in all parts of the world and occurs all over the year, with non seasonal variation. The prevalence of infection varies with socioeconomic status, living condition, and hygienic practices , antibody prevalence may be moderate (40-70%) inadults, in high socioeconomic group in developed countries in contrast to prevalence of 90% in children and adults in developing nations and in low socioeconomic group in developed countries. Human are only known host for cytomegalovirus (Jawetz et al., 2004). And infection rate are higher in non whites than in whites Human (knipe et al.,2001).

Transmission and spread of HCMV

CMV is not high contagious agent and transmission appears to be requiring direct contact with infectious material. Following initial acquition of CMV, infectious virus is present in urine, saliva, tears, semen and cervical secretion from month to years. High rates of CMVinfection occur in settings where close contact with body fluid is expected, Such as between sex partners, among

children in day care centers, and between preschool-aged children. But transmission of CMV through the sexual contact is an important means of spread, and the prevalence of CMV infection is higher among populations with other markers of sexual activity; such as greater number of partner and sexually transmitted diseases and among sexually active adolescent (knipe et al ., 2001). CMV virus is unique among the human herpesviruses in the transmission from mother to fetus or newborn so that it plays an important role in maintaining CMV in population. Human CMV spread from mother to baby by three major routes; transplacental, intrapartum and human milk (knipe et al., 2001). The virus can also be transmitted by organ transplants and by blood transfusion (William et al., 2001).

Cytomegalovirus and immunocompromised patient

Individuals at greatest risk for cytomegalovirus disease are those receiving organ transplants, those with malignant tumors who are receiving chemotherapy and those with AIDS. Both morbidity and mortality rate increased with primary and recurrent cytomegalovirus infection in immunocompromised individual. Interstitial pneumonitis caused by cytomegalovirus occurs in 10 to 20 % of bone marrow transplant recipients.

Virus associated leucopenia is common in solid organ transplant recipient, graft atherosclerosis after heart transplantation and cytomegalovirus related rejection of renal allograft. Cytomegalovirus often causes disseminated disease in untreated AIDS patient; gastroenteritis and chorioretintis are common problem (Jawetz et al., 2004).

Human cytomegalovirus and renal transplant patients

Symptomatic CMV infection occurs in 20 to 60% of all transplant recipients and is a significant cause of increased morbidity and mortality in this population (Brennan and Singer, 1999; Sai and Patel, 2000). The incidence may be somewhat lower in kidney transplant recipients who do not receive

antilymphocyte therapy, highdose mycophenolate, or the potent combination of mycophenolate and tacrolimus without adequate antiviral prophylaxis.

Historically, concern has focused mainly on avoiding CMV infection in the CMV D+/R- group because this group has been atgreatest risk for severe "primary" infection during the first 3 months after transplantation. However, the indirect effects of CMV infection on graft and patient survival have been increasingly recognized in recent years. The analyses of data from the United States Renal Data System and United Network of Organ Sharing revealed that by 3 years, it is the D+/R+ group and not the D+/R- group that have the worst graft and patient survival (Schnitzler *et al*., 1997). The reason for this is not entirely clear but may reflect the prevalence of multiple CMV virotypes and that the D+/R+ patients may have a double CMV exposure with reactivation of differing latent donor and recipient CMV. CMV can predispose to rejection as well. Subclinical CMV may also mimic and or predispose to late acute rejection.

Late subclinical infections are common and associated with relatively rapid graft loss (Kern *et al*., 1996). CMV has been associated with both atherosclerosis and chronic rejection, and the two most common causes of late graft loss are cardiovascular death and chronic rejection. Chronic rejection is also known as chronic allograft nephropathy and is characterized by myointimal thickening, which is a form of atherosclerosis. Latent CMV infection has been associated with a markedly increased rate of restenosis (1200% higher) after coronary angioplasty in non-transplant seropositive individuals compared with seronegative individuals (Zhou *et al*., 1996). Furthermore, the histologic lesion of coronary restenosis is diffuse atherosclerosis characterized by myointimal hyperplasia resembling chronic rejection. effects of p53 (Speir *et al*., 1994). However, more recent evidence suggests that the myointimal increase is not from proliferation but rather from migration of smooth muscle cells (SMC) mediated by virally encoded chemokine receptors that require tryosine kinase for expressions (Streblow *et al*., 1999). SMC migration could be blocked by

