

Sudan University of Science and Technology

College of Graduate Studies

**Serological Detection and Molecular Characterization of
Cytomegalovirus and its Glycoprotein B (UL55) among
Sudanese Renal Transplant Recipients**

**الكشف المصلي والتوصيف الجزيئي للفيروس مضخم الخلايا و الجين السكري gB
(UL55) لغارسي الكلي السودانيين**

**A thesis Submitted in Fulfillment for the Degree of Ph.D in Medical
Laboratory Science (Microbiology)**

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DECLARATION

I declare that this thesis is hereby submitted to Sudan University of Science and Technology for the degree of doctor of philosophy and has not been previously submitted by me for a degree at this or any other university.

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DEDICATION

To My faMil y with I ove

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ABSTRACT

This is analytical cross sectional study conducted at Kidney transplanted association hospital and Ahmed Gassim teaching hospital in Khartoum state in the period from 2013 to 2015, was aimed to detect CMV using ELISA and quantitative real-time PCR (qRT PCR), estimate viral loads and to determine the distribution of gB genotypes among Sudanese renal transplant recipients by DNA sequencing.

One hundred and four renal transplant recipients were included in this study. Blood and serum samples were collected from them, their age ranged from 11 to 72 years with mean age of 37 years. Males were 72(69.2%), while females were 32(30.8%). In this study, 50(48%) of them received their organs in local hospitals, while 54(52%) received their organs in abroad. Most of the renal transplant recipients' received organs from relative donors 79(76%) and only 25 (24%) received organs from non-relative donors. The mean post-transplantation duration was 54 months, ranged from < than month - 204 months. The majority of post-transplantation duration varies from < than month - 12 months which represents 53(51%), 13 to 24 months 18(17.3%), 25 to 36 months 9(8.8%), 37 to 48 months 8(7.7%), and more than 48 months was 16(15.4%). The majority of recipients under study 79(75.9%) received triple immunosuppressive drugs, 24(23.1%) received two drugs while only one (1%) received one drug.

CMV IgM was detected using ELISA technique in 27(26%) of recipients, while 103(99%) recipients had CMV IgG in their serum. CMV DNA (viremia) was detected in 40/104 (38.5%) of renal transplant recipients using quantitative real time PCR with viral loads ranging from 62 copies /ml ($1.8 \log_{10}$) to 1.43×10^8 copies/ml ($9 \log_{10}$) and average of 358×10^4 copies/ml ($6.5 \log_{10}$). Symptomatic recipients with CMV disease were 17/104 (16.3%) while asymptomatic with CMV infection were 23/104 (22.1%). CMV viremia showed no significant difference ($P.value > 0.05$) with sex and types of immunosuppressive therapy received by transplant recipients, while there is a significant difference ($P.value = 0.05$) between high viral loads and types of immunosuppressive therapy

received by transplant recipients. At the same time the correlation between high viral loads (>1000 copies /ml) and development of CMV disease observed significant difference ($P.value = 0.00$), in which, 14/17 (82%) of patients had clinical symptoms of CMV disease with high viral loads and 22/23 (95.7%) of patients had no clinical symptoms of CMV disease with low viral loads (<1000 copies/ml). The most common presenting symptoms of CMV disease were fever, fever and leucopenia, and gastrointestinal disease. The distribution of gB genotypes in Sudanese renal transplant recipients observed that gb3 was the most frequent genotype (80%) while gB4 was (20%) and no mixed genotypes were detected.

In conclusion, qRT-PCR from plasma samples is very sensitive for detection of CMV replication and was more sensitive than ELISA technique in detecting CMV in renal transplant patients. Viral loads were lower with asymptomatic patients. CMV gB3 was considered the most predominant glycoprotein B genotype in Sudanese renal transplant recipients with CMV disease.

المستخلص

هذه دراسة تحليلية مقطعية أجريت في مستشفى جمعية زارعي الكلي السودانية ومستشفى أحمد قاسم التعليمي في ولاية الخرطوم في الفترة من 2013 إلى 2015. هدفت هذه الدراسة لاكتشاف فيروس مضخم الخلايا باستخدام تقنية إلبسا وتفاعل البلمرة التسلسلي الكمي في الوقت المناسب وتعداد نسخ الفيروس ولتحديد التوزيع الجيني لجينات البروتين السكري (gB) لغارسي الكلي السودانيين باستخدام التسلسل الجيني. شملت هذه الدراسة مائة وأربعة من غارسي الكلي. وتم اخذ عينات الدم ومصل الدم، كانت أعمارهم تتراوح بين 11 و 72 سنة ومتوسط الأعمار 37 سنة. وكانت نسبة الذكور منهم (69.2%) ونسبة الإناث (32.8%). في هذه الدراسة 50 (48%) من الغارسين تم نقل الأعضاء لهم في مستشفيات محلية بينما 54 (52%) نقلت لهم الأعضاء في مستشفيات خارج البلاد. معظم الغارسين تلقوا أعضاء من أقربائهم 79 (76%) بينما 25 (24%) منهم فقط تلقوا أعضاء من أشخاص غرباء. وكان متوسط وقت ما بعد الغرس يمثل 54 شهرا في مدي يتراوح بين اقل من شهر- 204 شهرا، حيث ان معظمهم يقع في الفئه اقل من شهر-12 شهر التي تمثل 53 (51%) وفئة 13-24 شهر 18 (17.3%) و25-36 شهر تمثل (8.8%) و37-48 شهر تمثل (7.7%) وأكثر من 48 شهر تمثل (15.4%). معظم الغارسين 79 (75.9%) تلقوا علاجات ثلاثيه مثبتة للمناعة و24 (23%) منهم تلقوا علاجات ثنائيه مثبتة للمناعة و1 (1%) فقط منهم تلقى علاج واحد مثبت للمناعة.

تم الكشف عن القلوبولين المناعي (IgM) باستخدام تقنية إلبسا في 27 (26%) من الغارسين؛ بينما 103 (99%) منهم لديه القلوبولين المناعي (IgG) في مصل الدم. الحمض النووي لفيروس مضخم الخلايا اكتشف في دم 104/40 (38.5%) من غارسي الكلي باستخدام تفاعل البلمرة التسلسلي الكمي في الوقت المناسب بعدد نسخ بين 62 نسخة/مليتر ($1.8 \log_{10}$) و 1.43×10^8 نسخة/مليتر ($9 \log_{10}$) وبمتوسط نسخ فيروس $10^4 \times 358$ ($6.5 \log_{10}$)، ووجد ان 104/17 (22.1%) منهم لديهم أعراض المرض بينما 104/23 (16.3%) لديهم أصابه بالمرض.

أظهرت الدراسة عدم وجود فرق ذو دلالة إحصائية ($P.value > 0.05$) بين وجود الحمض النووي لفيروس مضخم الخلايا لدي غارسي الكلي والجنس وأنواع العلاجات المثبطة للمناعة ووجد فرق ذو دلالة إحصائية ($P.value = 0.05$) بين نسخ الفيروس العالية وأنواع العلاجات المثبطة للمناعة. في نفس الوقت لوحظ ان هناك فرق ذو دلالة إحصائية ($P.value = 0.00$) في العلاقة بين نسخ الفيروس العالية (أكثر من 1000 نسخة/مليتر) مع تطور مرض فيروس مضخم الخلايا والذي فيه 14/17 (82.4%) مريض لديه أعراض مرض فيروس مضخم الخلايا ونسخ عالية للفيروس. وان 23/22 (95.7%) من المرضي الذين ليس لديهم أعراض المرض ولديهم نسخ متدنية من الفيروس (اقل من 1000 نسخة/مليتر). الأعراض الأكثر شيوعا بيترضري فيروس مضخم الخلايا هي ألحمي، الحمي ونقصان كريات الدم البيضاء والأمراض المعوية. اظهر التوزيع الجيني لجينات البروتين السكري (gB) غارسي الكلي في السودان ان ألجين (gB3) ويمثل (80%) هو الأكثر شيوعا في السودان بينما (gB4) يمثل (20%) وليس هناك أنواع مختلطة.

ونتيجة لذلك نجد ان تفاعل البلمره التسلسلي الكمي في الوقت المناسبين عينات البلازما مفرط الحساسية للكشف عن تكاثر الفيروس مضخم الخلايا, كما انه أكثر حساسية من تقنية اليبسا في اكتشاف فيروس مضخم الخلايا. الاختلاف الكبير ظهر في نسبه وجود نسخ فيروس مضخم الخلايا مع وجود وعدم وجود الأعراض عند المرضى؛ نسب الفيروس المتدنية وجدت في المرضى الذين ليس لديهم أعراض. أعتبر جين البروتين السكري (gB) الأكثر شيوعا بين غارسي الكلي في السودان المصابين بمرض فيروس مضخم الخلايا.

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LIST OF ABBREVIATIONS

PCR: Polymearase Chain Reaction.

RT- PCR : Real time PCR.

CMV: Cytomegalovirus.

gp : glycoprotein

gB: Glycoprotein B.

gH: Glycoprotein H.

gN: Glycoprotein N.

gO : Glycoprotein O.

SOT : solid organ transplant.

ESRD : End-stage renal disease.

HLA: Human leucocyte antigen.

Cs A: Cyclosporine A.

CD: Cluster of differentiation.

MMF: Mycophenolate mofetil.

TLR: Toll like receptor.

FKBP: FK-binding protein.

TAC : Tacrolimus.

NK: Natural Killer cell.

PTLD: Post-transplant lymphoproliferative disorder.

C5b: Complement component 5b

ATP: Adenosine Tri –Phosphate

DAMPs : Damage-activated molecular patterns.

TCR : T-cell receptor.

RTRs: Transplantation recipients.

HHV: Human herpesvirus.

BKV : BK polyomavirus.

EBV: Epstein-Barr virus.

JCV: JC polyomavirus .

HSV : Herpes Simplex Viruses.

HCMV: Human Cytomegalovirus.

UL : Unique long region

US : Unique short region.

TRL : Terminal repeat long.

IRL: Internal repeat long.

IRS : Internal repeat short

MCP :Major capsid protein.

SCP: Smallest capsid protein.

TRS :Terminal repeat short.

gC: Glycoprotein complex.

ERGIC : Endoplasmic reticulum -Golgi intermediate compartment.

AD : Antigen domain.

gM : glycoprotein M.

gL : glycoprotein L.

IE : Immediate-early.

MIE: Major Immediate-early.

HIV: Human Immunodeficiency Virus.

AIDS: Acquired Immunodeficiency Syndrome.

MHC: Major histocompatibility complex.

TH: T helper cell.

D/R: Donor/Recipient.

TNF- α : Tumor necrosis factor alpha.

ALA : Anti-lymphocyte antibodies.

BAL : Broncho-Alveolar Lavage.

EDTA: Ethylene diamine tetra acetic acid.

VZV: Varicella Zoster.

TCID₅₀ : The tissue culture infective dose 50

CPE: Cytopathic effect.

IFA : Immune-fluorescence assay.

ELISA: Enzyme-linked immunosorbent assay

PBLs: Peripheral blood leucocytes.

qRT-PCR: Quantitative Real Time PCR.

CFT : Complement fixation test.

RIA : Radioimmunoassay.

RFLP : Restriction fragment length polymorphism.

TWBCs: Total white blood cells.

CHAPTER ONE

INTRODUCTION

1.1. Introduction

Chronic kidney disease is usually progressive and may lead to renal failure. Most of the signs and symptoms of renal failure can be relieved by dialysis but renal transplantation is the only real cure for patients with end-stage renal failure. It may restore complete health and function (Rhoades and Bell, 2009).

Human cytomegalovirus (HCMV) is one of the eight herpesviruses that are pathogenic for humans (Olyae *et al.*, 2005). It is a beta-herpesvirus with a large dsDNA genome (~ 235 000 bp) (Renzette *et al.*, 2014). It has most complex viral genomes, composed of double stranded linear DNA. The virus has a capsid surrounded by a tegument and a glycoprotein envelope. The virus is highly species specific and shows tropism for specific cell types (Dar, 2007).

HCMV is an opportunistic pathogen is ubiquitously distributed in human population (Zhang *et al.*, 2010) and the primary infection is usually asymptomatic (Polz-Dacewicz *et al.*, 2013) and usually causes a latent infection (Zhang *et al.*, 2010).

The seroprevalence of HCMV ranges from 40 to 100% worldwide, with lower rates in Europe, parts of North America, and Australia, and higher rates in Africa and Asia (Kim and Kim, 2011). The viral intrauterine transmission to fetus can lead to stillbirth, abortion, and mental retardation (Zhang *et al.*, 2010).

CMV is a common pathogen which complicates treatment of immunosuppressed patients (transplantation, HIV patients) (Kotton *et al.*, 2013) and chemotherapy recipients (Zhang *et al.*, 2010).

CMV is a major infectious complication of renal transplantation and CMV disease in renal transplant recipients. It has a significant impact on morbidity and mortality and graft survival (Olyae *et al.*, 2005). In the absence of any form of prophylaxis treatment, HCMV infection develops in 50% to 90% of organ transplant recipients, resulting in symptomatic disease in 7% to 33% of them (Madi *et al.*, 2011b). Three major patterns that observed in solid organ

transplantation recipients are: primary infection develops when a CMV seronegative individual receives cells latently infected with the virus from a seropositive donor, secondary infection or reactivation infection develops when endogenous latent virus is reactivated in CMV-seropositive individual post transplantation. Superinfection or reinfection occurs when a seropositive recipient receives latently infected cells from a seropositive donor and the virus that reactivates post transplantation is of donor origin (Cukuranovic *et al.*, 2012).

Many infected patients develop symptomatic CMV disease, manifested by pneumonia, hepatitis, gastrointestinal ulcers, a non-specific febrile illness associated with leucopenia and thrombocytopenia, or less commonly retinitis. Patients with CMV pneumonia or disseminated infection often die (Olyae *et al.*, 2005). In addition to direct consequences, CMV acts as an immunomodulator increasing the risk for other opportunistic infections (Kotton *et al.*, 2013) and potentially making CMV infection an important risk factor for the development of acute and chronic allograft rejection (Coquette *et al.*, 2004), resulting in significant morbidity, graft loss, and adverse outcomes (Kotton *et al.*, 2013).

Quantitative measurement of CMV-DNA levels including PCR testing of plasma or whole blood the best method for diagnosis of CMV infection in solid organ transplant (Kim and Kim, 2011). The gold standard for diagnosis of CMV is the quantitative nucleic acid testing (QNAT). QNAT-CMV has been performed preferentially by real time PCR, using plasma or total blood (Requião-Moura *et al.*, 2015) which is widely considered as an efficient and highly sensitive technique for the evaluation of HCMV DNA kinetics (Habbal *et al.*, 2009).

Whole blood assays often have higher viral loads than plasma assays (Kim and Kim, 2011), but plasma viral load monitoring is of modest clinical utility for prediction of CMV disease (Garrigue *et al.*, 2008). The presence of CMV DNA in plasma suggests active viral replication due to spread of the virus from the leukocyte into the plasma (Rangbar-Kermani *et al.*, 2011) The highest viral

loads are associated with tissue-invasive disease, while the lowest are observed with asymptomatic CMV infection (Kim and Kim, 2011).

The HCMV genome encodes numerous glycoproteins, of which gB, gH and gN are the most abundant. Genotyping of HCMV is mostly based on sequence variation in surface glycoprotein genes, which often show genetic polymorphism. g B gene, the gH gene and the gN gene have all been utilized for genotyping HCMV, with the gene for the gB glycoprotein having been used most frequently. There are four major gB genotypes (gB1 to gB4). In addition to its important role in viral entry into host cells, cell-to-cell transmission and membrane fusion of infected cells, the gB glycoprotein is also a major target for neutralizing antibodies (Dar, 2007).

Two strategies are commonly used for CMV prevention: universal prophylaxis and preemptive therapy. Universal prophylaxis involves giving antiviral therapy to all “at risk” patients beginning at or immediately after transplant for a defined time period. In preemptive therapy, patients are monitored at regular intervals for early evidence of CMV replication prior to the onset of clinical symptoms by use of a laboratory assay (Cukuranovic *et al.*, 2012).

Today, ganciclovir (GCV) and valganciclovir (VGCV) are the treatment of choice of HCMV in case of infection in solid organ transplant (SOT) recipients, which lead to a decline in the CMV disease and associated morbidity in SOT recipients (Madi *et al.*, 2011a).

1.2. Rationale

Renal failure is a common disease in Sudan; the estimated incidence is 70 -140/ million inhabitation/year in the world. Improved graft survival has made renal transplantation the ultimate choice for the patients with end stage renal disease (ESRD). The prevalence of treated ESRD reported as 106 patients/million population with renal transplantation of 28.4% of them (Banaga *et al.*, 2015).

CMV infection is one of most frequent infectious complications after renal transplantation and a significant risk factor for the development of graft failure and death after renal transplantation (Requião-Moura *et al.*, 2015). In the absence of prophylactic treatment, about 50% renal transplant with active CMV infection will progress a potentially fatal end-organ disease. Early diagnosis and detection of CMV infection and disease is a curial factor that lead to more effective methods of prevention and treatment of the disease (Madi *et al.*, 2011a).

Despite advances in this field in the world, still there is a difficulty in the diagnosis of this disease among transplants in Sudan. Numerous studies have focused on the distribution of CMV gB polymorphism and it is relation to clinical findings of the disease in transplant recipients internationally. However, few studied were performed on CMV in renal transplant recipients in Sudan. No published data were found about CMV genotyping neither in renal transplant recipients nor in other population.

This study helps in detecting CMV infection and disease in Sudanese renal transplant recipients and helps renal centers to avoid a lethal outcome of CMV and to start preemptive therapy at the earliest stage is of extreme significance to avoid loss of human resources by graft loss and death.

1.3. Objectives

1.3.1. General objective

To perform serological detection and molecular characterization of cytomegalovirus and its glycoprotein gB (UL55) among Sudanese renal transplant recipients.

1.3.2. Specific objectives

1. To detect CMV viremia in plasma of renal transplant recipients and; to estimate the viral loads using quantitative real –time PCR assay.
2. To detect CMV IgM and IgG antibodies in serum of renal transplant recipients using ELISA technique.
3. To correlate between CMV viremia, plasma viral loads and sex, post transplantation time, immunosuppressive therapy, and symptoms of CMV disease.
4. To determine the frequency rate of different gB (UL 55) genotypes using CMV DNA sequencing.

CHAPTER TWO

LITERATURE REVIEW

2. Literature review

2.1. Renal transplantation

The urinary system consists of two kidneys, two ureters, the urinary bladder, and the urethra. The formation of urine is the function of the kidneys, and the rest of the system is responsible for eliminating the urine. Body cells produce waste products such as urea, creatinine, and ammonia, which must be removed from the blood before they accumulate to toxic levels and to maintain the normal composition, volume, and pH of both blood and tissue fluid. Kidneys form urine to excrete these waste products (Scanlon and Sanders, 2007).

Chronic kidney disease is usually progressive and may lead to renal failure. Most of the signs and symptoms of renal failure can be relieved by dialysis but renal transplantation is the only real cure for patients with end-stage renal disease (ESRD). It may restore complete health and function (Rhoades and Bell, 2009).

Kidney transplantation is the treatment of choice for the majority of patients with end-stage kidney disease (Sharif, 2016). It offers improved survival and quality-of-life benefits compared with dialysis (Ishibashi and Suzutani, 2012).