tyrosine kinase inhibitors and suggests another potential future therapeutic strategy. Finally, human CMV infection may also cause atherosclerosis and chronic rejection by increasing oxidized low density lipoprotein uptake by vascular SMC (Zhou et al., 1996). This process is mediated by the IE gene IE72 and does not require viral replication, which may explain why angioplasty patients who were infected latently with CMV had higher rates of restenosis after angioplasty. CMV has been associated with several other vascular injuries that may explain why the D+/R+ group has the worst overall survival. One such vascular injury associated with CMV is transplant glomerulopathy. However, the frequency and clinical significance of this lesion are uncertain (Birk and Chavers, 1997). The lesion of the hemolytic uremic syndrome/thrombotic microangiopathy is one of the more common vascular pathologies associated with CMV and may be confused with or present with cyclosporine or tacrolimus toxicity (Miller et al., 1997; Hochstetler et al., 1994). CMV-associated hemolytic uremic syndrome/thrombotic microangiopathy may respond to Ig infusion (Hochstetler et al., 1994). CMV has been implicated as an important contributor to restenosis of coronary arteries after angioplasty in nontransplant patients (Zhou et al., 1996). CMV has also been associated with transplant renal artery stenosis. In a study of more than 900 renal transplant recipients, 75 were diagnosed with renal artery stenosis via angiography (Pouria et al., 1998).

Congenital Human cytomegalovirus

Fetal and newborn infection with cytomegalovirus may be sever. About 1% of live birth annually in the United States has congenital Cytomegalovirus infection and about 5-10% of those will suffer Cytomegalic inclusion diseases. A high percentage of babies with These diseases will exhibit developmental defects and mental Retardation. The virus can be transmitted with both primary and Reactivated maternal infection. About one –third of pregnant women With primary infection transmit the virus congenital infection may result in death of fetus in utero.

Cytomegalic inclusion disease of newborns is characterized by involvement of central nervous system and the reticuloendothelial system. Clinical features include intrauterine growth retardation, jaundice, heptosplenomegaly, thrombocytopenia, microcephaly, and retinitis. Mortality rates can approach 30%. The majority of survivors will develop significant central nervous system defect within 2 years; sever hearing loos, ocular abnormalities, and mental retardation is common. About 10 % of subclinical congenital cytomegalovirus infection will develop deafness (Jawetz *et al*., 2004). Ten percent of these infants are born symptomatic, manifesting various neurologic, hematologic, and developmental abnormalities (Demmler, 1991).

Stability of Human cytomegalovirus

CMV is a labile virus and readily inactivated by lipid solvents, pH below 5, heat (37°C for 1 h or 56°C for 30 min), and ultraviolet light for 5 min. It can survive on environmental surfaces for several hours. CMV can be stored at 4°C for a few days without loss of infectivity. Storage at –70°C without loss of infectivity is possible for several months. CMV can be stored at –190°C (liquid nitrogen) indefinitely (Daniel, 2001).

Persistence of Human cytomegalovirus

Human CMV persists within its host indefinitely; there is growing evidence that cells in bone marrow and peripheral blood are a key reservoir for CMV through latent infection. A small percentage of peripheral blood monocytes from subjects with the past infection harbor CMV DNA. But viral gene expression is limited to early genes. However; tissue macrophage early and late CMV antigens. Kondo *etal* (1994). showed that granulocyte macrophage progenitors from human fetal liver and bone marrow could be latently infected in vitro; the cells have a CD14-, CD15-, CD33-, phenotypic and expressed {E} early transcripts. Soberberg – naucler *et al* (1997) reported reactivation of CMV in vitro by allogenic stimulation from peripheral blood mononuclear cells. The experimental evidence

supports a model of CMV persistence which myelomonocytes stem cells resident in bone marrow maintain latent infection, and latently infected CD14+ monocytes circulate in peripheral blood, differentiation latently infected monocytes into tissue macrophages result of allogenic stimulation (transciplantation) or triggering of proinflammatorycytokines as intercurrent infection leads to productive infection (knipe *et al* ., 2001).

Reactivation of HCMV infection

Reactivation is believed to be more important than primary infection as a virus causing disease in immunocompromized host and is though to occur as a result of at least three collaborating events:

a. Cytokine stimulation of latently infected cells causing differentiation into a permissive cell type that supports viral replication (Hahn *et al*., 1998).
b. Viral amplification caused by reduced immune surveillance that leads to wide spread systemic infection with readily detectable viraemia (Farrel *et al.*, 1999) and virus in many tissues.
c. Disease state develops as a result of poorly understood collaboration of immune-organ and virus species determinants.

The three stages of reactivation help illuminate on important interplay of the immune response in reactivation and pathogenesis (Farrel *et al*., 1999). Immunocompromized individuals who support persistent infection or who frequently reactivate and shed viruses may be predisposed to more rapid amplification and clinical disease because the first event is unnecessary. Most clinical manifestation of reactivation results from second events, after virus replication reaches high systemic levels prior to and the absence of disease is the basis of preemptive antiviral or adoptive CMV specific CTL therapies (Riddel and Greenberg, 1997). As well as being the basis for the dramatic reduction in incidence of CMV retinitis and gastroenteritis resulting from immuno reconstitution that follows highly active retoviral therapy in AIDS patients (Donniger *et al*., 1999). The earliest event in reactivation is fascinating area

virus biology. Cytokine mediated differentiation of peripheral blood monocytes into macrophages under strongly proinflammatory conditions is undoubtedly key to this process (Meier and Stinski, 1996).