Since the first successful kidney transplant in 1954, the scientific advances over the subsequent decades have led to significant improvements in patient/graft survival and quality of life for kidney allograft recipients (Sharif, 2016). Donor organs are obtained from cadavers and living relative donors (*e.g.*, parent, sibling). The success of transplantation depends primarily on the degree of histocompatibility, adequate organ preservation, and immunologic management (Ramanathan *et al.*, 2001).

2.2. Host–graft adaptation

The term “host–graft adaptation” describes the decrease in both donor-specific responsiveness and the risk of rejection in the months after a successful transplantation that is maintained by immunosuppression. Changes in the organ, a loss of donor dendritic cells and a resolution of injury contribute to the adaptation. Regulatory T cells may also be able to control alloimmune responses, by analogy with their ability to suppress autoimmunity (Halloran, 2004).

2.3. Immunosuppressive treatment after renal transplantation

Immunosuppressive agents are used to control the immune response after transplantation of an HLA-mismatched graft. If no immunosuppression is used, the graft will be rejected (Afzali *et al.*, 2010).

Chronic allograft injury has replaced acute rejection as the major cause of graft loss in renal transplantation. The more potent immunosuppressive therapy that has successfully reduced the incidence of acute rejection has also resulted in a higher incidence of viral infection (Smith *et al.*, 2010). Triple immunosuppressive regimen, consisting of corticosteroids, azathioprine and cyclosporine A, became the standard immunosuppressive protocol for many transplant centers throughout the world (Sing-Leung, 2001).

2.3.1. Azathioprine (Imuran)

Azathioprine is a purine analogue. It inhibits purine metabolism, leading to DNA inhibition and finally cell proliferation is thereby inhibited. It causes impairing of a variety of lymphocyte functions (Piedras *et al.*, 2013) by blocking CD28 costimulatory signaling (Piedras *et al.*, 2013; Hartono *et al.*, 2013).

2.3.2. Corticosteroids

Steroids are a cornerstone of immunosuppressive therapy in kidney transplantation. More than 95% of transplant recipients are treated with steroids as a usual component of clinical immunosuppressive regimens. Prednisone, prednisolone, and other glucocorticoids are used alone and in combination with other immunosuppressive agents for treatment of transplant rejection and autoimmune disorders. Most immunosuppressive regimens are currently based on the combination of calcineurin inhibitors (cyclosporine A, tacrolimus) with antiproliferative agents (azathioprine, mycophenolate mofetil) and steroids (prednisone) (Piedras *et al.*, 2013).

2.3.3. Cyclosporine A

Cyclosporine (CsA) is the cornerstone of immunosuppression for many years. CsA is a calcineurin inhibitor. Calcineurin is a protein phosphate that is critical for T-cell activation. The effect is exerted through binding to cyclophilins (Watson and Dark, 2012); it inhibits the transcription of pro-inflammatory cytokine genes (e.g. Interleukin-2, interferon- γ) in T cells and thus prevents the proliferation of T cells. High cyclosporine

levels in blood have been associated with an increased risk of developing HCMV infection (Al-Alousy *et al.*, 2011).

2.3.4. Tacrolimus

Tacrolimus (TAC) is a macrolide antibiotic. Like cyclosporine A, tacrolimus binds to an immunophilin, FK-binding protein (FKBP) in the cytoplasm. The resultant tacrolimus-FKBP complex then interacts with calcineurin and inhibits its action in the same manner as cyclosporine A.

2.3.5. Polyclonal anti-lymphocyte globulins and OKT3

Anti-lymphocyte globulins (ALG) and thymoglobulin are polyclonal anti-lymphocyte globulins. The effect of is mediated mainly through interacting with a variety of surface markers (e.g. CD45, CD3, CD4) on the lymphocytes. OKT3 is a murine monoclonal antibody against the CD3 complex of molecules on the surface of T lymphocytes. Polyclonal anti-lymphocyte globulins and OKT3 are mainly used for the treatment of severe acute rejection. As both polyclonal anti-lymphocyte antibodies and OKT3 significantly impairs cell-mediated immunity, patients receiving these antibodies are predisposed to opportunistic infections especially CMV infections and malignancies (Sing-Leung, 2001).

2.3.6. Mycophenolate mofetil (Cellcept)

Mycophenolate mofetil has been developed as a replacement for azathioprine for maintenance immunosuppression. It acts by inhibiting inosine monophosphate dehydrogenase, a key enzyme in the *de novo* purine synthesis pathway, thereby limiting the proliferation of B and T lymphocytes. It reduces the incidence of acute rejection in the first year after transplantation and the need for intensive immuno-suppression to treat rejection. Mycophenolate mofetil may also be useful in treating acute cellular rejection and reversing refractory acute rejection in renal transplant patients and may help to prevent chronic allograft rejection. The major side effect of MMF is increased risk of tissue invasive cytomegalovirus infection (Sing-Leung, 2001).

2.3.7. Rapamycin

The mammalian target of rapamycin (mTOR) inhibitors, everolimus and sirolimus has been used for the prophylaxis of rejection in kidney transplant patients. Rapamycin binds to the same immunophilin as tacrolimus, namely FKBP, to become active. It acts by

inhibiting a key enzyme known as target of rapamycin (TOR), this results in the inhibition of proliferation of activated lymphocytes (Kahan, 2000), NK, and B cells (Johansson, 2014). Rapamycin is reducing acute rejection in renal transplant patients more than azathioprine (Kahan, 2000). Rapamycin may prevent the development of graft atherosclerosis, a hallmark of chronic rejection (Halloran, 2004).

2.3.8. Other immunosuppressive drugs

Other immunosuppressive therapy includes; daclizumab, basiliximab and alemtuzumab (Issa and Braun, 2013). Also aelatacept and alefacept (amevive) (Sing-Leung, 2001), eculizumab (soliris) (Kumar and Gaston, 2011). FK778, Malononitrilamide, FTY 720, CP-690, 550 and Tyrphostin AG 490 (Halloran, 2004).

2.4. Acute and chronic rejection in renal transplant recipients

Acute rejection seems to be a risk factor for chronic rejection in renal transplants (Caltenco-Serrano *et al.*, 2001), which is the major cause of graft loss in renal transplantation (Smith *et al.*, 2010). Chronic rejection is seemed to be a result of the production of cytokines and growth factors by different cell types, leading to a proliferative remodeling of graft vessels along with structural changes in the parenchyma, and gradual deterioration of graft function (Yilmaz *et al.*, 1996).

Late Acute Rejection is often severe and difficult to reverse, with a high risk of subsequent graft loss (Nankivell and Alexander, 2010).

Chronic renal allograft rejection was defined as the gradual deterioration in graft function in the absence of any other disease. It occurs during a span of months to years due to unresponsive to current treatment. It thought to be the end result of uncontrolled repetitive acute rejection episodes or a slowly progressive inflammatory process, its onset may be as early as the first few weeks after transplantation or any time thereafter (Shaikewitz and Chan, 1994). Characterized by interstitial fibrosis, glomerulosclerosis, tubular atrophy and arterial narrowing, is the major reason for the loss of renal allografts after the first post-transplant year (Yilmaz *et al.*, 1996). It's the most important cause of long-term graft failure and it is a common reason for retransplantation (Ishibashi and Suzutani, 2012).

From a tissue compatibility point of view between the donor and the recipient, that is, through the class I and II MHC antigens, there is a very important determining factor, as

for example the presence of receptor antibodies, specific against the MHC class I donor antigens. In these cases, the grafts are rapidly and irreversibly rejected (Caltenco-Serrano *et al.*, 2001).

2.4.1. Rejection mechanism

The donor kidney is comprised resident immune cells such as dendritic cells that are easily activated to engulf fragments of damaged tissue and pathogens. Resident dendritic cells become activated is through low blood flow to the kidney and to process of removing the kidney, from either a live or a deceased donor (McKay and Steinberg, 2010).

Activation of donor innate immune cells associated with transplantation begins with ischemia/anoxia induced death of donor kidney cells. Dead and dying cells contain immunologically active molecules called damage-activated molecular patterns (DAMPs), like heat-shock proteins, ATP, uric acid, RNA, DNA, as well as proteins. Donor kidney cells contain receptors for DAMPs including toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), they are thought to trigger the immune events that cause acute rejection. This newly identified innate immune system plays an essential role in the earliest events associated with rejection (McKay and Steinberg, 2010).

The recipient's innate immune cells (such as neutrophils, natural killer cells, and macrophages) vigorously infiltrate the donor tissue and add to the ischemia-induced tissue injury. The encounter between donor dendritic cells and recipient T cells is the key initiating event of cellular rejection. The two cell types engage each other using cell surface receptors – the human leukocyte antigen (HLA) molecule on the dendritic cell and T-cell receptor (TCR) of the T cell. The T-cell receptor (TCR) and several associated molecules called the CD3 chains. HLA molecules are highly polymorphic and important in transplantation, it allowing presentation of a diverse number of “foreign” peptides. The foreign peptides are derived from the allograft. Foreign proteins can be presented by HLA molecules to either CD4 T cells or CD8 T cells. If the foreign protein is adjoined to HLA class II molecules it will be presented to CD4 T cells. On the other hand, if adjoined to HLA class I molecules, it will be presented to CD8 T cells. Both CD4 and CD8 T cells participate in the rejection process (McKay and Steinberg, 2010).

T helper cells secrete different cytokines, in the case of graft rejection, they function to recruit the army of recipient immune cells aimed at destruction of the foreign allograft. When activated, cytotoxic T cells directly destroy target cells by releasing cytotoxins such as perforin and granzyme. Perforins form pores in the target cell membranes and granzymes enter the target cell and destroy it. Cytotoxic T cells play a role in graft rejection as well as destruction of virally infected tissue (McKay and Steinberg, 2010).

2.5. Viral infections after renal transplantation

Viral infections are a major problem in allograft recipients, most commonly 1 to 6 months after transplantation. Clinical disease can take place later, especially after intensification of immunosuppression or physiologic insults that increase the net state of immunosuppression (Ortiz and André, 2011). The more potent immunosuppressive therapy, the higher incidence of viral infection (Smith *et al.*, 2010).

Opportunistic viral infections make an important threat to renal transplantation recipients (RTRs), and with the use of more intense newly-developed immunosuppressive drugs, the risk of renal allograft loss due to reactivation of these viruses has increased considerably (Al-Obaidi *et al.*, 2015). Infection rates increased in adult kidney transplant recipients of >50 years from 48% to 69% during the first year post-transplantation (Egli *et al.*, 2007).

Some of viral infection result of community exposures (influenza, adenovirus), whereas some are commonly transmitted with the allograft (CMV, Epstein-Barr virus (EBV)) (Kotton and Fishman, 2005), also Human BK polyomavirus (BKV) (Adrian *et al.*, 2007), JC polyomavirus (JCV), and Simian virus (Cukuranovic *et al.*, 2012). Others are the result of more distant exposures reactivated in the setting of immune suppression (chicken pox and varicella zoster (VZV) as shingles) (Kotton and Fishman, 2005), as well as Human herpesvirus 1, Human herpesvirus 6, Human herpesvirus 7, and Human herpesvirus 8 (Cukuranovic *et al.*, 2012). Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection rates increased recently (Ortiz and André, 2011).

CMV infection produces a profound suppression of a variety of host defenses, predisposing to secondary invasion by such pathogens as *Pneumocystis carinii* (jiroveci), *Candida*, and *Aspergillus* (Ortiz and André, 2011). Multiple simultaneous infections,

viral and non-viral, are also common, such as CMV and human herpes virus 6 or CMV and *Pneumocystis* (Kotton and Fishman, 2005).

Reactivation of these viruses especially CMV and BKV in these chronically immunosuppressed renal transplant recipients can lead to renal impairment and subsequently allograft loss, unless it is treated (Al-Obaidi *et al.*, 2015).

Viral infections in donor are considered as the risk of infection to the organ recipient, and contraindicated to organ donation such as herpes simplex encephalitis, West Nile virus infection, rabies, HIV, and active hepatitis A, B and C (Hariharan, 2007).

CMV infection is one of most frequent infectious complications after renal transplantation (Requião-Moura *et al.*, 2015). It is a significant risk factor for the development of graft failure and death after renal transplantation. (Yilmaz *et al.*, 1996). CMV disease, but not asymptomatic infection, is an independent risk factor for biopsy-proven acute rejection, particularly in the first 12 months following renal transplantation (Costa, 2011).

2.6. Cytomegalovirus (CMV)

2.6.1. History of Cytomegalovirus

Cytomegalic inclusion disease (CID) was known as an infectious disease recognizable by the owl's eye cytopathology in salivary gland, liver, lung, kidney, pancreas, and thyroid autopsy materials from infants by the early 1930s (Knipe and Howley, 2007).

CMV was initially called “salivary gland virus” or “salivary gland inclusion disease virus”. In 1960, Weller *et al.* proposed the use of the term *Cytomegalovirus*. Klemola and Kaarianinen firstly described CMV mononucleosis, the principal presentation of previously healthy individuals, in 1965. CMV was first isolated in a renal transplant recipient in 1965 (Brennan, 2001).

HCMV infects man, but there are other Cytomegaloviruses that are specific for other animal species (e.g. murine CMV) (Greenwood *et al.*, 2012). The name means ‘large cell virus’ and derives from the swollen cells containing large intranuclear inclusions that characterize these infections (Collier and Oxford, 2006).

2.6.2. General characteristics

HCMV is a ubiquitous virus infection with worldwide distribution and associated with opportunistic disease that has been recognized in more highly developed areas of the

world. CMV acute disease occurs in only a small proportion of infected individuals, and it is restricted to settings where the ability to mount a cellular immune response is compromised, such as transplacental transmission during pregnancy leading to fetal damage and reactivation or primary infection of immunocompromised individuals (Murray *et al.*, 2007).

2.6.3. Physical prosperities

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) (Brennan ., 2001), cycles of freezing and thawing (Murray *et al.*, 2007), 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. It can be stored at 190°C (liquid nitrogen) indefinitely (Brennan, 2001).

2.6.4. Cytomegalovirus virology

CMV is a member of the genus Herpesvirus and belongs to the family Herpesviridae. There are 8 known human herpes viruses. The HHV are divided further into three subfamilies: the α -herpesvirinae, the β -herpesvirinae, and the γ -herpesvirinae. The α -herpesvirinae includes Human Herpes Simplex Viruses (HSV) 1 and 2 and Varicella Zoster virus. The β -herpesvirinae includes Cytomegalovirus (CMV), Human Herpes virus (HHV-6), and Human Herpes virus HHV-7. The γ -herpesvirinae includes Epstein-Barr virus (EBV) and Human Herpes virus HHV-8 (Brooks *et al.*, 2007).

2.6.5. Morphology

Morphologically Herpes viruses are indistinguishable from one another. The complete virion is 150 to 200 nm in diameter and icosahedral in shape and consists of an inner core, a capsid, and an envelope. The inner core (genome) of the CMV virus is a 64-nm linear double-stranded DNA molecule. The capsid is 110 nm in diameter and consists of 162 protein capsomers. The envelope contains lipoproteins and at least 33 structural proteins, some of which are glycosylated (glycoproteins). The glycoproteins determine the strain of CMV (Brennan, 2001).

The central DNA-containing core is surrounded by a capsid composed of 162 capsomeres. , The capsid is in turn surrounded the tegument, which is itself surrounded by a loosely-applied envelope (Zuckerman *et al.*, 2009).

2.6.6. Cytomegalovirus structure

2.6.6.1. Nucleic acid

CMV is a DNA virus containing 230-kb double-strand DNA (Ishibashi and Suzutani., 2012). It has the largest genetic content of the human herpesviruses (Brooks *et al.*, 2007), in that long and short unique sequences are bounded by terminally repetitive segments.

The viral genome is divided into two unique components, unique long (UL) and unique short (US) regions (Heli., 2004) (Figure 2.1). A pair of inverted repeats (terminal/internal repeat long TRL/IRL and internal/terminal repeat short IRS/TRS) flanks the unique regions (Sijmons *et al.*, 2014).

Each long and short sequence can be orientated in one of two directions, so that four DNA isomers are produced by cells in culture (Zuckerman *et al.*, 2009). Many proteins encoded by the virus (over 200) have been characterized (Brooks *et al.*, 2007). By international agreement, the proteins they encode are designated by p (for protein), gp (glycoprotein) or pp (phosphoprotein), followed by the gene number. This formal terminology may then be followed by a trivial name, for example gpUL75 (gH) is glycoprotein H, the product of gene number 75 in the unique long region.

Productively -infected cells produce linear genomes from concatameric precursors. Cleavage is accomplished by an endonuclease (terminase) coincident with packaging some areas of the genome are homologous with regions of human chromosomal DNA, which has practical importance for the selection of CMV DNA probes. The DNA can be digested with restriction endonucleases so that, following gel electrophoresis, oligonucleotide patterns characteristic of distinct CMV strains are produced (Zuckerman *et al.*, 2009).

2.6.6.2. Virion structure

CMV virion structure consisting of viral DNA, capsid, tegument and envelope (Ishibashi and Suzutani, 2012). The virion is 200 to 300 nm diameter. Virions contain a 125 nm icosahedral nucleocapsid composed of five herpesvirus core proteins: major capsid protein (MCP, the UL86 gene product), the minor capsid protein (TRI1, the UL46 gene product) together with the minor capsid protein binding protein (TRI2, the UL86 gene product). The smallest capsid protein (SCP, the UL48A gene product) that decorates MCP tips; and a portal protein (PORT, the UL104 gene product) that constitutes one

specialized penton used for encapsidation of viral DNA. The nucleocapsid encloses an S (oriLyt)-associated RNA. The nucleocapsid itself is embedded in a tegument (or matrix) (Knipe and Howley, 2007).

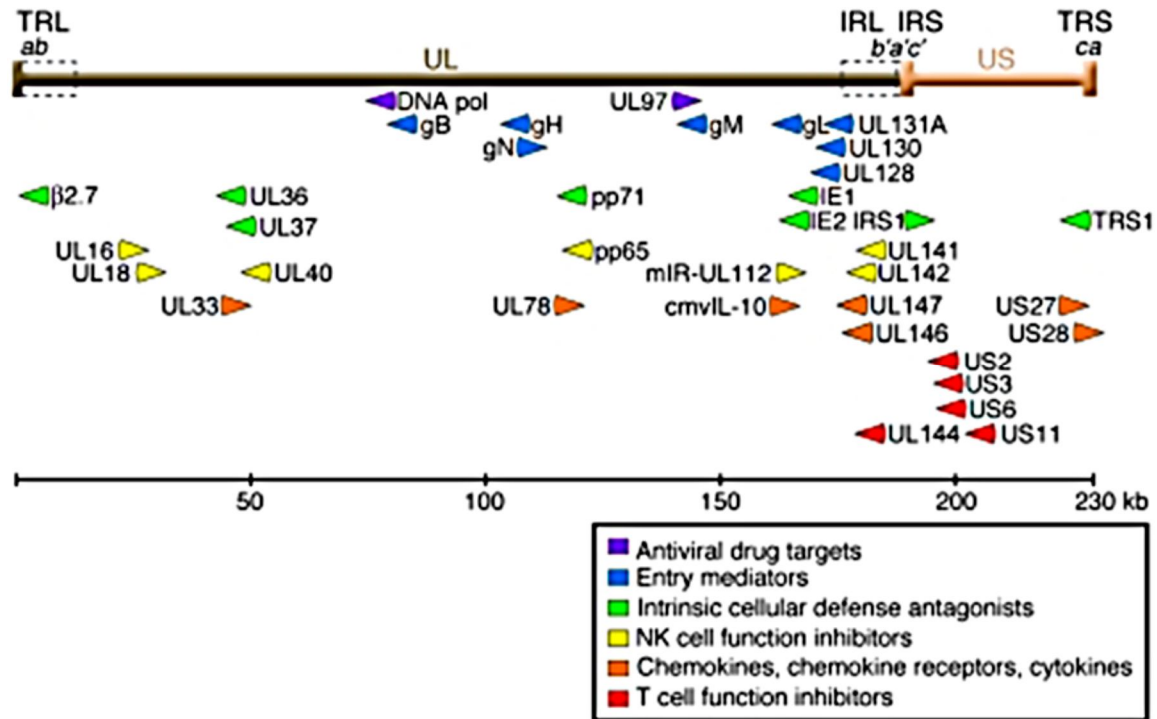


Figure (2.1): Genome structure of HCMV

2.6.6.3. Capsid structure

The CMV nucleocapsid exhibits icosahedral symmetry with 162 capsomeres and is approximately 130 nm in diameter. Capsid assembly intermediates as well as aberrant particles are formed during. Within infected cells, three distinct mature capsid forms are observed, termed A, B, and C capsids, but only DNA-containing HCMV nucleocapsids, termed C capsids, have completed maturation (Knipe and Howley, 2007).

2.6.6.4. Tegument

The nucleocapsid is embedded in a tegument (or matrix). The tegument appears is amorphous virion region located between the capsid and envelope. It contains most of the virion proteins (at least 27) as well as a selection of viral and cellular RNA and makes up about 40% of the total virion mass. Tegument proteins carry out a remarkably diverse

range of activities during infection and tend to be phosphorylated and highly immunogenic (Knipe and Howley, 2007).

The most abundant tegument proteins are the beta herpesvirus-conserved UL82 family members, including the most abundant HCMV tegument protein pp65 (lower matrix protein, UL83 gene product is the antigen that is detected in the antigenemia assay. Tegument is surrounded by a host cell endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC)-derived lipid bilayer envelope containing at least 20 virus-encoded glycoproteins. Tegument proteins play important roles during entry or maturation (Knipe and Howley, 2007).

2.6.6.5. Envelope

HCMV encodes more than 50 proteins that are potentially glycosylated or contain predicted transmembrane domains. The most functionally critical HCMV envelope glycoproteins are members of the herpesvirus core set which form three separate complexes that have been termed gC-I, gC-II, and gC-III on the cell surface and on the viral envelope, but are better known by common nomenclature glycoprotein (g)B, gH:gL, and gM:gN, respectively. All of these genes are required for CMV replication, including gM:gN, which is dispensable in many other herpesviruses (Knipe and Howley, 2007).

2.6.6.6. Glycoproteins of Cytomegalovirus

At least 57 potential glycoproteins are known to be encoded by the laboratory strain of CMV AD169 (Britt and Mach, 1996). These glycoproteins associate in high molecular weight complexes and the mature complexes are referred to as glycoprotein complex I (gC-I), glycoprotein complex II (gC-II) and glycoprotein complex III (gC-III). The genes encoding glycoproteins often show genetic polymorphism (Ishibashi and Suzutani, 2012).

2.6.6.6.1 Glycoprotein H (gH)

The disulphide-bond tripartite gC-III envelope complex consists of gH, gL and gO (Huber and Compton, 1999). The gH-gL dimer, which is essential for entry into all cell types and is postulated to be important for triggering gB fusion at the plasma membrane (Griffiths *et al.*, 2015). Glycoprotein H is one of the immunologically dominant glycoproteins in the CMV envelope, and is encoded by open reading frame (ORF) unique long (UL) region 75 (Britt and Mach, 1996). Although the UL75 is highly conserved among multiple CMV strains, sequence variations were found in the first 37 aa. Based on

the sequence analysis of UL75 from multiple strains, it was estimated that CMV gH has two genotypes (Chou, 1992). gH mediates viral/host cell membrane fusion in the initial step of infectivity. Anti-CMV gH antibodies show virus neutralizing activity and gH is considered a major antigen for the humoral immune response (Urban, *et al.*, 1996).

2.6.6.6.2. Glycoprotein B (gB)

Glycoprotein B, a component of the envelope complex gC-I, is the most abundant glycoprotein in the CMV envelope. gB is one of the most highly conserved components among all members of the herpesvirus family (Ishibashi and Suzutani, 2012). After budding of the envelope through the inner nuclear membrane, the particles of the virus go through the trans-Golgi network, where the virus particle becomes pathogenic through proteolytic cleavage of a consensus furin site to form glycoprotein B (gB). Furin was the first proprotein convertase to be identified. It is localized mainly in the trans-Golgi network (Brennan, 2001). gB is encoded by UL55 and exhibits genetic polymorphism. The 906 aa polypeptide of the AD169 strain gB is cleaved at position 460 by a cellular endoprotease. Nucleotide and peptide sequence analysis revealed that variations were most frequent between positions 448 and 480, which include the cleavage site (Chou and Dennison, 1991). Restriction enzyme analysis has identified four main gB groups (gB-1, gB-2, gB-3 and gB-4). While variations were found in gB, substantial conservation of the peptide sequence is observed in this region. The closely regulated variations in gB may suggest its important role in the viral life cycle (Chou and Dennison, 1991). Genetic variations of gB have been used for epidemiologic purpose. gB has a role in binding to cell surface receptors, and neutralizing gB-specific antibodies can inhibit the binding (Ohizumi *et al.*, 1992). The deletion of glycoprotein B (gB) renders HCMV incapable of entering cells unless a chemical fusogen (ie: polyethylene glycol) is added and show that gB is important for virus fusion (Griffiths *et al.*, 2015). gB also serves a role in the initial attachment to the cell via interactions with heparan sulphate glycosaminoglycans (Griffiths *et al.*, 2015).

The antigen domain 1 (AD1), which is located between positions 560 and 640 of gB, is a major neutralizing epitope (Schoppel, *et al.* 1996) and is the most highly conserved region among viral strains. The second antibody-binding site on gB is the antigen domain 2 (AD2), which is located between aa 28 and 84 of gB (Meyer *et al.*, 1992). Within the

AD2 domain, two antigenic sites have been identified. Site I is located between aa 68 and 77, and this region is conserved among CMV wild-type strains and is the target of neutralizing antibodies. Site II, another binding sequence in the AD2, is located between aa 50 and 54. Site II binds non-neutralizing antibodies and is strain-specific (Meyer *et al.*, 1992; Ishibashi and Suzutani, 2012).

2.6.6.6.3. Glycoprotein N (gN)

Glycoprotein N is a component of the envelope complex gC-II (Mach *et al.*, 2000; Dal Monte *et al.*, 2001). gN has been recognized as one of the major antigens together with gH and gB (Shimamura *et al.*, 2006). It is encoded by the ORF UL73, and antibodies against gN neutralize virus infectivity. UL73 has four main genomic variants, known as gN-1, gN-2, gN-3 and gN-4 (Pignatelli, *et al.*, 2001). The gN genomic variants are related to the immunopathogenesis of CMV in immunocompromised hosts and in congenitally infected infants (Pignatelli *et al.*, 2003a; Pignatelli *et al.*, 2003b).

2.6.6.6.4. Other glycoproteins

The large CMV genome encodes many additional glycoproteins other than gH, gB and gN, UL100 encodes glycoprotein M (gM), which, together with gN, is a component of gC-II. gM is essential for viral replication (Hobom *et al.* 2000), and seems to be highly conserved (Lehner *et al.*, 1991). It was shown that most sera failed to react with either gM or gN alone (Mach *et al.* 2000). Virus neutralizing antibodies were shown to be directed at the gN component of the gM–gN complex. In addition to gH, the gC-III envelope complex contains glycoprotein O (gO) and glycoprotein L (gL) (Huber and Compton 1999). gO is encoded by the UL74 ORF. The sequence analysis of UL74 showed a high degree of variability at the N-terminal end (Paterson *et al.*, 2002). The analysis of clinical isolates identified four major phylogenetic groups, known as gO-1, gO-2, gO-3 and gO-4 (Mattick *et al.*, 2004). gL is encoded by the UL115 ORF. Four major phylogenetic groups were identified and denoted gL-1, gL-2, gL-3 and gL-4 (Rasmussen *et al.*, 2002). gL is essential for the transport of the gH glycoprotein to the cell surface (Kaye *et al.*, 1992; Spaete *et al.*, 1993).

The large number of gH-gO-gL combinations suggests that gC-III has an immunological potential, and has implications for viral tropism and spread (Rasmussen *et al.*, 2002).

2.6.6.7. Noninfectious particles

When CMV is propagated in cell cultures, two additional morphological forms are produced from the virus-specific proteins and envelope. The first is a dense body and appears as a large amorphous structure without nucleocapsid or DNA. The second is a noninfectious enveloped particle and consists of an empty capsid surrounded by a lipid envelope (Zuckerman *et al.*, 2009). Whereas 1% of viral progeny are infectious, 99% or more is noninfectious because of three factors: instability of HCMV virion following release from cells, production of noninfectious enveloped particles and production of dense bodies, which can constitute more than 50% of a virion preparation. Purified virion preparations are predominantly noninfectious. Dense bodies are more heterogeneous in size than other particles; they lack an organized capsid or viral DNA, which composed entirely of tegument surrounded by an envelope, and are composed predominantly of the UL83-coded pp65 tegument protein (Knipe and Howley, 2007).

2.6.7. Virus replication

CMV replication is much slower than for HSV, and CPE may not be seen for 7 to 14 days. This may facilitate the establishment of latent infection in myeloid stem cells, monocytes, lymphocytes, the stromal cells of the bone marrow, or other cells. Fusion of the virion envelope and the cell membrane occurs, after the attachment of the virus to the cell surface. The cellular receptor (s) for HCMV is still unclear, but it has been suggested to be widely distributed in cells. The virus capsid is rapidly transported to the cell nucleus and, after the proteolysis of the capsid proteins; the viral DNA is released into the nucleus (Heli, 2004).

Viral gene expression in productive replication occurs in a temporally ordered cascade (Zuckerman *et al.*, 2009). The first proteins to be synthesized (α or immediate-early) are required for the transcription of the messenger ribonucleic acid (mRNA) for the second group of proteins (β or early). The early proteins allow DNA replication to proceed and this is followed by the appearance of the last proteins (γ or late) (Zuckerman *et al.*, 2009). Immediate-early (IE) gene expression is first activated followed by early and late gene expression which ultimately leads to virus assembly and release from the infected cells (Heli, 2004).

IE antigens appear in the nucleus of CMV-infected cells 1 to 3 hours after infection and remain present even in latent infection. IE antigen gene products direct production of both viral and cellular genes. Early antigens appear in the cytoplasm or membrane approximately 3 hours after infection. Early antigen gene products direct viral DNA synthesis. Late antigens appear in the nucleus and cytoplasm within 6 to 24 hours after infection late antigen gene products direct production of structural nucleocapsid proteins (Bernnan, 2001), and therefore are associated with active infection (Requião-Moura *et al.*, 2015).

The most abundantly expressed IE genes are transcribed from the major IE (MIE) locus, located in the UL region. Differential splicing of a primary transcript generates the gene products of UL122/123, IE1 and IE2. The IE proteins are transactivators of gene expression and play an important role in controlling both viral and cellular gene expression. One function of these proteins is to optimize the cellular environment for viral replication and viral gene expression. The early (E) proteins are involved in the replication of the viral DNA, whereas late (L) gene expression provides the structural proteins/glycoproteins of the viral capsid, tegument and envelope (Heli, 2004).

DNA replication, capsids formation and packaging of viral DNA occur in the nucleus. Subsequently, nucleocapsids acquire a primary envelopment by budding at the nuclear membrane. They further mature through a de-envelopment/re-envelopment process in the cytoplasm before leaving the cell via an exocytic-like pathway (Mocarski and Courcelle, 2001). Enveloped virions are found within vesicles in the cytoplasm and these appear to fuse with cellular membranes to allow egress of the mature virus particles. Dense bodies also mature and are released from the infected cell in the same way as virions, so that they contain virus-specific glycoproteins (Zuckerman *et al.*, 2009). The whole replication cycle of human CMV is slow, requiring approximately 48-72 hours. The cytopathic effect (CPE) in response to HCMV is characteristically cell enlargement with intranuclear inclusions (Heli, 2004).

2.6.8. Epidemiology

2.6.8.1. Transmission of CMV

HCMV has a worldwide distribution and infects humans of all ages, with no seasonal or epidemic patterns of transmission (Murray *et al.*, 2007). Initial infection with HCMV

commonly occurs during childhood (Harvey *et al.*, 2013) and often already during the first year or early in life, mainly during the first two decades (Heli, 2004).

CMV can be transmitted vertically and horizontally (Murray *et al.*, 2007). Most infections are acquired by direct close personal contact with individuals who are shedding virus. CMV is transmitted via bodily secretions (Kudesia and Wreghitt, 2009), including saliva, urine, breast milk, tears, stool, vaginal and cervical secretions, blood, and semen, it is obvious that transmission can occur in a variety of ways (Murray *et al.*, 2007). Thus, hygiene and virus shedding patterns remain important determinants of virus transmission patterns (Knipe and Howley, 2007). HCMV can cross the placenta and infect a fetus in utero. The virus is also present in breast milk, and neonates can be infected by this route. Child-to-child transmission has occurred especially in day care centers. Once infected, such children can transmit CMV to their parents (Murray *et al.*, 2007). By age 18 months, up to 80% of infants in a day care center are infected and actively excreting virus in saliva and urine (Ryan and Ray, 2010).

Developing areas of the world typically exhibit widespread transmission early in life, individuals may escape infection early in life and remain susceptible during the childbearing years (Knipe and Howley, 2007).

2.6.8.2. Prevalence of CMV

Prevalence of CMV immunity, in primary or secondary infection, varies significantly by geographic region, socioeconomic status, and ethnicity (The American College of Obstetricians and Gynecologists, 2015).

Antibodies of IgG class, representing past infection, are found in approximately 60% of adults in developed countries and 100% in developing countries (Griffiths *et al.*, 2015), with lower rates in Europe, parts of North America, and Australia, and higher rates in Africa and Asia (Kim and Kim, 2011). The seroprevalence of CMV increases with age in all populations and ranges from 40% to 100% (Murray *et al.*, 2007), while 35% to 90% of the population have antibody against the virus by adulthood (Harvey *et al.*, 2013). Primary CMV infection is acquired by 40–60% of persons by mid-adult life, and by more than 90% of those with multiple intimate exposures (Greenwood *et al.*, 2012).

The seroprevalence of CMV in general population ranging from 30 % to 97% according to (Paya, 2001; Preiksaitis *et al.*, 2005). Prevalence is higher among people of low

socioeconomic status, in whom CMV prevalence can be as high as 100% (Festary *et al.*, 2015).

In different countries the seroprevalence varies between 30% to 100%, in Scandinavian countries seroprevalence is as high as 60- 80 %, and in Finland even 70-80 % (Heli, 2004), CMV seroprevalence is 93.8% in Japan, 86.7% in Chile (Lagasse *et al.*, 2000), 82.5% in the United States (Fowler *et al.*, 2003), 49.5% in France (Lepage *et al.*, 2011).

The prevalence of CMV IgG antibodies in organ transplant recipients reflects their socio-economic grouping. Homosexual males with HIV-positive have a very high prevalence of CMV IgG antibodies (typically 95%) (Murray *et al.*, 2007).

2.6.8.3. Infectious period

The incubation period is about 3–6 weeks (Haaheim *et al.*, 2002). The infectious period in immunocompetent people, the virus is present for a few weeks in saliva, blood and some other body fluids after primary infection. In immunocompromised people, prolonged infectious period may be after primary infection (Kudesia and Wreghitt, 2009).

2.6.9. Pathogenesis and clinical syndromes

CMV can cause disease by a variety of different mechanisms, including direct tissue damage and immunologic damage (Ryan and Ray, 2010).

CMV is an excellent parasite and readily establishes persistent and latent infections rather than an extensive lytic infection. CMV is highly cell associated and is spread throughout the body within infected cells, especially lymphocytes and leukocytes (Murray *et al.*, 2013).

In any case, leukocyte-associated and endothelial cell-associated viremia appears. Viremia may last for several weeks during the primary infection. Hematogenous spreading typically results in the infection of ductal epithelial cells at the initial site. HCMV can infect a wide range of tissues including salivary glands, gastrointestinal tract, lung, liver, brain, kidney, spleen, pancreas, eye, heart, adrenals, thyroid and genital tract and then replicate in various cell types, including fibroblasts, epithelial cells, macrophages, vascular smooth muscle and endothelial cells (Heli, 2004). It can infect most renal cell types, including glomerular, tubular, and endothelial cells (Brennan, 2001).

CMV infects vascular endothelial cells and leukocytes and produces characteristic inclusions in the former. In vitro, CMV DNA can be demonstrated in monocytes showing no cytopathology, indicating a restricted growth potential in these cells. It is conjectured that these are the cells of latency for CMV (Ryan and Ray, 2010).

2.6.9.1. Primary CMV infection

Primary CMV infection is defined as the detection of CMV infection in an individual previously found to be CMV seronegative (Ljungman *et al.*, 2002).

In healthy individuals, primary HCMV infection is usually subclinical (no apparent symptoms) (Harvey *et al.*, 2013). In older children and adults is usually asymptomatic but occasionally causes a spontaneous infectious mononucleosis syndrome (Brook *et al.*, 2007), that identical to that caused by EBV (Harvey *et al.*, 2013). It is characterized by malaise, myalgia, protracted fever, liver function abnormalities, lymphocytosis (Brook *et al.*, 2007), and lymphadenopathy are characteristic infectious mononucleosis symptoms (Harvey *et al.*, 2013). CMV mononucleosis is a mild disease, and complications are rare. Subclinical hepatitis is common. In younger children (under 7 years old), hepatosplenomegaly is frequently observed (Brook *et al.*, 2007). About 8 % of infectious mononucleosis cases are caused by HCMV (Brook *et al.*, 2007; Harvey *et al.*, 2013), and HCMV is cause 20–50% of heterophil-negative (non-Epstein-Barr virus) mononucleosis cases. They thought that HCMV could infect the vascular wall and play a role in the development of atherosclerosis (Bruggeman, 2000).

2.6.9.2. Congenital infection

CMV infection is the most common congenital viral infection worldwide (de Vries *et al.*, 2011). Primary infection, reinfection and reactivation for the duration of pregnancy can all lead to in utero transmission to the developing fetus. CMV infection was relatively common among women of reproductive age, with seroprevalence ranging from 45 to 100% (Cannon *et al.*, 2010).

HCMV infection at any stage of pregnancy can give rise to congenital infection even in women who have no symptoms. The risk of transmission is greatest in the third trimester among pregnant women. Transmission rates for primary infection are 30% in the first trimester, 34–38% in the second trimester, and 40–72% in the third trimester (The American College of Obstetricians and Gynecologists, 2015). The major source of

HCMV for such women is young children, especially toddlers, whose saliva and urine contain high levels of HCMV. Forty percent of women with primary infection will transmit infection to their babies (Kudesia and Wreghitt, 2009).