Diagnosis of Human cytomegalovirus

There are several methods for diagnosing CMV. Until fairly recently, the available techniques for diagnosing CMV were limited to histologic identification of CMV inclusion bodies, viral culture, and serology. These techniques are labor intensive and not completely sensitive, and the time from primary infection or reactivation to detection of CMV is protracted, allowing for undetected and untreated disease progression. In addition, transplant recipients may fail to produce an antibody response despite other evidence for viremia, making the newer techniques preferable to serology for detection and monitoring. Newer techniques include shell vial culture, pp65 antigenemia assay, PCR, and the hybrid-capture RNA-DNA hybridization assay, which has recently received FDA clearance.

Histopathology

Characteristic large cells with intranuclear and, on occasion cytoplasmic inclusion can be seen in routine section of biopsy or autopsy material. Wright –Giemsa stained touch imprints of lung or other biopsy speciment may demonsrate such cells. Although the presence of characteristic cytological changes suggests CMV infection. Virological serological conformation is suggested since CMV can infect tissues without producing morphological changes. Failure to find typical cytomegalic cells does not exclude the possibility of CMV infection (Hodinka et al., 1991). The large inclusions are intranuclear and have a characteristic owl-eye appearance in haematoxylin and eosin stained tissue specimens. The positive results correlate well with active HCMV infection of the organ, e. g. hepatitis, but the sensitivity of the histopathological finding is relatively low (Colina et al., 1995; Mattes et al., 2000). Immunostaining with specific polyclonal or monoclonal

antibodies against HCMV antigens has increased the sensitivity of the method compared to conventional staining (Barkholt et al., 1994; Paya et al., 1990). Although the histological methods may have an important role in detecting HCMV organ involvement, they are not suitable for the early diagnosis of HCMV infection. In addition, these assays are very laborious, and biopsy specimens are only taken when an end-organ disease is suspected.

Serology

Serological tests for antibody to CMV are useful to determining whether a patient had CMV infection in the past, a determination of great clinical importance for organ and blood donors, and in pretransplant evaluation of prospective transplant recipients. In addition, serologic test for seroconvertion and for IgM antibody to CMV are commonly used to establish whether or not infection in the normal host occurred recently. A variety of different laboratory methods have been used successfully to measure antibody to CMV. Traditionally, serologic evidence of recent primary CMV infection depended on the demonstration of conversion from IgG antibody negative to positive are demonstration of IgM antibody (Kinpe *et al*., 2001).

Enzyme linked immunsorbent assay (ELISA)

Enzyme linked immunsorbent assay (ELISA) is the most commonly available serologic test for measuring antibody to CMV.

The result can be used to determine whether it is due to acute infection, previous infection, or passively acquired maternal antibodies in an infant. ELISA also is proved to be a useful technique when recombinant antigens are used to differentiate between primary and secondary or past CMV infection in pregnancy, it was found that IgM response to recombinant proteins and PP150 in primary infected women varies. Predominant IgG response to P52 was observed in early sera less than 4 weeks after seroconversion, whereas IgG response to recombinant proteins PP150 was delayed and appeared 2-7 weeks later. On the

other hand women with secondary and those with post primary infection had IgG antibodies to PP150. ELISA for CMV specific IgM may give false positive result unless steps are taken to remove rheumatoid factor (Daiminger *et al.*, 1998).

The immune system and CMV

Because most immunocompetent individuals do not suffer from CMV disease during either primary or recurrent infections, the immune system must be effective in controlling replication. However, this simplistic assumption masks a level of complexity between the virus and host that is still being unraveled. The B cell response to CMV is largely dominated by an anti-glycoprotein B cell response, and most of the neutralizing antibodies are also directed against this protein (Wagner *et al.*, 1992; Ohlin *et al*., 1993). In contrast, the catatonic T cell (CD8) response is almost exclusively directed against the pp65 (UL83) protein and epitopes have been mapped for human major histocompatibility complex (HLA) types A (Wills *et al*., 1996; Mclaughlin *et al*., 1994). In the face of such an aggressive immune response, how does the virus survive. It is now clear that CMV encodes a range of gene products and uses several mechanisms to manipulate the host immune system (Ploegh, 1998). Thus, the presentation of immediate–early antigens is suppressed by phosphorylation by a virion phosphoprotein, which leads to a lack of processing within the proteasome (Gilbert *et al.*, 1996). In addition, the HLA class I display pathway is interrupted at multiple time points after infection via interaction with the TAP system, the recycling system, and terminal maturation by genes US2, US3, US6, and US11 (Ploegh, 1998). The consequences of reduced HLA class I display should result in an increased susceptibility to natural killer (NK) cell activity, because cells require presentation of the signal peptides derived from HLA class I A, B, or C to be protected against NK cell lysis (Borrego *et al.*, 1998). However, the CMV genome contains an HLA class I homologue (UL18), which is synthesized late in the viral replicative cycle, when HLA class I downregulation is maximal