Cytomegalic inclusion disease ranging from varying degrees of damage to liver, spleen (hepatosplenomegaly), blood-forming organs, and components of the nervous system to fetal death. Damage to the nervous system is a common cause of hearing loss and mental retardation (Harvey *et al.*, 2013). Other Symptom includes chorioretinitis, petechial rash, (Kudesia and Wreghitt, 2009), Jaundice, thrombocytopenic purpura, myocarditis, and pneumonitis (Murray *et al.*, 2007). Approximately 30% of severely infected infants die, and 65–80% of survivors have severe neurologic morbidity (The American College of Obstetricians and Gynecologists, 2015).

2.6.9.3. Perinatal infection

This is mainly acquired through three routes, from infected maternal genital tract secretions, from breast-feeding (Murray *et al.*, 2013) or through transfusions of CMV seropositive blood (Ross *et al.*, 2011). About 10% of women shed CMV in the genital tract at or near the time of delivery, and virus is transmitted to approximately 50% of the newborns. Such infants begin to excrete of the virus at 3 to 4 weeks of age, but usually remain asymptomatic (Murray *et al.*, 2007), or a mild course. Nevertheless, sporadic cases of pneumonia or multiorgan infections are also described (Figlerowicz *et al.*, 2011). Perinatal infection causes no clinically evident disease in healthy full-term infants (Murray *et al.*, 2013).

2.6.9.4. Postnatal infection

This can be acquired in many ways. Saliva containing CMV is spread among young children, and, at older ages, by kissing (Greenwood *et al.*, 2012). Semen can contain high titres of virus, and may be a source of sexual transmission or artificial insemination-associated infection. Whole blood transfusion used to be (and donated organs remain) an important source of CMV. The infections are usually subclinical, but infectious mononucleosis may occur. The disease is characterized by malaise, myalgia, protracted fever and liver function abnormalities (Haaheim *et al.*, 2002), sore throat, swollen glands, abdomen pain and jaundice can occur (SA Health, 2012). Atypical, peripheral

lymphocytes may resemble those of EBV mononucleosis. Lymphadenopathy is usually not prominent, and heterophile antibodies are not present (Haaheim *et al.*, 2002).

2.6.9.5. CMV latency

After primary infection, CMV can establish latency. The virus may persist at specific sites in the host without any detectable viral infection (Sinclair and Sissons, 2006), in which the genome of the virus is present in cells, but infectious virus is not recovered. During latent infection of cells, viral DNA is maintained as an episome (not integrated), with limited expression of specific virus genes required for the maintenance of latency (Ryan and Ray, 2010).

Latently infected blood leukocytes are an important reservoir for transmission of the virus in organ transplantation and blood transfusion mainly peripheral blood mononuclear cells (Heli, 2004). HCMV genome establishes a latent infection in the nucleus of these infected CD34+ haematopoietic cell population in the bone marrow (Sinclair and Reeves, 2013), and CD14+ monocytes. Viral DNA has also been detected in the common precursors of dendritic and myeloid cells (CD33+/CD14+ and CD33+/ CD15+, along with the dendritic cell markers CD1a and CD10) (Heli, 2004). It can also persist in kidneys for years (Levinson, 2010).

The major immediate early promoter (MIEP) is extremely suppressed in these cells and that this is achieved through cellular transcriptional repressors directing histone-modifying enzymes to impart repressive post-translational modifications of MIEP-associated histones (Sinclair and Reeves, 2013). CMV can be reactivated when cell-mediated immunity is decreased (Brennan, 2001).

2.6.9.6. Transmission via blood transfusion

CMV can be transmitted by blood, only 1–5% of blood units taken from seropositive donors lead to infection of seronegative recipients (Zuckerman *et al.*, 2009), but if occur it often results in an asymptomatic infection (Murray *et al.*, 2013). Transmission via blood products has been virtually eliminated by the routine use of filters to remove leukocytes during transfusion (Zuckerman *et al.*, 2009).

2.6.9.7. CMV and malignancy

The detection of CMV-DNA, mRNA, or antigens in tumor tissues in some studies suggests a possible role of CMV infection in the pathogenesis of several human

malignancies. HCMV nucleic acids and proteins have been discovered in a high percentage of low- and high-grade malignant gliomas. CMV is found within the breast epithelial cells, which suggests that it may play a role in the neoplastic process. US28 is a costimulatory chemokine receptor encoded by CMV. Transgenic coexpression of the US28 ligand, CCL2, which is an inflammatory chemokine, increases intestinal endothelial cell proliferation and the development of intestinal neoplasia (Ardalan, 2012)

2.6.9.8. CMV infections in immunosuppressed and immunodeficient patients

The group of patients potentially affected by CMV infection includes patient with solid organ transplantation, hematopoietic stem cell transplant recipients and patient with HIV/AIDS. The latter two groups can exhibit the most severe CMV disease due to severely impaired cellular immunity (Ross *et al.*, 2011). Another group is cancer patients (particularly those with leukemia and lymphoma receiving chemotherapy) (Murray *et al.*, 2007).

2.6.9.8.1. CMV Infection and AIDS patients

CMV remains among the main causes of secondary infections to AIDS because it seems that acts as an inducer or co-factor in the progression of HIV infection pathogenesis (Silva *et al.*, 2015). Most infections are caused by a reactivation of latent CMV infection (Kudesia and Wreghitt, 2009).

Before the use of highly active antiretroviral therapy (HAART), approximately 20% to 40% of adults with AIDS developed CMV disease. The risk of CMV disease among persons with HIV infection, is linked closely with immune impairment as reflected by low CD4 T-cell count (Knipe and Howley, 2007) declined below 100/ μ L. In 85% of HIV with coinfection with CMV, the end-organ disease was retinitis (Griffiths *et al.*, 2015). CMV disease is associated with colitis, encephalitis, falling white blood cell counts (Kudesia and Wreghitt, 2009), fever, polyradiculomyelopathy, gastritis, ulcerative colitis (Murray *et al.*, 2007), oesophagitis (Greenwood *et al.*, 2012), viremia and high CMV virus load (Zuckerman *et al.*, 2009).

Transactivation of the promoter in the HIV-1 is the proposed mechanisms through which CMV could enhance replication or reactivation from latently infected cells (Knipe and Howley, 2007). CMV can also down regulate HIV replication, but CMV is more likely to stimulate HIV. HIV has an integrated provirus and when CMV is not actively replicating

(Zuckerman *et al.*, 2009), when CMV early genes transactivate other viral and cellular genes leading to the production of HIV from latently infected cells (Greenwood *et al.*, 2012).

2.6.9.8.2. CMV infection in solid organ transplantation

I. Risk factors

The specific risk factors for CMV infection after transplant include, CMV donor-recipient mismatching and the use of lymphocyte-depleting preparations induction for rejection therapy, episodes of allograft rejection, comorbid illnesses, potentially coinfection with HHV-6 and -7 (Hartmann *et al.*, 2006; Karuthu, and Blumberg, 2012), neutropenia (Hartmann *et al.*, 2006) and older donors (>60 years) (Karuthu, and Blumberg., 2012).

In SOT recipients, the risk of CMV disease is the result of the balance between the degree of viral replication and the recipient's level of cellular competence and humoral immune response (Aguado *et al.*, 2012).

The effect on CMV replication is especially intense when using high doses of methyl prednisolone or agents (such as antilymphocyte globulin (ALG) and antithymocyte globulin (ATG)), antilymphocyte antibodies (such as OKT3), mycophenolate mofetil and azathioprine (Aguado *et al.*, 2012). Mycophenolate mofetil (MMF) has also been variably reported to be associated with an increased incidence of CMV viremia and CMV disease (late CMV) as reported by (Pereyra and Rubin, 2004).

II. Impact of CMV infection in solid organ transplant

CMV infection in transplant recipients is a significant cause of direct (caused by the virus) and indirect (caused by virus interactions with the immune system) (Greenwood *et al.*, 2012).

The frequency and severity of CMV infection in organ transplant recipients will vary, depending on; the type of transplant, the source of the donated organ, the immune status of the recipient, and the duration of the immunosuppressive therapy. The major symptoms in these patients usually include fever, malaise, lethargy, myalgia or arthralgia, leukopenia, thrombocytopenia, and hepatitis. Specific organ damage may lead to pneumonitis in recipients of lung or heart-lung transplants; the development of myocarditis, retinitis, or accelerated vascular damage and atherosclerosis after cardiac

transplantation; hepatitis and pancreatitis in liver and pancreas transplant recipients, respectively; and gastrointestinal disease. These lead to an increased risk of graft rejection in solid-organ transplants. CMV is also responsible for the failure of many kidney transplants. This may be the result of virus replication in the graft after reactivation in the transplanted kidney or infection from the host (Murray *et al.*, 2013). Death may occur as a result of various complications, including bacterial and fungal superinfections (Murray *et al.*, 2007).

2.6.10. Immunity to CMV

The protective immunity against the virus to primary CMV includes is both humoral (IgM, IgG) and cellular (T lymphocyte) responses (Greenwood *et al.*, 2012). The responses in people with normal immunity keep CMV suppressed into a latent state in most individuals for most of the time (Zuckerman *et al.*, 2009). Natural maternal immunity present prior to conception provides 69% protection against delivering a subsequent neonate with congenital CMV infection (Zuckerman *et al.*, 2009).

2.6.10.1. Humoral immunity

Antibodies to CMV occur in most human sera. CMV-specific antibodies of the IgM, IgA, and IgG classes have all been detected (Brook *et al.*, 2007).

Antibodies of IgG class are produced at the time of primary infection and persist for life. IgM-class antibodies are produced on primary but not recurrent infection of immunocompetent individuals and persist for three to four months (Zuckerman *et al.*, 2009). The range of outstanding antibodies are those against surface glycoproteins (glycoproteins gB and gH) that almost certainly participate in the blockage of cell infection (Caltenco-Serrano *et al.*, 2001).

Many HCMV proteins are immunogenic and nearly all immune human sera have antibody to envelope glycoproteins gB (UL55), gH (UL75), and the tegument phosphoprotein pp150 (UL32) as well as to a nonstructural DNA binding phosphoprotein, pp52 (UL44). Neutralizing antibody has been detected against principal envelope glycoproteins gB, gM:gN, and gH:gL, and may be associated with protection from infection and disease. Neutralizing antibody may play important roles in controlling transmission (Knipe and Howley, 2007).

Immunocompromised patients may fail to produce IgM antibodies with primary infection and one third of them have IgM detectable with recurrent infections. With intrauterine infection, IgM antibodies are produced by the fetus, together with an IgG-class response (Zuckerman *et al.*, 2009).

2.6.10.2. Cell mediated immunity

Cell-mediated immunity is essential for resolving and controlling the outgrowth of CMV infection (Murray *et al.*, 2013) especially disseminated infection in the immunocompromised host (Greenwood *et al.*, 2012). CMV infection alters the function of lymphocytes and leukocytes. Because CMV infects mononuclear cells, there is a degree of immunosuppression associated with the acute infection (Greenwood *et al.*, 2012). The virus prevents antigen presentation to both CD8 cytotoxic T cells and CD4 T cells by preventing the expression of MHC I molecules on the cell surface and by interfering with cytokine-induced expression of MHC II molecules on antigen-presenting cells (including the infected cells) (Murray *et al.*, 2013).

Antigens induce the response of the CD8+ memory lymphocytes during the primary infection in about 90% of immunocompetent individuals (Caltenco-Serrano *et al.*, 2001). A viral protein also blocks natural-killer-cell attack of CMV-infected cells. CMV also encodes an interleukin-10 analogue that would inhibit TH1 protective immuneresponses (Murray *et al.*, 2013).

2.6.11. CMV Infection in renal transplant recipients

CMV infection is the most common opportunistic infection in kidney transplant recipients (Karuthu, and Blumberg, 2012).CMV activation and CMV disease in the early period after renal transplantation are independent risk factors for chronic allograft nephropathy and kidney allograft loss in the late post transplant period. CMV disease is an additional factor in the pathogenesis of chronic allograft nephropathy in the presence of acute rejection (Bernarde *et al.*, 2007).

CMV infection can be classified as primo-infection, when the transmission occurs through the graft, or reactivation, when the recipient is CMV seropositive (Requião-Moura *et al.*, 2015).

After transplantation, CMV can appear as an infection, when the patient presents with evidence of viral replication without symptoms or disease, which has two clinical spectra,

typical viral syndrome or invasive disease, which is a less common form. Their effects can be classified as direct, while the disease is developed, or indirect, with an increase of acute rejection and chronic allograft dysfunction risks (Requião-Moura *et al.*, 2015).

A symptomatic infection with CMV is associated with renal allograft dysfunction, mortality, and graft loss, in spite of early detection and treatment of asymptomatic infections. Poor outcomes are associated with poor HLA matching and may reflect increased immunogenicity, inflammatory response, or failure to treat CMV (Bohl *et al.*, 2007). Finding of McLaughlin and Wu (2002), proposed that the D+/R- group have an increased risk of early allograft loss due to acute rejection, which have poorer allograft function 3 years post-transplant

2.6.11.1. Direct effects of CMV infection after kidney transplantation

The effects of CMV are classified as direct and indirect. This classification serves to separate the effects of invasive viral infection (cellular and tissue injury) from effects mediated by inflammatory responses (*e.g.*, cytokines) or by alterations in host immune and inflammatory responses (Kotton and Fishman, 2005).

The direct clinical effects of CMV include CMV infection, CMV disease and end-organ diseases (Ljungman *et al.*, 2002). Symptoms of CMV disease are largely nonspecific, such as fever, fatigue, body aches, and myelosuppression. CMV disease in some patients is manifested as tissue-invasive disease. The gastrointestinal tract is the most common site for tissue-invasive CMV disease, independent of the type of allograft transplant (Corte *et al.*, 2010), which can cause abdominal pain and diarrhea. In severe cases, CMV ulceration of the gastrointestinal tract can lead to hemorrhage and perforation. Other organs that may manifest tissue invasive disease include the liver, lungs, heart, pancreas, and kidneys, and may present with allograft dysfunction easily misdiagnosed as acute or chronic rejection (Couzi *et al.*, 2010).

2.6.11.1.1. CMV infection

CMV infection is defined as isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen. It is recommended that both the source of the specimens tested (*e.g.*, plasma, serum, whole blood, peripheral blood leukocytes, CSF, urine, or tissue) (Ljungman *et al.*, 2002) and there is evidence of CMV replication regardless of symptoms (Kotton *et al.*, 2010; Kim and Kim, 2011).

2.6.11.1.2. CMV recurrent infection, reinfection and reactivation

The latent virus has been detected in most tissues in the body. It can infect most renal cell types, including glomerular, tubular, and endothelial cells (Brennan, 2001).

Recurrent infection is defined as new detection of CMV infection in a patient who has had previously documented infection and who has not had virus detected for an interval of at least 4 weeks during active surveillance. Recurrent infection may result from reactivation of latent virus (endogenous) or reinfection (exogenous) (Ljungman *et al.*, 2002).

Reinfection is defined as detection of CMV strain that is distinct from the strain that was the cause of the patient's original infection. Reinfection may be documented by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic. Reinfection with exogenous virus may introduced by the transplanted organ. It diagnosed if the tow strains are distinct (Ljungman *et al.*, 2002). Symptoms have a tendency to be most severe after primary infection; however, reactivation infection or reinfection in a severely immunocompromised host may also cause serious illness (Murray *et al.*, 2007).

Sporadic reactivation events may occur, but they are generally controlled by cell mediated immunity, cytotoxic T-cells, and NK cells (Kim and Kim, 2011). Reactivation is common, and virus is shed in body secretions. Mononuclear cells carry the latent virus genome and viral RNA transcripts of early genes have been detected in such cells. Bone marrow progenitor cells of the myeloid line once their descendants have been activated to differentiate into tissue macrophages, the virus can enter the replication cycle (Greenwood *et al.*, 2012).

Reactivation of CMV is usually associated with less severe disease, but can be fatal in severely immunocompromised patients (Kudesia and Wreghitt, 2009), especially those with altered cellular immunity, have frequent reactivations of herpesviruses that can lead to clinically severe disease (Ryan and Ray, 2010).

The virus is reactivated by immunosuppression (e.g., corticosteroids, infection with HIV) and possibly by allogeneic stimulation (i.e., the host response to transfused or transplanted cells) (Murray *et al.*, 2013).

In renal transplant recipients, viral reactivation, and the primary risk factors identified are the use of anti-lymphocyte antibodies (ALA), type of immunosuppression protocol used (type of drug, dose and duration), and treatment of acute rejection. A few factors related to the recipient, such as age, co-morbidities, and the development of neutropenia. Reactivation is related to reduction of cellular immune activity, especially of CD8⁺ cells, as result of the immunosuppressed state, also due to activity of cytokines that induce the virus to move from the state of latency, especially tumor necrosis factor alpha (TNF- α) and interleukin-1 β (IL-1 β). The use of ALA, besides causing intense and prolonged lymphopenia, is related to the release of cytokines, especially TNF- α . Acute rejection, in addition to requiring increase of immunosuppression, causes an increased expression of IL-1 β , which is a cytokine that stimulates viral replication (Requião-Moura *et al.*, 2015).

2.6.11.1.3. CMV disease and CMV syndrome

The CMV disease is defined as detection of CMV in a clinical specimen, accompanied by either CMV syndrome with fever (Chakravarti *et al.*, 2009), the temperature $>38^{\circ}\text{C}$ for at least 2 days within a 4-day period (Ljungman *et al.*, 2002). Muscle pain (Chakravarti *et al.*, 2009), leucopenia (white blood cell count (WBC) $< 3 \times 10^3/\text{cmm}$) (Liang *et al.*, 2013) and /or thrombocytopenia. Moreover, organs involvement, such as hepatitis, nephritis, myocarditis, retinitis, cystitis, or pancreatitis (Chakravarti *et al.*, 2009) as well as gastrointestinal invasion with colitis, gastritis, ulcers, bleeding, or perforation (Ortiz and André, 2011). When a CMV syndrome is complicated by specific organ involvement, the term CMV disease is used (Dirk *et al.*, 1998).

2.6.11.1.4. CMV end-organ disease

Patients receiving immunosuppressive drugs to allow allo-transplantation are, therefore at risk of developing CMV end-organ disease (Griffiths, and Lumley, 2015). CMV causes direct damage in the form of viral syndrome or end-organ disease (Aguado *et al.*, 2012). In solid organ transplant recipients, CMV end-organ diseases can present as lung, liver, gastrointestinal, renal or retinal disease and is considered the most important infectious complication in those patients (Kalpoe, 2007). A high viral load is required for CMV end-organ disease to develop (Griffiths, and Lumley, 2015).

2.6.11.2. Indirect effects of CMV infection after kidney transplantation

CMV is associated with a variety of indirect effects due to the virus' ability to modulate the immune system (Couzi *et al.*, 2010). Indirect effects of viral infections include responses to viral infections such as release of cytokines, chemokines, or growth factors. These effectors are immunomodulatory, results in further immune suppression and increasing the risk of other opportunistic infections from other viruses, such as CMV and hepatitis C in a form of viral "cross-talk." (Kotton and Fishman, 2005). Secondary infections might develop through different mechanisms in which CMV could disrupt mucosal surfaces, predisposing the patient to superinfection, or it could cause alterations in humoral and cell-mediated immunity (Ljungman *et al.*, 2002).