(Hassan Walker *et al*., 1998). In addition to encoding HLA class I dysregulatory genes, the genome encodes four potential G coupled protein receptors, one of which (US28) acts as a broad specificity ß chemokine receptor and can act as a coreceptor for the uptake of HIV (Pleskoff *et al* .,1997). The genome also contains two genes with homology to the CXC chemokine family (Chat *et al.*, 1996). And one of these has been shown to stimulate neutrophil migration with the same potency as other cellular chemokines (Penfold *et al.*, 1999).

Vaccination against HCMV

Although attempts to develop vaccine for prevention of maternal and congenital CMV have been made for over 25 years no vaccine is currently licensed or near licensure. However, investigational vaccines employing different formats have been evaluated in the clinical trials; these include attenuated live virus, recombinant protein in fowl pox vector and recombinant protein given with anew adjuvant. The Twne strain of CMV was passaged more than 125 times in vitro to achieve attenuation. Subsequent study showed that the Twne strain of CMV was attenuated in that it did not produce a febrile illness in challenge studies wheras allow passageToledo strain did. Immunization with Twine strain CMV vaccine induces both antibody responses and lymphocyte proliferative response to CMV. An efficacy trial in renal transplant recipient patients showed that pretransplantation immunization of seronegative patients with Twne strain CMV vaccine protected than from sever disease although it did not prevent infection (Knipe *et al*., 2001).

Treatment

The three antiviral drugs that are currently licensed for the treatment of HCMV infection are ganciclovir (GCV), foscarnet and cidofovir. Ganciclovir is the drug of choice in the treatment of symptomatic infections. Ganciclovir is a specific nucleoside analog which first is phosphorylated before it gains antiviral

activity (Littler *et al*., 1992; Sullivan *et al*., 1992). The initial phosphorylation is mediated by virus encoded phosphotransferase (product of *UL97*).

The monophosphate form is further phosphorylated by the host's cellular kinases into the active triphosphate form, which inhibits viral DNA polymerase by competing with deoxyguanosine triphosphate (Noble and Faulds, 1998). Foscarnet is an inorganic pyrophosphate analog and directly inhibits viral DNA polymerase (Crumpacker,1992). However, foscarnet is nephrotoxic, which limits its use in kidney transplantation. Intravenous cidofovir may also be used, but experience of this agent in solid organ recipients is still very limited. Cidofovir is a cytosine analog which does not require the virus encoded phosphotransferase activity. Instead host cellular enzymes carry out the phosphorylations needed for activation (Lalezari *et al*., 1995; Snoeck *et al*., 1988). Valganciclovir, a pro-drug of ganciclovir, is a new promising compound which is administered orally and has an increased bioavailability compared to that of oral ganciclovir (Pescovitz *et al*., 2000). Nowadays valganciclovir is used in therapy of HCMV disease; however, more clinical trials are needed. The HCMV hyperimmunoglobulin may also be used in some patient groups in combination with antiviral drugs.

Prevention

Specific control measure is not available to prevent cytomegalovirus spread. Screening of transplant donors and recipient for cytomegalovirus may prevent some transmission of primary cytomegalovirus. The cytomegalovirus seronegative transplant recipient population represents a high risk group for CMV infection (Jawetz *et al.,* 2004). In organ transplantation the common source of CMV infection is reactivation of transplant recipient latent virus, and there is no means to prevent this infection. Although selection of seronegative donors for seronegative recipients will prevent primary CMV infection in solid organ transplants recipient (Knipe *et al*., 2002). The leukocyte free blood products are used for transplant patients. WBC is main source of CMV

infection in peripheral blood products have been shown to reduce the Risk of CMV transmission.

There is no effective vaccine against HCMV. Before the development of suitable antiviral drugs against HCMV, a reduction in the immunosuppressive regimen was used to prevent HCMV disease in transplant patients. However, this often led to an increased incidence of graft rejection (Sia and Patel, 2000; Knipe *et al*., 2002). CMV immunoglobulin were also used then administration of human Ig prepared from plasma pools obtained from healthy persons with high titer of CMV antibodies has given discordant results in tests to\ decrease the incidence of the viral infection in the transplant recipients (Jawetz *et al*., 2004). Currently, good availability of antiviral agent allows maintenance of the level of immunosuppression required to prevent graft rejection. Two different strategies, prophylaxis and preemptive therapy, are used for the prevention of symptomatic HCMV infection after transplantation. In prophylaxis, antiviral drugs are administered before any evidence of the virus, and in pre-emptive therapy, antiviral drugs are administered when there is laboratory evidence of active but asymptomatic infection Also hyper immunoglobulin in combination with ganciclovir has been used in certain patient groups, e.g. lung transplant recipients (Weill *et al*.,2003).In community use of good hand washing and careful hygiene have been recommended as mean to prevent CMV infection in precare workers and parents of young children (Knipe *et al*., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Study design

This is prospective hospital base study.