CMV infection may trigger HHV-6 and HHV-7 reactivation (Kotton and Fishman., 2005; Cukuranovic *et al.*, 2012) and Epstein-Barr virus-related post-transplant lymphoproliferative disease (Razonable *et al.*, 2003), As well as secondary bacterial infections (e.g., *Nocardia* spp.) (Peleg *et al.*, 2007) and fungal infection (e.g., *Aspergillus* spp) (Husni *et al.*, 1998). A higher rate of allograft failure and death in renal transplant recipients considerably associated with CMV infection, in part due to increased opportunistic infections as well as acute and chronic allograft rejection and reduced allograft function and survival after renal transplantation was associated with CMV persistence in the allograft (Sagedal *et al.*, 2007).

Co-infection of BKV and CMV has been reported in renal transplant recipients, CMV has been shown to induce polyomaviruses amplification and DNA replication in vitro (Al-Obaidi *et al.*, 2015).

In addition, CMV infection may alter expression of surface antigens (*e.g.*, histocompatibility antigens), provoking graft rejection, the patients with CMV infection are more likely to experience acute and chronic rejection (Cukuranovic *et al.*, 2012). In addition, new-onset diabetes mellitus has been reported in patients with CMV infection or disease after renal transplantation (Hartmann *et al.*, 2006; Rodrigo *et al.*, 2006).

2.6.11.3. Prevalence and incidence of CMV infection in renal transplant recipients

Incidence of CMV infection and disease during the first 100 days post-transplantation being 60% and 25%, respectively, when no CMV prophylaxis or preemptive therapy is

given (Chakravarti *et al.*, 2009). Lower incidence rates of CMV is estimated in the renal transplant population between 8 % and 32 % (Hartmann *et al.*, 2006).

Dar (2007), found that although 60-100 % of renal transplant recipients develop HCMV infection, only 20-30% has symptomatic infection.

In a study of Requião-Moura *et al.*, (2015) conducted on 477 renal transplant patients. The prevalence of acute rejection is 38% confirmed by biopsy, 64% of infection by CMV, and 24% of disease, the study observed that infection and the disease by CMV increased the risk of acute rejection by 1.6- and 2.5-fold, respectively.

Sixty to 90% of all renal transplant candidates have latent CMV infections, but symptomatic infection occurs only in 20-60% of them. CMV is a significant cause of increased morbidity and mortality in this population (Chakravarti *et al.*, 2009). CMV infection occurs in 35%–55% in renal transplant recipients (Sia and Paya, 1998). Sagedal and his collage (2002), were found that among 451 patients, 290 (64%) had CMV infection and among these 108 (24%) had CMV disease.

CMV infections have been reported to occur in 65–88% of recipients, in which 48–60% of them develop CMV disease (Hartmann *et al.*, 2006).

Finding of Cordero *et al.*, (2012), showed that incidence of CMV disease was 5.8% with fever as the most common presenting symptom and 55% developed CMV disease \leq 3 months post transplantation. Coinfection occurred in 53% and case fatality rate in 11%. Risk factors for developing CMV disease included CMV donor+/recipient- .

2.6.11.4. CMV serostatus in donors and recipients

The serological status should be conducted by means of identifying IgG class antibodies (Requião-Moura *et al.*, 2015).

Primary infection is defined as a new-onset infection in seronegative recipients (D+/R-), in whom the viral infection is transmitted by the transplanted organ. This led to the highest risk without prophylaxis (Requião-Moura *et al.*, 2015). The latent virus can be easily transmitted from a transplant donor to recipient by either the leukocytes, or possibly even tissue cells, of the kidney. Transplant patients' cell mediated immunity is impaired and cannot control the virus, resulting in reactivation without immunity to CMV (D+/R-), as well as in CMV-seropositive (R+). Other recipients undergo reactivation of their own latent virus (Kim and Kim, 2011).

In D+/R+ cases, approximately 20% of recipients experienced CMV disease in the absence of any prophylaxis (Sagedal *et al.*, 2000). The incidence of CMV infection is 50-75% in D+/R- groups in the study of Chakravarti *et al.*, (2009).

The D+/R+ not the D+/R- groups had the worst graft and patient survival by 3 years. This may reflect the prevalence multiple CMV virotypes, and that D+/R+ recipients may have double exposure to different CMV strains (Kim and Kim, 2011).

In a study of Emery *et al.*, (2012), showed that CMV syndrome/disease in SOT occurred in 20.5% of the D+/R- patients, whereas the incidence was only 8.1% and 9% in the D+/R+ and D-/R+ group, respectively ($p < 0.001$ compared to the D+/R- group). CMV viremia in the D+/R- group was associated with a high probability (65%) of CMV syndrome/disease in renal transplant recipients.

The incidence of CMV disease in D-/R- is <5% in Karuthu, and Blumberg, (2012) studies.

In study of Requião-Moura *et al.*, (2015) conducted on 20.000 transplanted patients found the distribution of serological matchings to IgG status, D+/R+=47.7%, D-/R+=24.1%, D+/R-=18.2%, and D-/R-= 10.3%. When D+/R- are compared with D-/R-, there is a 28% increase in risk of graft loss, 36% in the risk of death due to all causes, and eight-fold the risk of dying by a viral infection.

2.6.12. Laboratory diagnosis of CMV

There are two potential strategies for providing a diagnosis of CMV: the detection of virus or the demonstration of a specific immune response (Zuckerman *et al.*, 2009).

Variety of methods is available for use in the diagnosis and management of patients infected with CMV include isolation of the virus in cell culture (Murray *et al.*, 2007), shell vial culture, pp65 antigenemia test, and qualitative and quantitative nucleic acid (Khoury and Brennan, 2005), or viral proteins detection assays (Murray *et al.*, 2007).

2.6.12.1. Specimens collection for direct detection

Tissue specimens, respiratory secretions, urine sediment, cerebrospinal fluid (CSF), amniotic fluid, and peripheral blood leukocytes have been used for the direct detection of CMV antigens or nucleic acids (Murray *et al.*, 2007).

For PCR, from blood acid-citrate-dextrose or EDTA (Zuckerman *et al.*, 2009), plasma obtained from anticoagulated whole blood, serum obtained from clotted blood, or

purified peripheral blood leukocytes have all been used to quantitate CMV DNA in molecular amplification assays (Mengelle *et al.*, 2003). Delays in preparation of whole blood samples, purified leukocytes, or plasma samples after blood collection, can result in lysis of leukocytes, which may result in inaccurate quantitation of CMV DNA (Sanche and Storch, 2002). Plasma or serum may be preferable in neutropenic patients who may have inadequate numbers of leukocytes for testing (Murray *et al.*, 2007).

Immunocompromised patients should be investigated by means of surveillance samples, taken preferably twice weekly, of blood and possibly urine or saliva must be done as routine on all patients, rather than waiting for symptoms to develop. CMV excretion from urine and saliva is very common in allograft recipients so the relative risk for future disease is typically (Zuckerman *et al.*, 2009).

2.6.12.2. Specimens for serologic testing

Serum specimens are useful in screening of CMV IgG for evidence of past infection and for identifying individuals at risk for CMV infection. Detection of IgM in a single serum specimen may be beneficial. In patients with CMV neurologic disease, CSF may be tested for viral antibody if paired with a serum specimen collected on or close to the same date (Murray *et al.*, 2007).

2.6.12.3. Direct examination

Several specific definitions for CMV detection in blood are recommended. Viremia is defined as the isolation of CMV by culture that involves the use of either standard or shell vial techniques. Antigenemia is defined as the detection of lower matrix phosphoprotein pp65 in leukocytes and nucleic acid testing including CMV-PCR and hybrid-capture DNA (Weikert and Blumberg, 2008). DNAemia is defining as the detection of DNA in samples of plasma, whole blood, and isolated peripheral blood leukocytes or in buffy-coat specimens. RNAemia is defined as the detection of RNA (e.g., by nucleic acid sequence-based amplification or noncommercial reverse transcriptase-PCR) in samples of plasma, whole blood, or isolated peripheral blood leukocytes or in buffy-coat specimens (Ljungman *et al.*, 2002).

2.6.12.3.1. Histopathologic testing

The demonstration of CMV inclusions in tissues in the setting of a compatible clinical presentation is the “gold standard” for diagnosis (Ortiz and André, 2011). It can be

identified by the characteristic of cytomegalic cell, which is an enlarged cell (25 to 35 mm in diameter) containing a dense, central, “owl’s eye,” basophilic intranuclear inclusion body (Murray *et al.*, 2013). These intranuclear inclusions have a surrounding halo and marginated chromatin (Zuckerman *et al.*, 2009). These cytomegalic cells are present in the epithelial or endothelial cells of most viscera (Murray *et al.*, 2013). They can be found in kidney tubules, bile ducts, lung and liver parenchyma, gut, inner ear and salivary gland but are less prominent in brain tissue (Zuckerman *et al.*, 2009). The presence of characteristic cytologic changes suggests CMV infection and correlates with active disease in most cases. The inclusions are readily seen with Papanicolaou, hematoxylin-eosin staining (Murray *et al.*, 2013) or Wright-Giemsa (Murray *et al.*, 2007). The sensitivity of the histological examination has been enhanced by using immunostaining or *in situ* hybridization (ISH). However, these techniques have limited usefulness with transplant patients (Heli., 2004), because the secretion of CMV into lung fluids or urine is common in immunosuppressed individuals in the absence of invasive disease (Kotton and Fishman, 2005) , and they are mainly used for diagnosing HCMV in an organ involvement (Heli, 2004).

2.6.12.3.2. Electron microscopy

Samples of urine from infants infected congenitally or perinatally contain high titres (10^3 – 10^6 TCID₅₀/ml) of CMV. Using the pseudoreplica electron microscopy technique has been possible to demonstrate this viruria. The viral specificity of the technique has been reported at 100%. Electron microscopy cannot be used in immunocompromised patients because the titre of CMV found in clinical samples from adults is generally lower than that found in infants, also human herpesviruses frequently infect immunocompromised patients and cannot be distinguished from each other by electron microscopy (Zuckerman *et al.*, 2009).

2.6.12.3.3. Cell culture (conventional tube method)

Human fibroblasts best support the growth of CMV and serially passaged diploid human fetal lung strains such as WI-38, MRC-5, or IMR-90 (Murray *et al.*, 2007). CMV can be propagated only in human fibroblasts, in which it gives rise to foci of swollen multinucleate cells with characteristic intranuclear inclusion (Collier and Oxford, 2006). The virus stays cell-associated (Brooks *et al.*, 2007), such changes may take at least 4 to

6 weeks to appear, because the characteristic CPE develops very slowly in specimens with very low titers of the virus. The time to appearance of cytopathic effect in vitro is long. CMV replication in vivo is a highly dynamic the doubling time/half-life of CMV in blood is ~1 day (Emery *et al.*, 1999). Viral culture is a qualitative method with a limited role in the diagnosis of HCMV infection (Heli, 2004). Isolation of CMV is especially reliable in immunocompromised patients, who often have high titers of virus in their secretions (Murray *et al.*, 2013).

Tubes are examined for CPE daily for the first 5 days and then twice a week for at least 4 weeks for most specimens (6 weeks for leukocyte specimens). More commonly, foci of CPE, consisting of enlarged, rounded, refractile cells, appear during the first week. In cultures inoculated with urine or respiratory specimens from older individuals, CPE usually appears within 2 weeks. Leukocyte cultures may not become positive until after 3 to 6 week (Murray *et al.*, 2007).

Identification of CMV isolates is made by observing of characteristic CPE. Furthermore, the suspected CMV isolates are best confirmed by an immune-fluorescence assay (IFA) using monoclonal or polyclonal antibodies (Murray *et al.*, 2007).

2.6.12.3.4. Spin-amplification shell vial assay

The technique of detection of early antigen fluorescent foci was developed as a means of retaining the specificity and sensitivity of cell culture without having to wait for the production of CPE as a diagnostic end point (Zuckerman *et al.*, 2009). More rapid results are achieved by centrifuging a patient's sample at a low speed (Akhter and Bronze, 2015), inoculated onto confluency grown MRC-5 fibroblast cells on 12-mm round coverslips in 1-dram (3.7-ml) shell vials (Murray *et al.*, 2007). Following inoculation CMV is absorbed into the cell within minutes and rapidly starts to produce α - and β -proteins but CMV DNA synthesis is delayed and protracted until several days after infection. This explains the long delay seen in conventional cell cultures, since CMV needs to replicate, produce daughter virions and infect neighboring cells in order to produce the CPE. Infection can be diagnosed as early as 24 hours after inoculation by indirect immunofluorescent staining with monoclonal antibody (Collier and Oxford, 2006). After 24 and 48 hours, the tissue culture medium is removed and the cells are stained using a fluorescein-labeled anti-CMV antibody. The cells are read using a

fluorescent microscope (Akhter and Bronze, 2015) for the presence of one or more of the immediate early viral antigens (Murray *et al.*, 2013).

2.6.12.4. The antigenemia assay

The antigenemia assay is the detection of lower matrix phosphoprotein pp65 (which is encoded by UL83 gene) in leukocyte (peripheral blood mononuclear cells as targets) (Cukuranovic *et al.*, 2012). It is a semi-quantitative immunofluorescent assay based on detection of infected cells in the peripheral blood (Kim and Kim, 2011) and to estimate of the systemic viral load (Aguado *et al.*, 2012). This assay has far higher sensitivity and specificity than culture-based methods, and is comparable in sensitivity to CMV PCR (Kim and Kim, 2011). A pp65 is a protein located in the nucleocapsid of CMV and can be identified within infected leukocytes using fluorescein-labeled monoclonal antibody specific for pp65 (Levinson, 2010).

2.6.12.5. Molecular methods

2.6.12.5.1. Polymerase chain reaction (PCR assay)

The molecular assays by direct DNA polymerase chain reaction, hybrid capture, or amplification assays are highly specific and sensitive for the diagnosis of CMV disease associated with viremia and to monitor response to antiviral therapy (Ortiz and André, 2011). PCR successfully used in a variety of clinical specimens from organ transplant recipients, patients with AIDS, and infants with congenital infection. It has replaced virus isolation for routine detection of CMV infections, especially, in immunosuppressed patients. It designed to detect replicating virus, not latent viral genomes (Brooks *et al.*, 2007).

The sensitivity of the assay was increased by amplifying genomic regions from both the immediate-early and the late CMV genes or by using nested primers to a single gene fragment (Murray *et al.*, 2007).

2.6.12.5.2. Other nucleic acid-based techniques

Successfully been used in the detection of viral mRNA is nucleic acid sequence based amplification (NASBA), nucleic acid sequence based amplification have been developed, and the diagnostic value of monitoring the expression of CMV immediate-early and late pp67 mRNA has been evaluated for blood leukocytes and CSF specimens. Assays used to detect CMV mRNA are usually less sensitive than other methods, but they have a high

specificity for diagnosing CMV disease. Reverse transcriptase PCR (RT-PCR) assays have been used instead quantitative assay to demonstrate that qualitative amplification of specific CMV mRNA transcripts that are expressed only during active infection may make it possible to identify the patients at greatest risk for developing symptomatic infection (Murray *et al.*, 2007). In addition, signal amplification hybrid capture assay uses RNA probes to detect and quantify viral DNA in an ELISA-type format where the resulting signal is measured (Ross *et al.*, 2011).

2.6.12.5.3. Qualitative PCR assay for detection of HCMV in renal transplant recipients

Detection of the virus from the blood of immunocompromised patient is a sign of viremia and is always considered as a serious finding. Additionally, positive virus isolation from any site of a seronegative recipient is a clear indication of primary infection (Heli, 2004). CMV DNA has been successfully detected in whole blood, purified peripheral blood leukocytes, plasma, and serum by PCR (Murray *et al.*, 2007), but cannot differentiate active disease or latent infection (Ortiz and André, 2011) especially, when peripheral blood leukocytes, whole blood are used. This is due to the capability of these tests to detect HCMV DNA even in the case of latent infection and in monitoring the success of antiviral therapy in immunocompromise patients (Murray *et al.*, 2007). The most common assay for the detection of HCMV DNA is plasma specimen, because the results correlate more closely with disease than that in leukocytes or whole blood (Heli, 2004). The results provided by qualitative PCR is appears to be useful in detection of CMV in urine, tissue, amniotic fluid, or fetal blood for diagnosis of congenital CMV infection. In addition to the aqueous or vitreous humor in patients with CMV retinitis, and in the blood of patients at high risk of severe infection, such as CMV D+/R- transplant patients (Murray *et al.*, 2007).

Finding of Olyae *et al.*, (2005), showed that 12/37 renal transplant recipients had positive samples containing HCMV DNA in PBLs (32.4%), whereas, 5 of them showed symptomatic CMV disease (13.5%) and 7 of them did not show symptomatic CMV disease, 25 patients had negative PCR results, and all of them did not have symptomatic CMV disease.

Lashini and his collage (2011), reported that CMV DNA was detected in 33(25.9%) samples of 127 renal transplant patients using PCR. From 33 patients with positive PCR test, 20 patients had clinical symptoms and 13 (33.4%) of the patients had no clinical symptoms of disease.

The prevalence of CMV was detected in plasma and/or urine by PCR in renal transplant recipient in Serbia. CMV DNA was detected in 14/112 (12.5%), EBV DNA in 4/49 (8.16%), BKV DNA in 10/31 (32.26%) and JCV DNA in 3/31 (9.68%) renal transplant recipients. These results show that CMV infection is more often present in renal transplant recipients compared to other investigated viral infections (Ćupic *et al.*, 2012).

CMV viremia was detected by Khalafkhany *et al.*, (2016) in Iran, in 68 (15.9%) of renal transplant recipients in Iran. The mean post-transplantation time in recipients was 50 months, ranging from 1 to 354 months. Viremia was detected in 31.2%, 30.7%, 17.5%, 10.2%, and 6.4% of the recipients in 0-3, 4-6, 7-12, 13-24, and more than 24 months post-transplantation, respectively.

2.6.12.5.4. Quantitative Real Time PCR (qRT-PCR) for detection and quantitation of CMV

The gold standard for diagnosis of CMV is the quantitative nucleic acid testing (QNAT). QNAT-CMV has been performed preferentially by real time PCR (RT-PCR), using plasma or total blood (Requião-Moura *et al.*, 2015). Whole blood assays often have higher viral loads than plasma assays. The highest viral loads are associated with tissue-invasive disease, whilst the lowest are seen with asymptomatic CMV infection (Kim *et al.*, 2011; Kim and Kim, 2011).

Real-time PCR is widely considered as an efficient and highly sensitive technique for the evaluation of CMV DNA kinetics (Habbal *et al.*, 2009), and are currently the primary choice for the surveillance of active CMV infection in the SOT setting. Because of their extreme sensitivity, simplicity, accuracy, superior reproducibility, and dynamic linear measuring range, and short turnaround time (Aguado *et al.*, 2011).

The qualitative standard indicates CMV in activity, but has no direct relation with the presence of the disease, which requires quantification. For that reason, the viral load detected by PCR has high predictive power for the disease (Requião-Moura *et al.*, 2015), at the same time understanding pathogenesis of the disease (Zuckerman *et al.*, 2009).

Opposing to antigenemia, sensitivity is not altered by blood storage, and can be transported for its use in distant centers (Requião-Moura *et al.*, 2015).

Different type of quantitative PCR assays both in-house and commercial ones have been employed. Commonly used assays include PCR testing of plasma or whole blood, which is commercially available. CMV viral load testing using PCR techniques (including real-time PCR) or hybrid capture assays can detect and quantify CMV DNA or DNA-RNA hybrids in clinical specimens, including the CSF (Cobo, 2012).

I. Principle of quantitative Real Time PCR

In real-time PCR (kinetic PCR), the accumulation of the PCR products is monitored continuously during the PCR run, compared with the end-point measurements that quantitate the final PCR product. Chemistries for the detection of PCR products during real-time PCR can be classified into amplicon sequence specific or non-specific detection methods. The most commonly used detection methods in the virus diagnostic assays are based on the use of specific fluorogenic oligoprobes. These methods depend on fluorescence resonance energy transfer (FRET), which is the interaction of two fluorescent dyes. TaqMan probes, also called 5' nuclease or hydrolysis oligoprobes, were the first ones used in special real-time instruments (Heli, 2004).

For target amplification, oligonucleotide primers and probes for amplification and detection, respectively, of nucleic acid are selected from conserved nucleotide sequences within a viral gene; these products represent the first level of sensitivity and specificity for quantitative real-time PCR. The assay is subsequently adjusted to allow the polymerase enzyme to function optimally and to produce sensitive and specific signals from labeled probes that are proportional to the amount of target DNA present in the blood sample. Three to 5 commercial quantitative standards are included in the quantitative test. The software for the real-time PCR instrument generates a standard curve with use of these quantitative standards. This plot relates the cycle number in which the amplified nucleic acid target from the standards is detected (by measuring fluorescence) to the amount of target present in the standards. The quantitative level of viral nucleic acid in a test specimen is then determined by comparing the cycle number (crossover point) of the specimen with the standard curve generated with the known levels of the target nucleic acid (Smith *et al.*, 2007).

2.6.12.5.5. Quantitation of CMV from renal transplant recipients

In qRT-PCR assays both plasma as well as whole blood allows a good idea of the viral expansion processes due to *de novo* infection or reactivation that a transplant patient is experiencing (Reyes-Pérez *et al.*, 2016). Concentrated leukocytes may be more productive for detecting CMV DNA than cell free specimens. Reactivated CMV infection in the transplant recipient may result in predominately cell-free (e.g., plasma) virus (Smith *et al.*, 2007). There is a very close correlation between viral load present in whole blood and in plasma, at least in immunosuppressive patients (Ortiz and André, 2011).

According to the study of Kalpoe *et al* (2004), correlation between CMV DNA loads in plasma and whole blood showed correlation coefficient (*r*) of 0.962 indicated is a high correlation between CMV DNA loads in plasma and whole blood. In other prospective study, CMV DNA was quantified in blood samples of 255 kidney recipients with and without CMV-related symptoms in Kuwait. CMV DNA was detected in 54/255 (24%) patients; of these, 17 (31.5%) were asymptomatic, and 37 patients (68.5%) had symptomatic CMV infection (Madi *et al.*, 2007)

In study of Zhang *et al* (2012), which uses qRT-PCR, CMV-DNA was detected in plasma of 29/77 recipients, yielding a positive rate of detection of 37.7%. Twelve of 21 recipients (57.1%) who suffered acute rejection had positive CMV-DNA. Among the 56 recipients suffered from chronic or mild rejection, 17(30.4%) had positive CMV-DNA plasma. Moreover, of the 29 recipients who had detectable CMV-DNA after transplant, 12 (41.4%) suffered from acute rejection; of the 48 recipients with undetectable CMV-DNA, only nine (18.8%) developed acute rejection. Post-transplant patients with acute rejection had a higher rate (57.1% vs. 30.4%, $P = 0.03$) of post-transplant CMV infection than those with chronic or mild rejection.

2.6.12.5.6. Plasma viral load

Plasma viral load monitoring is of modest clinical utility for prediction of CMV disease (Kotton and Fishman, 2005; Garrigue *et al.*, 2008), because CMV is highly cell associated, samples of whole blood or leukocyte preparations provide for more sensitive detection of virus than assays that exclude leukocytes (Sanchez and Storch, 2002). However, the presence of CMV DNA in plasma suggests active viral replication due to spread of the virus from the leukocyte into the plasma (Rangbar-Kermani *et al.*, 2011).

On the other hand, virus release into plasma from multiple pools, including endothelial cells and the reticuloendothelial system (Garrigue *et al.*, 2008), and thus could be more associated with clinically significant disease (Sanchez and Storch, 2002). In addition, plasma viral load and its incremental rate could be used as suitable diagnostic tools in R+ recipients (William *et al.*, 2000).

Quantification of CMV by real-time PCR showed that 3×10^3 genome equivalents per milliliter of whole blood or 1×10^3 genome equivalents per milliliter of plasma correlated with the presence of CMV disease (Knipe and Howley, 2007).

Huurman *et al.*, (2006) adopted another opinion of view that viremia is only marker of active CMV infection. CMV viremia was defined as detection of two consecutive CMV DNA loads of more than $2.7 \log_{10}$ (= 500) copies/ml plasma.

In a study of Enan *et al* (2011) HCMV DNA was detected in 32/98 (32.7%) from plasma samples of Sudanese renal transplant recipients, with viral loads ranging from <200 to 42932 copies/ml.

Another study of Rangbar-Kermani *et al.*, (2011) aimed to investigate CMV-DNA plasma viral load in active CMV infected renal transplant patients in Iran using Real - Time CMV PCR technique. The average of CMV DNA viral load was 7.61×10^7 copies/ml (min. load: 1.38×10^2 copies/ml and max load: 1.9×10^9 copies/ml).

In a prospective study of Helanterä *et al.*, (2009), showed that CMV infection developed in 12/25 patients a mean of 107 days (range 26-330 days) after prophylaxis ended. Two were asymptomatic. In 10 patients symptoms include fever (N = 7), gastrointestinal (N = 5), upper respiratory tract (N = 3) and hepatopathy (N = 2). One patient with infection had prophylaxis terminated after 5 months (leukopenia). The mean viral load at diagnosis was 49 517 (range 490 -325 300)

In observational prospective cohort study of Silva Junior (2015), was conducted to assess CMV replication antigenemia and viral load in 200 renal transplant recipients receiving preemptive therapy. Antigenemia reference: zero Positive Cells /200.000 cells. Viral load using Real Time PCR - TaqMan Result: < 50 copies/mL Log: < 1.70. Detection Limit: 50 copies/mL. The viral loads above 100 copies /mL should be considered as active replication. The range of viral load from 50 to 109 copies/ml.

The prediction of HCMV plasma load from evaluation of CMV whole-blood load in samples from renal transplant recipients. According to the model, the plasma viral load was >500 copies/ml when the whole-blood load was >3,170 or >4,000 copies/ml with (95% confidence intervals, 73.5 and 80.5%) which is positive in patients with or without treatment, respectively (Garrigue *et al.*, 2008).

2.6.12.6. Tissue immunofluorescence assay (IFA)

In tissue immunofluorescence assay an immunostaining with specific polyclonal or Monoclonal antibodies may be used for the detection of HCMV (Heli, 2004) in biopsy material (e.g liver, lung) (Zuckerman *et al.*, 2009) and blood leucocytes or cell obtained by bronchoalveolar lavage (Haaheim *et al.*, 2002). These biopsy samples may contain cells infected with HCMV, which can be visible by staining frozen sections with antisera to HCMV. Alternatively, the tissue can be disrupted and the cells fixed to glass slides before staining. Tissues should be brought to the laboratory in transport medium or fixed in ethanol (Haaheim *et al.*, 2002).

2.6.12.7. *In situ* hybridization (ISH)

In situ hybridization (ISH) methods have been employed to improve the histological diagnosis of infection. HCMV specific probe, usually labeled by biotin, is used in these assays (Heli, 2004). The concentration of CMV infected cells per ten high-power fields (HPF) was assessed by counting the total number of cells showing definite CMV staining in each biopsy fragment or representative section dividing this number by the total number of HPFs present in the slide (Moreira *et al.*, 2010).

2.6.12.8. Serological diagnosis (serological tests)

Humoral response to primary HCMV infection is manifested by the production of IgG and IgM antibodies (Heli, 2004). Detection of viral IgM antibodies suggests a current infection (Requião-Moura *et al.*, 2015) and IgM antibodies can also persist for a long time after infection in some healthy individuals (Heli, 2004).

Serologic tests are useful before transplantation to predict risk for disease (Requião-Moura *et al.*, 2015). HCMV IgG antibody testing has a role in the evaluation of organ and recipient prior to transplantation.

Seroconversion may not occur until well after the resolution of symptoms and after renal transplantation occurs in the majority of seronegative recipients of seropositive donors

within 6 to 12 month. This may correlate with some degree of immunological protection against CMV (Kotton and Fishman, 2005). Specific IgM antibody may not be present especially during reactivation of virus (Ryan and Ray, 2010). Seroconversion is diagnostic but rarely occurs, especially in AIDS patients, because more than 95% of these patients are seropositive for CMV before infection with human immunodeficiency virus (HIV). Interpretation of the results may be confounded by the presence of passive antibody that may have been acquired from a blood or body-fluid contamination (KDIGO, 2009; Ortiz and André, 2011), and by antibody based therapy. In the other hand, increase or decrease in antibody levels, in general, does not provide an actual diagnosis of HCMV infection in the immunosuppressed patient population, due to frequent reactivations of the virus (Heli, 2004).

Detection of IgM antibodies may be helpful in cases of suspected CMV mononucleosis or in pregnant women (Zuckerman *et al.*, 2009).

2.6.12.8.1. IgG Antibody as a marker of past infection

The detection of IgG antibodies against CMV is hallmark of infection sometime in the past. The individual is said to be seropositive and is liable to experience reactivations of their latent infection. The presence of IgG antibodies against CMV is thus a marker of potential infectivity; although a seropositive individual is ‘immune’ in the immunological sense, this term doesn’t means protection from endogenous or exogenous infection (Zuckerman *et al.*, 2009).

2.6.12.8.2. Enzyme-linked Immunosorbent Assay (ELISA)

ELISA (enzyme-linked immunosorbent assay)-based methods can be easily automated; they are fast and convenient to perform. They usually have good sensitivity and they are also commercially available. Sensitive ELISA allows the detection of specific IgG and IgM antibodies (Haaheim *et al.*, 2002). The serologic diagnosis using ELISA techniques can distinguish primary from recurrent infection by demonstrating IgG seroconversion or the presence of HCMV-specific IgM (Harvey *et al.*, 2013). IgM antibodies peak early in the infection and are usually undetectable 12–16 weeks after the onset of subclinical infections. IgM persists for longer periods in symptomatic infections and especially in congenital infections. Low levels of IgM antibodies may be detected in recurrent CMV infections. Specific CMV serum IgG antibodies last for decades. A variable rise in the

IgG titre is seen in recurrent CMV infections. A pretreatment serological status is of great value in transplant patients and cancer patients receiving chemotherapy (Haaheim *et al.*, 2002).

However, the rise in serum antibody levels is an insensitive sign of actual HCMV infection in transplant patients. The seroprevalence is high and the presence of IgG antibodies is only informative of the patient's past history regarding HCMV infection. Furthermore, there is a time lag between primary infection and IgM antibody production (IgM level can remain undetectable because of delayed seroconversion owing to immunosuppressive agents). Therefore, serology has a limited diagnostic value in the transplant patient group ((Halling *et al.*, 2001).

In a study of Enan *et al.*, (2011), the sero-reactivity against HCMV in renal transplant patients in Khartoum state – Sudan using ELISA were 100% for IgG, while only 6% of them showed IgM antibodies against CMV. Another report in Sudan by Awad Alkareem *et al.*, (2013) found that the seropositivity of IgG were 98% and 95% in renal transplant and haemodialysis patients respectively, while 6% of renal transplant had IgM using ELISA technique.

Khairi *et al.*, (2013), conducted another study among pregnant women in Sudan, aimed to determine the seroprevalence of HCMV using ELISA technique. Out of the 200 pregnant women tested, 195 (97.5%) and 12 (6.0%) were CMV IgG and CMV IgM positive, respectively.

In study of Abd Alla *et al.*, (2015), the seroprevalence of IgM and IgG of CMV among hemodialysis patients in Gezira state, central Sudan using ELISA technique were 45.2% (42/93) and 95.7% (89/93), respectively.

Al- Khaweledy *et al.*, (2014) carried out a study in Iraq aimed to detect the HCMV among patients suffering from acute or chronic renal failure. The obtained results showed that HCMV IgG was detected by ELISA in (100%) of renal failure patients while IgM were (18.66%).

2.6.12.8.3. Indirect immunofluorescence assay (IFA)

Indirect immunofluorescence assay is commonly used method for detecting CMV antibodies, in the indirect IFA dilutions of test serum are incubated with virus-infected cells that have been fixed to a glass microscope slide. Specific antibody-antigen

complexes are detected using an anti-human antibody conjugated with fluorescein isothiocyanate and fluorescence microscopy. IFAs are useful for the qualitative and quantitative detection of CMV antibodies (Murray *et al.*, 2007).

2.6.12.8.4. Other serological tests

Varieties of laboratory tests with different degrees of sensitivity have been described for the measurement of HCMV antibodies in human sera. The methods include complement fixation (CFT), indirect hemagglutination, latex agglutination, radioimmunoassay (RIA) (Sia and Patel, 2000) and neutralization test are other serologic tests used to measure CMV antibody (Murray *et al.*, 2007).

2.6.13. Genotyping of CMV

The large CMV genome encodes several hyper variable loci. Many genetically different strains of CMV circulate in the human population. It has been suggested that difference in virulence, pathogenicity, progression and severity of disease in immunocompromised individuals, including transplant recipient may be attributed to variation between HCMV strains (Gandhoke *et al.*, 2013). They thought that the genetic variations not only affecting the viral pathogenicity but also clinical outcomes in immunocompromised patients (Madi *et al.*, 2011b).

Glycoprotein B (gB), gM, gN, gH, gL and gO are not only key targets for neutralizing antibodies but also are believed to be involved in viral egress and entry into cells (Yan *et al.*, 2008). The gH component induces virus-neutralizing antibodies and facilitates the penetration of the virus into the host cell. gL is necessary for transport of gH to the cell surface, whereas gO is dispensable for viral replication. It has recently been proposed that gH/gL binds cellular receptors before triggering gB, which is not required for binding ligands, and instead acts as the fusion protein (Paradowska *et al.*, 2014).

The HCMV gB, gH and gN are the most abundant. The gB gene, the gH gene and the gN gene have all been utilized for genotyping HCMV most frequently (Dar, 2007). Extensive attention has recently been focused on the analysis of strain variation among HCMV isolates. Some 20 different strains have been isolated and differentiated by restriction fragment length polymorphism (RFLP) (Gandhoke *et al.*, 2013).

2.6.13.1. Glycoprotein B genotyping

Glycoprotein B the most widely characterized polymorphic gene and it is encoded by UL55 (Sowmya *et al.*, 2007) is considered to be an important multifunctional envelope component, responsible of virus entry, cell-to cell spread, and the fusion of infected cells (Jun *et al.*, 2012). It is a critical factor in tissue tropism, viral pathogenesis (Sowmya *et al.*, 2007) and specific cytotoxic T-cell immune responses (Arista *et al.*, 2003). gB antibodies have been of interest because of their therapeutic potential for neutralization (Jun *et al.*, 2012).

CMV gB is expressed as a precursor molecule that is glycosylated and then cleaved at codon 461 to form a disulfide-linked complex of gp55 and gp116 (Coaquette *et al.*, 2004). HCMV gB genotyping is based on the highly variable region around the proteolytic cleavage site (Bhattarakosol and Chantaraarphonkun, 2007). Wild type CMV strains can be classified into four major gB genotypic variants (gB 1-4) (Cunha *et al.*, 2011) based on gB nucleotide sequence, which encodes a variable region that encompasses the protease cleavage site (Coaquette *et al.*, 2004). A fifth gB genotype (gB 5) was detected in several AIDS patients (Deckers *et al.*, 2009). Each of them has tropism for distinct cell lines, leading to different pathogenesis and severity of disease (Cunha *et al.*, 2011). It has been suggested that differences in virulence, pathogenicity, progression and severity of disease in immunocompromised individuals, including transplant recipients, may be attributed to variations between HCMV strains (Dar, 2007).

Several reports find a correlation between gB genotype and the occurrence of CMV infection or disease in organ transplant recipients (Ishibashi and Suzutani, 2012). Furthermore detecting gB antigen in patients with HCMV infection may facilitate the monitoring of the infection (Jun *et al.*, 2012). According to analysis of two envelope glycoproteins (gB and gH), clinical HCMV isolates have been shown to adopt one of four gB and two gH sequence configurations at certain variable loci (Madi *et al.*, 2011b). Reported data indicate that gB type 1 HCMV strains are less virulent than other gB genotypes strains. gB types 2 and 3 in bone marrow-transplant (BMT) recipients and AIDS patients are associated with greater virulence, at the same time as the gB type 4 virus is rarely found in transplant recipients. In congenitally infected infants, strains with the gB genotype 1 are frequently encountered (Arista *et al.*, 2003). Transplantation in a

D+/R+ setting is usually accompanied by multiple CMV strains in recipients after transplantation, with mixtures of gB and gH genotypes were commonly observed in organ transplant recipients (Ishibashi and Suzutani, 2012). Presence of multiple gB genotypes in immunocompromised patients is associated with a higher viral load, higher prevalence of HCMV disease and concomitant infection with other herpesviruses such as EBV (Sowmya *et al.*, 2007).

The genotyping of HCMV strains based on the gB gene have been determined by PCR-based RFLP, Single-stranded conformation polymorphism, heteroduplex mobility analysis, DNA sequencing (Renzette *et al.*, 2014).

Genotyping of HCMV among renal transplant recipients in study of Madi *et al.*, (2011b), revealed that HCMV gB1 and gH1 genotypes were the most the predominant HCMV genotypes ($P < .05$, $P < .05$, respectively). Both HCMV gB1 and gH1 genotype were significantly more often associated with the development of fever with leucopenia and severe HCMV disease than other gB or gH2 genotypes.

Another study conducted by Bhattarakosol and Chantaraarphonkun (2007), in which a total of 128 patients including renal transplant were undergoing CMV genotyping directly by using nested PCR and RFLP with restriction enzyme *HinfI* and *RsaI*. HCMV gB genotyping was successful in 113 (70%) samples. Mixed gB genotype was most frequently found (39 samples, 35%), followed by gB1 (37, 33%), gB3 (17, 15%), gB2 (12, 11%), and non-typed (8, 7%). No gB4 was observed.

In a large prospective cohort study of Manuel *et al.*, (2009) observed that organ transplantation have shown mixed infection to be associated with higher viral loads and delayed virological clearance according on the basis of gB distribution analysis . Mixed genotype infection was more likely shown in D+/R.

A prospective analysis of active HCMV infection was conducted on 33 pediatric renal or hematopoietic stem cell post-transplant patients in Brazil. The study evaluated the prevalence of different gB and correlation with clinical signs. Twenty (60.6%) patients demonstrated active HCMV infection. gB1 and gB2 genotypes were more frequent in this population. they observed that gB2 had correlation with reactivation of HCMV infection and that patients with mixture of genotypes did not show any symptoms of HCMV disease (Dieamant *et al.*, 2010).

Finding of study of Coaquette *et al.*, (2004) showed that patients infected with a single gB genotype, patients infected with multiple gB genotypes developed progression to CMV disease, had an increased rate of graft rejection, higher CMV loads. The presence of multiple gB genotypes, rather than the presence of a single gB genotype, could be a critical factor associated with severe clinical manifestations in immunocompromised patients.

Arista *et al.*, (2003) showed that predominant circulation of HCMV strains with gB type 2 and 3 was detected in both the immunocompetent host with a primary HCMV infection and the immunocompromised host (including renal transplant) with or without HCMV disease. No association between gB types and subjects with different risk of developing HCMV disease was found.