Study area

The study conducted in Doctor Salma Centre for Kidney Disease.

Study population

Subject investigated were chronic renal failure patients and renal transplant rejected patients on hemodialysis.

Data Collection Method

Data was collected as per a structural interview questionnaire specially designed to collect and maintain all information of each subject examined.

Ethical consideration

- Approval to run the research was taken from the faculty of post graduate study
- Permission was taken from the local authorities
- Verbal consent was taken from the patients studied.
- Positive and negative results were handed to all patients included in this study.

Data analysis

The collected data were analyzed using SPSS software.

Sample collection

A total of 100 blood samples were collected. 91with chronic renal failure patients and 9 with previous rejected renal transplant on hemodialysis in Khartoum state from Doctor Salma Center for Kidney Disease between March and June 2018.

Data on respondent background on various issues in from of questionnaire were collected (attached in appendix).

Venous blood samples were collected using vacationer and Needles. The blood was drown into the tube without any anticoagulant and the blood was allowed to clot at room temperature for no more than 1 hour. The serum was removed and

placed in sterile capped microfuge tubes and the tubes were centrifuged at 1000 rpm for 5 min at 4ºC. The serum was then removed without disturbing the pellet and the tubes were stored at -20ºC till used.

Study variables

Detecting CMV antibodies as well as assessing the sero-markers in relation to age and gender.

Samples

Human serum or EDTA, heparin or citrate plasma.

Method of ELISA and principle

Attached in appendix

ELISA kit (EUROIMMUM)

CHAPTER FOUR

RESULTS

4. RESULTS

The result reveal the analysis of 100 participants data in this study, 56(56%) male and 44(44%) female participants who received hemodialysis.(see figure 4-2) They were divided by age into four groups from 20 to 30 years old as the first group, form 31 to 40, from 41 to 50 and over 50 years as the last group.(see Figure 4-1) Their mean age was 46.8(SD=13.9). Most of them are married with percentage of 80% VS 20% as single individuals.(see Figure 4-2)

The total of positive CMV are 74 individuals 56(75%) of them with chronic term of infection (positive for IgG CMV antibody) and 18 (24%) was in acute infection was found .(see table 4-1). for the DM incidence and other percentage of disease history.(see figure 4-3).

The highest incidence of acute CMV infection(CMV IgM positive) is within +50 ages as from 41 to 50 years old with 7(7%) individuals, and chi-squire test reveals that higher ages are associated with CMV infection(p value=0.02)(see table4-2) also the highest incidence of chronic CMV infection (CMV IgG positive) is within higher age over 50 years old with 17(17%) individuals, and chi-squire test reveals that higher ages are associated with CMV infection(p-value=0.02)(see table4-2). Also highest frequencies of recent infection was in male's gender with 10(10%) frequency and there was significant association between gender of individual and recent infection specially among men (p-value=0.02).(See table4-2). Also CMV correlation is established with disease history of DM, HBV and HCV, show there is no relation between CMV infection and those disease history.

Table (4-1): shows CMV sero- markers among participant

CMV infection	**Number**	**%**
positive (IgG)	56	75.7
positive (IgM)	18	24.3
negative	26	2
Total	100	100

Table (4-2): shows CMV sero-markers in association with Age groups.

Age groups	**No of screened**	**CMV sero-positivity IgG No (%)**	**CMV sero-positivity IgM No (%)**	**CMV sero-negativity No (%)**
20-30	16	4 (4%)	2 (2%)	10 (10%)
31-40	19	9 (9%)	3 (3%)	8 (8%)
41-50	24	8 (8%)	7 (7%)	9 (9%)
Over 50	41	17 (17%)	6 (6%)	18 (18%)

*p-value ≤ 0.05 considered significant

Table(4-3): shows CMV infection in association with past history of disease.

History of disease	No of screened	Sero-positivity IgG No (%)	Sero-positivity IgM No (%)	Sero-negativity No (%)
DM	15	6 (6%)	1 (1%)	8 (8%)
HBV	6	2 (2%)	3 (3%)	1 (1%)
HCV	4	2 (2%)	0 (0%)	2 (2%)

*p-value= less than0.05

*p-value ≤0.05 considered significant

the result is constant no p-vale produced

Table(4-4) : shows distribution CMV sero-markers among individual with rejected kidney transplant.

CMV sero-marker	**NO**	**percent**
positive (IgG)	2	22 %
Positive (IgM)	3	33 %
Negative	4	55%
Total	9	100 %

CHAPTER FIVE

DISCUSSION

5. DISCUSSION

Cytomegalovirus infection remains a major cause of morbidity and mortality in immunocompromised patients, and most serious problem in organ allograft recipients. The possibility of using specific antiviral therapy to treat CMV infection makes a timely diagnosis imperative (Peterson *et al* 1980).

This study was focused on the serologic diagnosis of human CMV in rejected renal transplant patients and hemodialysis patients in Doctor Salma For Kidney Disease . A number of 100 serum samples were collected from these two groups. We studied the prevalence of CMV antibodies among rejected renal transplant and hemodialysis patients by using ELISA.

We studied the frequency of CMV antibodies among rejected renal transplant and hemodialysis patients by using ELISA. We found that the chronic infection (IgG) frequency was 33% in rejected renal transplant and 56% hemodialysis patients, respectively, suggesting high incidence of previous infection in all groups tested. Eldowma (2004) was the first to detect IgG antibodies of CMV among blood donors and antenatal women in Sudan with 77% and 95%, respectively. Bushra (2006) detected 96% seropositivity for IgG in pretransplant kidney recipient, 17% in healthy candidate donors, and 84% in blood donors. Since no vaccination program is practiced against CMV in Sudan our results denotes for previous infections with the virus. Taken together, our results and the findings of Eldowma (2004) and Bushra (2006) it is obvious that CMV is endemic in the Sudan. The present high CMV seroprevalence in the two groups studied point of high spread of the infection in the population as a whole. This high prevalence of CMV may be attributed to the poor socioeconomic status and hygienic practice known to play an important role in transmission of CMV due to over-crowdness. According to Jawetz *et al* (2004) the prevalence of infection varies with socioeconomic status, living condition, and hygienic practices, antibody prevalence may be moderate (40-70%) in adults in high

socioeconomic group in developed countries in contrast We studied the prevalence of CMV antibodies among rejected renal transplant and hemodialysis patients in Khartoum state by using ELISA. We found that the chronic infection (IgG) frequency was 33% in renal transplant and 56% hemodialysis patients, respectively, suggesting high incidence of previous infection in all groups tested. Eldowma (2004) was detected chronic infection (IgG) of CMV among blood donors and antenatal women in Sudan with 77% and 95%, respectively. Bushra (2006) detected 96% seropositivity for IgG in pretransplant kidney recipient, 17% in healthy candidate donors, and 84% in blood donors. Since no vaccination program is practiced against CMV in Sudan our results denotes for previous infections with the virus. Taken together, our results and the findings of Eldowma (2004) and Bushra (2006) it is obvious that CMV is endemic in the Sudan. The present high CMV seroprevalence in the two groups studied point of high spread of the infection in the population as a whole. According to Jawetz *et al* (2004) the prevalence of infection varies with socioeconomic status, living condition, and hygienic practices, antibody prevalence may be moderate (40-70%) in adults in high socioeconomic group in developed countries in contrast to prevalence of 90% in children and adults in developing nations and in low socioeconomic group in develop countries.

For acute infection(IgM) ELISA was used to establish whether or not infection in the normal host has occurred recently. The prevalence of HCMV acute infection(IgM,) in the present study, was 18% in rejected renal transplant and hemodialysis. These might reflect an alarming picture of the disease in the population and indicate that seroconversion is an ongoning process in rejected renal transplant and hemodialysis group in the Sudan.

The presence of HCMV IgM antibodies probably indicate active infection as has been reported previously by Farrell *et al* (1999). ELISA can detect HCMV antibodies of both primary CMV and reactivation of latent infection. Accordingly, high titer of CMV IgM antibodies by ELISA suggests recent

exposure or reactivation of latent infection. Further studies are needed to determine the exact prevalence of CMV infection in the different parts of the Sudan.

CONCLUSION

1. Frequency of HCMV chronic infection (IgG) was found in the renal transplant patients and hemodialysis patients and probably the dialysis machines of dialysis have a role in the transmission of the virus.
2. The prevalence of CMV acute infection (IgM) antibodies is higher in the renal transplant group maybe due to reactivation or new infection.
3. The prevalence of CMV chronic infection (IgG) antibodies is higher in the two groups: renal transplant patients and hemodialysis patients.

RECOMMENDATIONS

1. Blood donors should screened for CMV sero-positivity to minimize spread of the virus.
2. Patients on hemodialysis should screened for CMV sero-markers initially and periodically.
3. Renal transplant patients should screened for CMV sero-markers periodically.
4. Patients sero-positive CMV on hemodialysis should be isolated in dialyzed separate machine.