In study of Khalafkhany *et al.*, (2016), the distribution of gB1, gB2, gB3, and gB4 genotypes in renal transplant recipients in Iran was detected as 26.5%, 20.5%, 17.6%, and 5.9%, respectively. Mixed genotype infection was observed in 29.4% of the recipients.

In study of Gandhoke *et al.*, (2012) in India, both RFLP and sequencing of gB gene fragment showed that gB 1, 2 and 3 genotypes were in circulation. gB 3 was the most prevalent genotype in symptomatic infants. Hepatosplenomegaly was the most common feature in gB-3 genotype of CMV. gB2 congenital CMV infection was more commonly associated with long term sequelae.

2.6.14. CMV sequencing

HCMV is highly diverse within humans (Renzette *et al.*, 2014). The complete genome of HCMV was elucidated almost 25 years ago using a traditional cloning and Sanger sequencing approach. Analysis of the genetic content of additional laboratory and clinical isolates has led to a better, albeit still incomplete, definition of the coding potential and diversity of wild-type HCMV strains (Sijmons *et al.*, 2014).

Extensive sequence variation is found in the gB and gH genes (5–10 %), a greater level is found in the gN and gO genes (40–50 %); the gL and gM genes are highly conserved among clinical strains (Yan *et al.*, 2008).

The most commonly used HCMV genotyping methods, such as traditional Sanger DNA sequencing, restriction fragment length polymorphism of PCR products, and genotype-specific PCR assays, lack sensitivity and are not quantitative. Even cloning of PCR

products and subsequent Sanger sequencing do not allow a sensitive assessment of the genotypes present unless a very large number of individual clones is sequenced. Improved genotype-specific real-time-PCR-based assays have been established recently for gB and gH genotyping, and these allow simultaneous detection and quantitation of distinct genotypes in mixed infections down to a level of 5% or even less (Görzer *et al.*, 2010).

Ultra-deep pyrosequencing, is the most sensitive method for quantitative HCMV genotypes, with which even low-abundance genotypes at a frequency of less than 1% of the population can be detected. It detects sequence variations over the entire amplified region, it is especially useful for genotyping highly variable regions that show a large number of distinct genotypes, as is the case for gO and gN. Novel genotypes or variants of genotypes can also be discovered with ultra-deep pyrosequencing, even when they are present in the mixture at low abundance (Puchhammer-Stöckl and Görzer, 2011). Ultra-deep pyrosequencing to study the gO, gN and gH loci from HCMV-infected transplant recipients. They found that all patients studied had mixed infections, with as many as six genotypes observed in a single patient (Renzette *et al.*, 2014). On the other hand, deep sequencing enables novel diagnostic applications for sensitive in detecting drug resistance mutation (Sijmons *et al.*, 2014).

2.6.15. Treatment and prevention of CMV

Treatment of established CMV disease requires a multifactorial approach, including reduction of immunosuppressive agents, antiviral agents, and in some cases adjuvant therapy. Intravenous ganciclovir has been considered the mainstay of therapy (Karuthu, and Blumberg., 2012). Both of these strategies effectively control CMV end-organ disease in transplant patients (Griffiths, and Lumley, 2015). There are two main methods for CMV prevention: universal prophylaxis and preemptive therapy (Helanterä *et al.*, 2010).

2.6.15.1. Universal prophylaxis

Universal prophylaxis involves giving antiviral medication at prophylaxis dose for a defined time to a cohort (i.e. when either donor and/or recipient are seropositive for CMV) or defined subset of a cohort (i.e. given only to the highest risk subset, D+/R-). D+/R- are at greatest risk of CMV infection, and without prophylaxis more than 50% of

these high-risk patients will develop symptomatic infection. Therefore, prophylaxis is recommended for D+/R- for at least 3 months after transplantation (Helanterä *et al.*, 2010). Primary antiviral prophylaxis appears to be more effective in preventing the indirect effects of CMV than pre-emptive therapy (Hodson *et al.*, 2005).

2.6.15.2. Preemptive therapy

Preemptive therapy is defined as serial testing done weekly or biweekly for the first few months after transplant or after treatment of rejection, with treatment dose antiviral therapy initiated once a certain defined positive threshold is reached (Kotton, 2013), with absence of signs and symptoms (Costa, 2011). Pre-emptive therapy is very effective at preventing HCMV end-organ disease, such as gastrointestinal ulceration, hepatitis, pneumonitis or retinitis, which are caused by viremic spread of virus (Griffiths *et al.*, 2015). Aguado *et al* (2011), proposed for initiation of pre-emptive therapy in SOT recipients at DNAemia levels, ranging from 1,000 to 5,000 copies/ml of plasma or from 1,000 to 300,000 copies/ml of whole blood, at low or intermediate risk for CMV end-organ disease.

Ganciclovir is a potent inhibitor of CMV replication and can be used either for prophylaxis or for pre-emptive therapy (Griffiths, and Lumley., 2015). Ganciclovir GCV is a synthetic acyclic nucleoside analogue, structurally similar to guanine. GCV requires phosphorylation to achieve antiviral activity. The enzyme responsible for its phosphorylation is the product of the CMV UL97gene, a protein that functions as a protein kinase and phosphotransferase which generate the triphosphate form. GCV triphosphate competitively inhibits DNA synthesis catalyzed by inhibition of the CMV polymerase (encoded by the UL54gene) (Karuthu, and Blumberg, 2012). Ganciclovir lead to improved graft function in patients with CMV-associated late acute rejection (Yilmaz *et al.*, 1996).

Valganciclovir (Val-GCV) is a mono-valyl ester pro-drug of GCV (Buonsenso *et al.*, 2012). Valcyte in CMV-Disease Treatment of Solid Organ Recipients (VICTOR) trial found that valganciclovir was as effective as intravenous ganciclovir at least, in some SOT recipients with mild to moderate disease. For patients with life-threatening CMV disease, high viral loads, leukopenia, and impaired absorption, intravenous ganciclovir is preferable and maintenance. Immunosuppression should be decreased despite the

potential risk of rejection (Karuthu, and Blumberg, 2012). Acyclovir antiviral also used for universal prophylaxis of CMV infection (Kotton *et al.*, 2013).

Number of factors are contributes to emerging of drug resistance including; treatment with GCV for prolonged periods, lack of specific CMV immunity, type of graft, potent immunosuppression, suboptimal antiviral drug levels, and delayed viral clearance during treatment (Menghi *et al.*, 2016). Resistance can be identified by genotype testing for mutations of the genes encoding UL97 and UL54 (Karuthu, and Blumberg., 2012) or sequencing of both genes (Madi *et al.*, 2011a). An alternate therapy for drug-resistant CMV; high-dose ganciclovir (for specific genetic mutations), foscarnet, and cidofovir have been used effectively (Karuthu, and Blumberg., 2012). In addition, three new drugs have recently completed phase II clinical trials (maribavir, brincidofovir and letermovir) (Griffiths and Lumley, 2015).

2.6.15.3. Vaccination of CMV

Several CMV vaccines are under development; none is currently available for routine clinical use. Types of vaccines includes live attenuated, DNA, subunit vaccine proteins with an adjuvant, and recombinant viral vaccines (Kotton *et al.*, 2013). A Towne strain vaccine study was also conducted in post-renal transplantation patients with high risk of CMV infection. After vaccination, cellular and humoral immune response was observed. A recombinant gB vaccine with MF59 adjuvant was shown to induce neutralizing antibodies and prevent infection (Kotton *et al.*, 2013). Passive immunoprophylaxis is still on trail state (British Transplantation Society Guidelines, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

Blood specimens were collected from renal transplant recipients.

3.2. Methods

3.2.1. Study design

This is an analytical cross sectional study, aimed to detect CMV infection among renal transplant recipients, in addition to genotype the CMV detected.

3.2.2. Study area and duration

This study was conducted in Kidney transplanted association hospital and Ahmed Gassim teaching hospital in Khartoum state from June 2013 to June 2015.

3.2.4. Study population

Renal transplant recipients in two hospitals in Khartoum State.

3.2.5. Inclusion criteria

Renal transplant recipients, who agreed to participate in this study, adults and children, of both sexes with or without signs and symptoms of CMV infection were included in this study.

3.2.6. Exclusion criteria

Other organ transplant recipients were excluded.

3.2.7. Sample size

One-hundred and four (n=104) renal transplant recipients were selected for this study

3.2.8. Sampling technique

This study is based on non-probability convenience sampling technique. Samples were taken from attended agreed recipients during their regular medical checkup.

3.2.9. Method of data collection

Data were collected through non self-administrated questionnaire from renal transplant recipients. The interview questionnaire consists of 10 opened and

closed -end questions. Some information were taken from patient's clinical reports.

3.2.10. Specimen collection

Five ml of blood specimens were collected in EDTA (for TWBCs, platelets count and DNA extraction) and plain container (For detection of IgG and IgM) from each individual. TWBCs, platelets count were done from EDTA samples then blood samples from EDTA and plain containers were centrifuged at 3000 rpm for 5 minutes. Plasma and sera were collected in aliquots of 3 plain sterile containers for each one and stored at -20° C until tested. EDTA sample for (TWBCs, platelets count) was tested immediately.

3.2.11. Total white blood cells count (TWBCs) and platelets count

Blood samples were aspirated and TWBCs, platelets count were measured by (Sysmex Corporation 2012-2014) Leucopenia was defined as total white cell count of < 4000 TWBCs/cmm. Persistent thrombocytopenia was defined as a platelet count <150.000 platelet/cmm.

3.2.12. Enzyme-Linked Immunosorbent Assay (ELISA) for detection of CMV IgG antibodies

The Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect the specific HCMV IgG and IgM antibodies. Commercial ELISA Kits (G.E.N.E.S.I.S diagnostics, Omega diagnostics group PLC, Cambridge shire, UK) were used as described by the manufactures. The sample diluent was diluted 1:14 in distilled water. A 100 µl of each of the following: the negative control, 3 U/ml standard, positive control and diluted samples (1:100) (all were in duplicate) were incubated in microplate well coated with CMV antigen at room temperature for 20 minutes. The wells were washed three times manually by washing buffer. Then 100 µl of conjugate reagents were added to each well and incubated at room temperature for 20 minutes. After four times washing, substrate solution (TMB Substrate) was added (100 µl /well) and the plate was incubated for 10 minutes then 100 µl of the stop solution were added. Optical densities (O.D) of controls, the standard and samples are measured using a microplate reader within 10 minutes at 450 – 630 nm.

3.2.12.2. Calculation and interpretation of the results

To determine the presence or absence of CMV-IgG, the measured O.D is compared to O.D mean of 3 U/ml standard as follow: Negative samples: $O.D < O.D$ of 3 U/ml standard and positive samples: $O.D \geq O.D$ of 3 U/ml standard.

3.2.13. ELISA for detection of CMV IgM antibodies

Commercial ELISA Kits (G.E.N.E.S.I.S diagnostics, Omega diagnostics group PLC, Cambridge shire, UK) were used as described by the manufactures. IgG- absorbent is prepared by addition of 40 ml of sample diluent to 10 ml IgG absorbent (1:4) and all samples were diluted 1:100. A100 μ l of the negative control, 10 U/ml standard, positive control and diluted samples were incubated in microplate well coated with CMV antigen at room temperature for 20 minutes. The wells were washed three times manually by washing buffer (which diluted firstly by distilled water 1:9). Then 100 μ l of conjugate reagent were added to each well and incubated at room temperature for 20 minutes. After another washing step to eliminate unbound material, substrate solution (TMB Substrate) was added (100 μ l / well) and the plate was incubated for 10 minutes then the stop solution (100 μ l) were added. The optical densities (O.D) of controls, 10 U/ml standard and samples are measured in a microplate reader within 10 minutes at 450 – 630 nm.

3.2.13.2. Calculation and interpretation of the results

To determine the presence or absence of CMV-IgM, the measured O.D is compared to O.D mean of 10 U/ml standard as follow: Negative samples: $O.D < O.D$ of 10 U/ml standard and positive samples: $O.D \geq O.D$ of 10 U/ml standard.

3.2.14. Quantitative Real Time PCR

3.2.14.1. DNA extraction from plasma samples

DNA is extracted from peripheral blood plasma using QIAamp DNA mini kit (Qiagen-Germany). Internal control (IC Glob- β -globin gene DNA), should be added to each sample during extraction. The preparation for extraction as follow: twenty (20 μ l) Qiagen protease (proteinase K) were pipette into the bottom of a 1.5 ml microcentrifuge tubes labeled by number of samples, (C-) and (Pos) (for negative control and positive control). Negative control and

positive control were included in each set of DNA extraction. A 10 µl of Internal Control (IC) were added to each tested tubes. Then 200 µl plasma samples were added to the corresponding microcentrifuge tubes, then 100 µl of negative control was added to tube labeled (C-), 90 µl of negative control, and 10 µl of positive DNA CMV/human (CMV Real –RT Quant - Sacace- Italy) was added to tube labeled (Pos). After this step 200 µl of AL buffer were added to all tubes, mixed by pulse vortexing for 15 second and incubated at 56°C for 10 minutes. Briefly, the tubes were centrifuged to remove the drops from inside of the lid. Then 200 µl of (98-100%) ethanol were added to all tubes and were mixed again by pulse vortexing for 15 second then briefly centrifuged. Carefully the mixture were applied to the QIAamp mini spin column (in 2 ml collection tube) then all tubes were centrifuge at 8000 rpm for 1 minute. Then spin column were placed in a clean 2 ml tubes, and the tubes containing filtrate were discarded. Carefully QIAamp mini spin column were opened and 500 µl Buffer AW1 were added then all tubes were centrifuge at 8000 rpm for 1 minute. Then spin column were placed in a clean 2 ml tubes, and the tubes containing filtrate were discarded. Carefully mini spin column were opened and 500 µl Buffer AW2 were then all tubes were centrifuge at full speed 14000 rpm for 3 minute. Then spin column were placed in a clean 2 ml tubes, and the tubes containing filtrate were discarded. Then centrifuged again at full speed for 1 minute. The spin column were placed in a clean 1.5 ml tubes and, tubes containing filtrate were discarded again. Carefully spin column were opened and 100 µl Buffer AE were added and incubated at room temperature (15-25°C) for 1 minute and then centrifuged at 8000 rpm for 1 minute. DNA yield were stored at -20°C for one month or at -70°C for one year.

3.2.14.2. qRT- PCR (detection and viral load estimation)

CMV amplification and quantification (estimation of viral load) was done using CMV Real – RT Quant kits (Sacace- Italy) (Appendix I) and Rotor –Gene Q series software 2.0.3 (build 2) Real time PCR machine (Qiagen-Germany). For reproducibility of qRT- PCR in measuring CMV viral load accurately in clinical

specimens, intraassay variability was included using a duplicates of CMV calibrator standard containing 10^2 and 10^4 .

The total reaction volume was 25 μ l. PCR-mix -2- FRT and polymerase (TaqF) mixture was prepared by transfer the content of the tube with polymerase (TaqF) (30 μ l) into the tube with PCR – mix -2-FRT (300 μ l). Test preparation for quantitative PCR amplification is described in table (3.1).

Table (3.1) Tested samples procedure for qPCR.

Sample	PCR-mix-FRT CMV	PCR-mix-2-FRT and Polymerase (TaqF)	QS1	QS2	Pos	C-	RNA-buffer	DNA
QS1*	10 μ l	5 μ l	10 μ l	-	-	-	-	-
QS2*	10 μ l	5 μ l	-	10 μ l	-	-	-	-
PCE	10 μ l	5 μ l	-	-	10 μ l	-	-	-
NCE	10 μ l	5 μ l	-	-	-	10 μ l	-	-
NCA	10 μ l	5 μ l	-	-	-	-	10 μ l	-
Sample	10 μ l	5 μ l	-	-	-	-	-	10 μ l

Key: - QS1: DNA calibrator 1, QS2: DNA calibrator 2, *: Each calibrator in duplicates, PCE: positive control of extraction, NCE: negative control of extraction, NCA: negative control of amplification.

3.2.14.3. Amplification

For rotor-type thermocycler instruments, the temperature profile program was created as described in table (3.2).

Table (3.2): Amplification profile program

Step	Temperature°C	Time	Fluorescent detection	Cycle repeats
Hold	95	15 min	-	1
2	95	5 sec	-	5
	60	20 sec	-	
	72	15 sec	-	
3	95	5 sec		40
	60	20 sec	FAM,HEX/JOE/Cy3,ROX/ Texas Red	
	72	15sec	-	

Fluorescent was detected on the second step (60°C) in FAM/Green, HEX/JOE/Cy3/Yellow and ROX/ Orange/ Texas Red fluorometer channels.

3.2.14.4. Calculation of the results

The real -time monitoring of the fluorescent intensities during the real –time PCR allows the detection of accumulating product. The fluorescent were detected at the annealing step (60°C) in cycling 2 of each cycle, and cycle threshold (Ct) for each sample was calculated by determining point at which the fluorescence exceeded threshold limit. CMV DNA (Positive control and human DNA) was detected in the HEX/JOE/Cy3/Yellow channel. Internal Control STI-87(IC) was detected in the ROX/ Orange channel. Fluorescent intensities detected in two channels: CMV DNA in the HEX/ Yellow/JOE/Cy3 channel, Internal Control (IC) DNA in ROX/ Orange/ Texas Red channel. Interpretation of the results of (Pos) and (NC) and (NCA) according to guiding sheet.

3.2.14.5. Estimation of viral load

For quantitative analysis (viral load copies for samples and positive control), the concentration of CMV DNA (^{KP} CMV DNA) per ml of sample for peripheral blood plasma samples was calculated by the following formula:

$K^P \text{ CMV DNA} = K \text{ CMV DNA} / K \text{ IC} \times \text{IC coefficient (copies / ml)}$ were as:

$K^P \text{ CMV DNA}$ = the number of the CMV DNA copies in DNA sample.

$K \text{ IC}$ = the number of IC DNA copies in DNA samples.

IC = coefficient corresponds to the number of IC DNA copies in DNA samples.

Coefficient was specific to each lot in data sheet (1.25×10^5) (Appendix II and III, IV).

3.2.15. Glycoprotein B genotyping

Genotyping of HCMV gB was carried out by nested PCR and sequencing of highly diverse region of glycoprotein B.

3.2.15.1. Nested PCR glycoprotein B genotyping

3.2.15.1.1. Primers

PCR amplification was performed using published primer pairs according to (Gandhoke *et al.*, 2012), which are shown in table (3.4).

3.2.15.1.2. Preparation of primers

10 μl from 100 pmol/ml from each primer was dissolved into 90 μl DW for preparing 10 pmol/ml as working primer.

Table (3.3): The selected primers for amplification of gB CMV genes

Gene	Primer
gB-1	5,CAAGARGTGAACATGTCCGA 3,
gB-2	5, GTCACGCAGCTGGCCAG,3
gB-3	5,TGGAAGTGGAAACGTTTGGC,3
gB-4	5,GAAACGCGCGGC AATCGG ,3

3.2.15.1.3. Preparation of reaction mixture for outer nested PCR

Reagents were used for each gene in the following volumes (total reaction volume was 25 μl) in 0.2 ml eppendorff tube. A 10 μl deionized sterile water and 5 μl Master mix (iNtRON, Seongnam, Korea), then 1 μl forward primer (Metabion, Germany) followed by 1 μl reverse primer (Metabion, Germany) finally 8 μl DNA (template DNA).