REFERENCES

Birk, P.E, Chavers, B.M. (1997). Does cytomegalovirus cause Glomerular injury in renal allograft recipients. *J. Am. Soc. Nephrol.* 8: 1801– 1808**.**

Boppana, S.B., Smith, R., Stagno, S., and Britt W.J. (1992). Evaluation of microtiter plate fluorescent antibody assay for rapid detection of HCMV infection. J.Clin. Microbiol. 30:721-623.

Brennan, D.C, Singer, G.G. (I999). Infectious complications in renal Transplantation. Clinical Nephrology Dialysis and Transplantation, edited by Malluche HH, Sawaya BP, Hakim RM, Sayegh MH, Ismail N, Bosch-Druck, Landshut, Germany

Britt, W.J., and Alford, C.A. (1996). Cytomegalovirus. In: Field's virology, 3Rd edition, Fields B.N., Knippe D.M.

Bushera, O. (2006). The prevalence of Cytomegalovirus antibodies in Kidney candidate, Kidney candidate donors and blood donors at Ahamed Gasim Renal Transplant Center. MSC Thesis U.of.K.

De Jong, M.G., Golasso, G. J., Gazzard B., Griffith P.D., Jabs, D. A., Kern E.R., and Specter. (1998). Summary of 11 international symptosium on cytomegalovirus. Antivral. Res. 39 (3): 141-62.

Eldowma, E.Y. (2004). Prevalence of Cytomegalovirus among Blood donors and antenatal women. MSc Thesis Sudan university for science and technology.

Emery, V.C. (2001). Investigation of CMV disease in immunocompromised patients: *J. Clin. Pathol*. 54:84-88.

Farrel, H.E., Degli-Esposti, M.A., and Davis-Poynter, N.J. (1999). Cytomegalovirus evasion at natural killer cell response. immno rev. 168: 187-197.

Hillyer, C.D., Emmens, R.K. (1994). Methods for the reduction of transfusion transmitted cytomegalovirus infection. Filtration versus the use of serovegative donor units. Transfusion., 34 (10): 929-934

Hochstetler, L.A, Flanigan, M.J, Lager DJ. (1994). Transplantassociated thrombotic microangiopathy: The role of IgG administration as initial therapy. Am. J. Kidney. Dis 23: 444–450.

Hodinka, R.L., and Freeman, H.M. (1991). Human cytomegalovirus in: manual of Clinical Microbiology. Hauslo, J.r.; Herman, K.L.; Isenbers, H.D. and Shadoom, H.J. (edtitors), pp. 829-837.

Jawetz, E., Melinck, Adberg, E.A. Broks, G.O., Butel, J.S., and Ornston, N.L. (2004). Medical microbiology. Applenton and lange:Norwalk. 23th edition.

Kern, F., Ode-Hakim, S., Nugel, H., Vogt, K., Volk, H.D. (1996). Reinke Peripheral T cell activation in long-term renal transplant patients:Concordant upregulation of adhesion molecules and cytokine gene transcription. J. Am. Soc. Nephrol. 11: 2476–2482.

Knipe, D.M., and Howley, R. (2001). Fields virology. Editor. Fourth edition (Volume 2), Lipincott, Williams and Wilkings. AWOLTERS Klumer Company, Philadelphia, USA, pp. 2381-2698.

Miller, B.W., Hmiel, S.P., Schnitzler, M.A., Brennan, D.C. (1997). Cyclosporine as cause of thrombotic microangiopathy after renal transplantation. Am. J. Kidney. Dis. 29: 813–814.

Pertal, P., Hirschtick, R., Phair, J. (1992). Risk of developing Cytomegalovirus retinitis in persons infected with the human immunodeficiency virus. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 5: 1069-1074.

Pouria, S., State, O.I., Wong, W., Hendry, B.M. (1998). CMV infection is associated with transplant renal artery stenosis. QJM. 91: 185– 189.

Schnitzler, M.A, Woodward, R.S, Brennan, D.C, Spitznagel Dunagan, W.C., Bailey, T.C. (1997). The effects of cytomegalovirus serology on graft and recipient survival in cadaveric renal transplantation: Implications for organ allocation. Am. J. Kidney. Dis. 428–434.

Smith, M.G. (1956). Propagation in tissue culture at cytopathogenic virus from human salivary gland virus (SGV) disease. Proc. Soc. Exp. Biol. Med. 92: 242-436.

Soderbery C., Larsson S,. (1993): Definition of a subset of human Peripheral blood mononuclear cells that are ermissive to human cytomegalovirus infection. J. Virol. 67 (6): 3166.

Speir, E., Modali, R., Huang, E.S, Leon, M.B, Shawl, F., Finkel, T., Epstein, S.E. (1994). Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. Science. 265: 391–394.

Weller, T.H.; Macaulay, J.C. and Craig, J.M. (1957): Isolation at intra-nuclear inclusion producing agents from infants with illnesses resembling cytomegatic inclusion disease. Proc. Soc. Exp. Biol. Med. 94: 4-12.

William, A.S., Harriet, R., and Bruce, D.F. (2001). Lipincotts illustrated Reviews: microbiology. Richard, A.H. and Pamela, C.C. London. Pp. 326-329.

Zhou, Y.F., Guetta, E., Y.u. Z.X., Finkel, T., Epstein, S.E. (1996). Human cytomegalovirus increases modified low density lipoprotein uptake and scavenger receptor mRNA expression in vascular smooth muscle cells. J. Clin. Invest. 98: 2129–2138.

Appendix

Questionnaires

Date: / / 2018 sample No…

. Name:…….

. Gender: male ☐ female ☐

. Marital status: single ☐ married ☐

1. First diagnosis of the disease:

Month ☐ year ☐

2. History of hemodialysis:

. The first time of hemodialysis?

……

. Duration of hemodialysis per week?

……

3. Chemotherapy: yes ☐ No ☐

4. medical history :

. Abortion : yes ☐ No ☐

Number of abortion ?.....

. Blood transfusion: yes ☐ No ☐

Number of transfusion?.....

. Pervious surgery: yes ☐ No ☐

. D.M: yes ☐ No ☐

. Hepatitis: yes ☐ No ☐

Type of hepatitis: HBV ☐ HCV ☐

. HIV: yes ☐ NO ☐

. Other disease?

…….

5. Clinical feature:

Prolonged fever ☐ Jaundice ☐

pneumonia ☐ Skin rash ☐

Other: ……..

6. Investigation result:

.CMV IgM: positive ☐ Negative ☐

. CMV IgG: positive ☐ Negative ☐

EUROIMMUN Medizinische Labordiagnostika AG

Qualitätskontrollzertifikat
Quality Control Certificate

Produkt: *Product:* **Anti-CMV ELISA (IgM)**

Best.-Nr.: *Order No:* **EI 2570-9601 M**

Ch.-B.: *Lot:* **E180511AC**

10-May-2019

		Referenzwert *Reference value*		Valider Bereich *Valid range*	
Kalibrator *Calibrator*		0,370	O.D.	> 0,140	O.D.
Pos. Kontrolle 1 *Pos. Control 1*	semiquantitativ *semiquantitativo*	2,7	Ratio	1,5 - 3,9	Ratio
Neg. Kontrolle *Neg. Control*	semiquantitativ *semiquantitativo*	0,2	Ratio	0 - 0,7	Ratio

EUROIMMUN Medizinische Labordiagnostika AG

Anti-CMV ELISA (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2570-9601 M	Cytomegalovirus (CMV)	IgM	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the immunoglobulin class IgM against CMV in serum or plasma for the diagnosis of the infection with cytomegalovirus.

Application: Infections with cytomegaloviruses (CMV) can be diagnosed by the detection of specific antibodies of classes IgG and IgM. Antibodies of class IgM quite reliably indicate an acute infection. However, their detection in CMV cannot be used for differentiation from a primary infection of a reactivation as they may occur in both conditions. In cases of positive IgM findings, the avidity determination of pathogen-specific IgG antibodies is therefore a suitable method to differentiate between primary infection and reactivation.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with CMV antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. **Microplate wells coated with antigens** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	—	12 x 8	STRIPS
2. **Calibrator** (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL
3. **Positive control** (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. **Negative control** (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. **Enzyme conjugate** peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
6. **Sample buffer** containing IgG/RF-absorbent (anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLE BUFFER
7. **Wash buffer** 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. **Chromogen/substrate solution** TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. **Stop solution** 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. **Test instruction**	—	1 booklet	
11. **Quality control certificate**	—	1 protocol	

LOT Lot description
IVD In vitro diagnostic medical device
CE 0197
Storage temperature
Unopened usable until

EUROIMMUN Medizinische Labordiagnostika AG

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.
- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.
- **Sample buffer:** Ready for use. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.
- ***Chromogen/substrate* solution:** *Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.*
- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrator and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

EUROIMMUN Medizinische Labordiagnostika AG

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors of class IgM from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM-negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The patient samples for analysis are diluted **1:101** with green coloured sample buffer. For example: add 10 µl sample to 1.0 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing. Incubate the mixture for at least **10 minutes** at room temperature (+18°C to +25°C). Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analysed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls are ready for use, do not dilute them.

Incubation

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode")

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.
Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	P 6	P 14	P 22								
B	pos.	P 7	P 15	P 23								
C	neg	P 8	P 16	P 24								
D	P 1	P 9	P 17									
E	P 2	P 10	P 18									
F	P 3	P 11	P 19									
G	P 4	P 12	P 20									
H	P 5	P 13	P 21									

The above pipetting protocol is an example of the **semiquantitative analysis** of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons **(cut-off)** recommended by EUROIMMUN.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

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

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.