3.2.15.1.4. Protocol for outer nested PCR of gB CMV gene

The amplification was performed by using primus 96 thermal cycle (BIO-RAD). The PCR mixture was subjected to initial denaturation step at 95 °C for 10 minutes. DNA was amplified for 35 cycles as followed: denaturation at 95 °C for 1 minute, primer annealing at 55 °C for 1 minute, followed by a step of elongation at 72 for °C 1 minute, the final elongation was at 72 °C for 7 minute, according to (Gandhoke *et al.*, 2012).

3.2.15.1.5. Preparation of reaction mixture for inner nested PCR

Reagents were used for each gene in the following volumes (total reaction volume was 25 µl) in 0.2 ml eppendorff tube. Volume of 17 µl deionized sterile water and 5 µl Master mix (iNtRON, Seongnam, Korea) then 1 µl forward primer (Metabion, Germany) followed by 1 µl reverse primer (Metabion, Germany) finally 1 µl product from outer nested PCR (template DNA).

3.2.15.1.6. Protocol for inner nested PCR of gB CMV gene

The PCR mixture was subjected to initial denaturation step at 95 °C for 10 minutes. DNA was amplified for 35 cycles as follow : Denaturation at 95 °C for 30 seconds, primer annealing at 54 °C for 45 seconds, followed by a step of elongation at 72 for °C 30 seconds, the final elongation was at 72 °C for 7 minute, according to (Gandhoke *et al.*, 2012).

3.2.15.1.7. Preparation of agarose gel

The PCR products (520bp, 305bp) were subjected to gel electrophoresis on 1.5% agarose. For preparation of 75 ml of 1.5% agroase gel, 1.12 gram of agarose powder were dissolved in 67.5 ml DW and 7.5 ml of 10X Tris base EDTA (TBE) buffer (Appendix V) and heated until became clear. Then the mixture was cool to 55 °C, 2.5 µl of (20mg/ml) ethidium bromide were added, mixed well and poured in a casting tray, any bubbles were removed and left to solidify at room temperature.

3.2.15.1.8. Visualization of PCR products

The gel casting tray was flooded by 1X TBE buffer (Appendix VI) near the gel cover surface, then 5 µl of PCR products of each sample was loaded into each well, in addition to control positive and negative. Then to the first well of

casting tray 5 μ l of DNA ladder 50 bp (marker) was injected for each run. The gel electrophoresis apparatus was connected to the power supply (CONSORT E865, Belgium). Then the electrophoresis was run at 80 volts for 40 minutes. Gel was removed by gel holder and visualized by U.V transilluminator (Upland, USA). Gel results were photographed using computer software.

3.2.16. DNA sequencing

Sequencing was carried out from the inner (305bp) PCR product. DNA sequencing was performed for 10 PCR products of CMV gB gene. DNA purification and standard sequencing was performed for both strands of gB genes by Macrogen Company (Seoul, Korea).

3.2.17. Bioinformatics Analysis

3.2.17.1. Sequences similarity and alignment

PCR product purification, direct sequencing for strands, as well as nucleotide and translated amino acid sequence analysis were performed. Before uploading the sequences to NCBI we proofread the nucleotides chromatogram to ensure that all ambiguous sites are correctly called and determined the overall quality of it. Then nucleotide sequences of gB CMV genes achieved were searched for sequence similarity using nucleotide BLAST (Atschul *et al.*, 1997) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Highly similar sequences were retrieved from NCBI and subjected to multiple sequence alignment using the BioEdit software (Hall, 1999). In GeneMark S version 4.25 (<http://exon.gatech.edu/genemark/genemarks.cgi>), the gene sequences were translated into amino acid sequence (John *et al.*, 2001). Sequence similarities were searched with BLASTp (<http://blast.ncbi.nlm.nih.gov/Blast.cgi> [CMD = Web and PAGE_TYPEB last Docs]), highly similar sequences were achieved from NCBI and subjected to multiple sequence alignment and evolutionary analysis using BioEdit software.

3.2.17.2. Mutant genes analysis

The mutant nucleotides were confirmed by their reverse strands. I-mutant version 3 (Capriotti *et al.*, 2008), was used to study stability of mutant protein.

Chimera software version 1.9 was used to predict the tertiary model of protein (Huang *et al.*, 2014).

3.2.17.3. Phylogenetic tree

Phylogenetic tree of CMV gB genes and their evolutionary relationship with well-characterized reference strains obtained from NCBI database (M60926.2, KR992839.1, AY186111.1, KR992940.1, AY186112.1 and KR992932.1) (Nogueira *et al.*, 2002; Chou, 2014; Barans *et al.*, 2015) was constructed by the neighbor-joining method with the Bootstrap test of phylogeny in Molecular Evolutionary Genetics Analysis (MEGA) program, version 6 (Tamura *et al.*, 2013). Bootstrap resembling strategy and reconstruction were carried out 100 times to confirm the reliability of the phylogenetic tree.

3.2.19. Data analysis

Data were analyzed using statistical package for social science software (SPSS v.11.5) Data was presented in form of tables and figures. Frequencies and mean were calculated. Chi square test were performed between qualitative variables. Correlation were performed between two quantitative variables. A *P.value* of < 0.05 was considered as significant for all statistical tests in the present study.

3.2.20. Ethical consideration

The study proposal was approved by Ethical Board of Sudan University of Science and Technology and approved from two hospitals administration.

Verbal consent was taken from each renal transplant recipients prior to enrolment into the study. Data and samples were collected after informing and agreement of renal transplant recipients about the purposes and importance of the study.

CHAPTER FOUR

RESULTS

A total of 104 renal transplant recipients were participated in this study, their age ranged from 11 to 72 years and mean age of 37 years \pm 14.37 (SD). Out of them, males recipients were 72 (69.2%), while 32 (30.8%) were females, as shown in figure (4.1).

In this study, 50 (48%) of renal transplant recipients had received their organs in localized hospitals, while 54 (52%) received their organs in abroad as shown in figure (4.2).

Most of the renal transplant recipients received organs form relative donors 79 (76%) and only 25 (24%) from non-relative donors as shown in table (4.1).

The mean post-transplantation time in renal transplant recipients was 54 months, ranged from < than one to 204 months. The majority of post-transplantation duration in our study group were < than 1 to 12 month which represents 53(51%), 13 to 24 month 18(17.3%), 25 to 36 month 9(8.8%), 37 to 48 were 8(7.7%), and more than 48 months 16(15.4%) of recipients (as shown in figure 4.3).

Maintenance immunosuppression is necessary to prevent immunologic rejection of the allograft. The given immunosuppressive drugs among the study group are as follows: 28(26.9%) of recipients received tacrolimus, prednisolone and imuran, 15(14.4%) received tacrolimus, prednisolone and cellcept. About 10(9.8%) received prednisolone, cyclosporine and cellcept, while 8(7.7%) received each of (cyclosporine, prednisolone and imuran) and (tacrolimus, cyclosporine and imuran). Seven (6.7%) received tacrolimus, cyclosporine and cellcept. Five (4.8%) received by each of (tacrolimus and prednisolone), (cyclosporine and cellcept), and (tacrolimus and cellcept). Each of (cyclosporine and imuran) and (tacrolimus and cyclosporine) were given to 3(2.9%) of recipients, 2(1.9%) given each of (tacrolimus and imuran), and (cyclosporine, cellcept and Imuran). One recipient 1(1%) received (tracrolimus, cellcept, and imuran), (prednisolone and immuran) and cellcept respectively. This reflects that the majority of population 79(75.9%) received triple therapy while

24(23.1%) received two drugs and only one (1%) received one drug as shown in table (4.2).

The mean total white blood cells count among renal transplant recipients was 7100 WBCs/cmm \pm 2586.669 (std) with minimum count of 3200 WBCs/cmm and maximum count of 18600 TWBCs/cmm. All the study group showed adequate platelet counts and no thrombocytopenia was observed.

One hundred and four serum samples of renal transplant recipients were examined by direct ELISA for detection of CMV IgM and IgG antibodies. Twenty seven 27(26%) were positive for CMV IgM and 77(74%) of them showed negative results, while 103(99%) had CMV IgG in their serum and only 1(1%) sample was negative as shown in figure (4.4), (4.5), respectively. All positive CMV IgM samples were also positive for IgG, while 76 of 77 (98.7%) negative CMV IgM were positive for CMV IgG and only one of 77 (1.3%) samples showed negative results for both IgM and IgG, as shown in table (4.3).

All plasma specimens (n=104) were investigated for the presence of CMV DNA and viral load were estimated using hot start quantitative real time PCR kits.. In real Time -PCR a data curve was constructed in which PCR cycles (x axis) are plotted against the fluorescence intensity (y axis) for both DNA samples and internal control. Each sample regarded as positive for tested DNA and internal control if exceeded cycle thresh old (Ct) as shown in figure (4.6).

Based on the constructed standard curve the correlation coefficient was at least 0.999, the amplification efficiency were varies between 97% to 100% and coefficient of variation (CV%) were from 0.00% to 8.5% for tested DNA and internal control for all trails, as shown in figure (4.7).

CMV DNA (viremia) was detected in 40/104 (38.5%) of renal transplant recipients, while 64/104 (61.5%) showed negative results, (figure 4.8).

The average of CMV DNA viral load was 358×10^4 copies/ml (6.5 log₁₀) with minimum viral load 62 copies/ml (1.8 log₁₀) and maximum viral load 1.43×10^8 copies/ml (9 log₁₀).

Table (4.4) displays that CMV DNA were detected in 26/104 (25%) males and 14/104(13.5%) females, while 46(44.2%) of male and 18(17.3%) of females

were negative for CMV DNA which revealed that there was no significant difference ($P.value > 0.05$) between sex and CMV viremia. Although most of recipients with positive CMV DNA 25/40 (65%) were males and 14/40 (35%) were females.

The sensitivity of ELISA was estimated to be 67.5% and specificity 100% in comparison to real time PCR as gold standard method.

Figure (4.9) demonstrated that CMV viremia was detected in (60%), (17%), (10%), (5%) and (8%) of the recipients in < than 1-12, 13-24, 25-36 37-48, and more than 48 months post-transplantation respectively, and there was no significant difference ($P. value = 0.296$) between CMV viremia and post renal transplantation time as shown in table (4.5).

Table (4.6) exhibits the association between immunosuppressive drugs and positive CMV viremia as follow: 10(66.7%) with positive CMV viremia and 5(33.5%) negative CMV received tacrolimus, prednisolone and cellcept, 6(21.4%) with positive and 22(78.6%) negative were given tacrolimus, prednisolone and imuran. Four (57.1%) with positive and 3(42.9%) with negative CMV viremia received tacrolimus, cyclosporine and cellcept. Four (40%) with positive and 6 (60%) with negative given prednisolone, cyclosporine and cellcept. While 3(37.5%) with positive and 5(62.5%) negative were received (tacrolimus, cyclosporine and imuran), and (cyclosporine, prednisolone and imuran). Only one recipient (100%) with negative CMV viremia received tacrolimus, imuran and cellcept, while tacrolimus and cyclosporine were taken by 3(100%) recipients with negative CMV viremia. Tacrolimus and prednisolone taken by 3(60%) with positive and 2(40%) with negative CMV viremia. Only 1(20%) recipient with positive and 4(80%) with negative CMV viremia taken tacrolimus and cellcept. While (tacrolimus and imuran) and (cyclosporine, prednisolone, and imuran) received by 1(50%) of recipient with both positive and negative CMV viremia. Cyclosporine and cellcept were taken by 3(60%) and 2(40%) with positive and negative CMV viremia respectively. One recipient (33.3%) with positive and 2(66.7%) with negative CMV viremia received cyclosporine and imuran. Only 1(100%) recipient with negative CMV

viremia taken each of (prednisolone and imuran) and cellcept respectively. This finding showed no significant difference ($P.value = 0.386$) between immunosuppressive drugs and positive CMV viremia.

According to Knipe and Howley, (2007) viral load of 1000 copies/ml from plasma, which is equivalent to 3000 copies/ml of whole blood, was selected to be the positive predictive value of the qRT-PCR for a group of symptomatic patients and correlate with presence of CMV disease.

Results of table (4.7) showed significant difference ($P. value = 0.05$) between the type of received immunosuppressive drug and high viral loads (>1000 copies /ml) which indicates CMV disease and < 1000 copies /ml that indicates CMV infection. Patients received tacrolimus, prednisolone and cellcept with viral loads of >1000 copies /ml were 9 (81.8%) and <1000 copies /ml were 2 (18.2%). Patients received tacrolimus, prednisolone and imuran with viral loads of > 1000 copies /ml were 2 (40%) and < 1000 were 3 (60%). Triple therapy (tacrolimus, cyclosporine and cellcept) and (cyclosporine, prednisolone and cellcept) were received by 2 (50%) recipients with >1000 and < 1000 copies /ml. One of recipients (33%) with >1000 copies /ml and 2 (67 %) with < 1000 copies /ml were received (tacrolimus, cyclosporine and imuran) and (cyclosporine and cellcept) respectively. Two (67 %) and 1(33%) of recipients received tacrolimus and prednisolone with >1000 and <1000 copies /ml respectively. Two (100%) of recipients received cyclosporine, prednisolone and imuran with >1000 copies /ml. One (50%) of recipients with >1000 and <1000 copies /ml respectively received cyclosporine and imuran. Only one recipient (100%) of >1000 copies /ml were received (tacrolimus and cellcept), (tacrolimus and imuran) and (cyclosporine, cellcept and imuran) respectively.

Figure (4.10) and table (4.8) displayed that recipients with positive CMV viremia and had symptoms of CMV disease were 17/104 (16.3%), while 23/104 (22.1%) were asymptomatic. Furthermore, 14/17(82.4%) patients with positive CMV viremia had clinical symptoms of CMV disease with viral loads >1000 copies/ml and 3/17(17.6%) had clinical symptoms of CMV disease with viral loads <1000 copies/ml. While 1/23(4.3%) had no clinical symptoms of CMV

disease with viral loads >1000 copies/ml and 22/23 (95.7%) had no clinical symptoms of CMV disease with viral loads <1000 copies/ml. The results revealed that the correlation between CMV loads of >1000 copies/ml and presence of symptoms of CMV disease were highly significant (*P.value* =0.000).

The medium CMV DNA viral loads copies/ml among symptomatic patients was (8.4×10^6 copies/ml = $6.9 \log_{10}$) and in asymptomatic patients was (316 copies/ml = $2.5 \log_{10}$). Individual DNA values for asymptomatic patients ranged between 62-1016 copies/ml (1.8 to $3 \log_{10}$), whereas for symptomatic patients they ranged from 537– 1.43×10^8 copies/ml (2.7 to $9 \log_{10}$). The discrepancies in three symptomatic patients showed viral loads of (537, 711, 690 copies/ml) which is relatively low and one patient was asymptomatic with viral loads of 1016 copies/ml.

The findings of this study indicated that fever 7(41%), fever and leucopenia 6(35%) and gastrointestinal disease 4(24%) were the most common presenting symptoms of CMV disease as shown in figure (4.11).

Successful sequencing of CMV encoding gB was determined for 10 samples of symptomatic Sudanese renal transplant recipients after performing nested PCR, with gB gene (UL55) product of 305bp as shown in figure (4.12). The nucleotide sequences of 10 isolates and their accession numbers were deposited in the GenBank database shown in table (4.9).

The result of CMV-genotyping by sequencing based on MEGA software revealed 8 cases (80%) for gB3, and 2 cases (20%) for gB4 genotypes among Sudanese renal transplant recipients. The most frequent genotype in HCMV-positive Sudanese renal transplant recipients was gB3 and no mixed genotypes observed as shown on table (4.10).

BLAST nucleotide search showed that isolate 24 and 149 were 99% identity with CMV gB genotype 4 (Genbank accession number M60926.2) from United State of America, Spain (Genbank accession number KR992839.1. and KR992940.1), Brazil (Genebank accession number AY186111.1 and AY186112.1). Isolates 230, 189, 164, 147, 135, 118, 10, 11 were 100% identity

with CMV gB genotype 3 (Genbank accession number KR992932.1) from Spain as shown in figure (4.13) (Appendix VII, VIII).

Multiple sequence alignment of obtained CMV gB sequences compared with reference sequences previously published in data base exhibited transversions mutation in isolates 230, 189, 164, 147, 135, 118, 10, 11. In which C was replaced by A at position 253 from reference CMV gB 3 (KR992932.1) as shown in figure (4.13). That resulted in substitution of the codon CGT Arginine (R), to AGT Serine (S) as shown in figure (4.14) and (4.15) (Appendix IX).

Resultant of substitution protein shown by tertiary protein structure of wild type (R), and mutant type (S) at position 85, figure (4.16) and (4.17). This substitution resulted in decrease of protein stability as illustrated by I-mutant software, table (4.11).

The Phylogenetic tree analysis was performed to compare the genetic distances and evolutionary lineage for all 10 isolates with well-characterized reference isolates from Genbank as shown in figure (4.18).

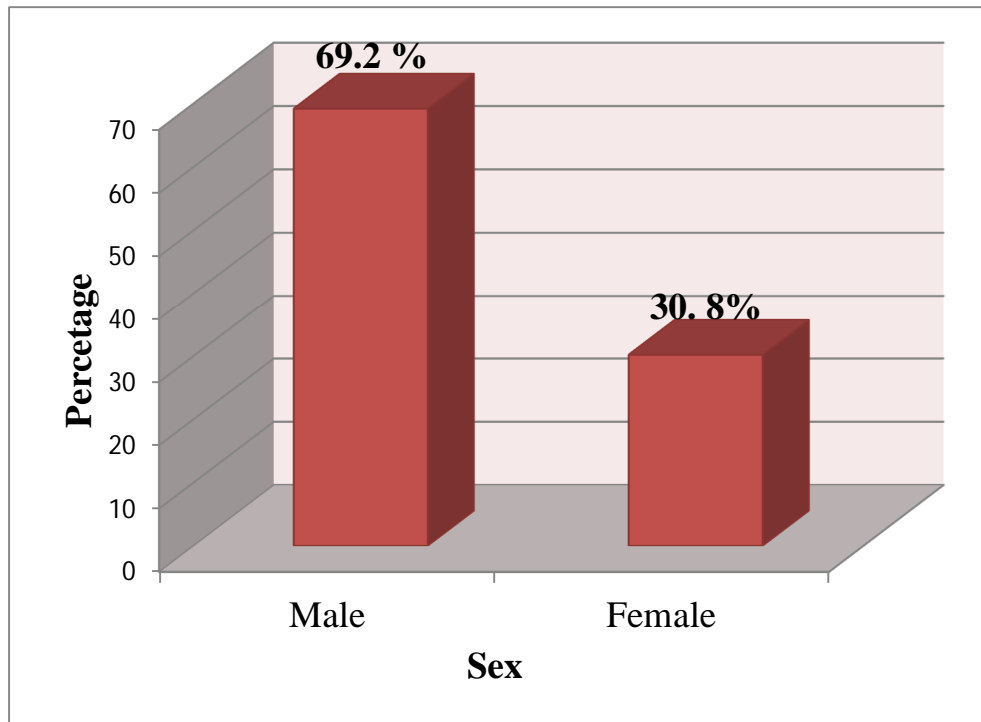


Figure (4.1): Frequency of sex among renal transplant recipients

Table (4.1): Frequency of renal transplant donors and recipients relationship

Donor recipient relationship	Frequency	Percentage
Relative	79	76%
Non-relative	25	24%
Total	104	100%

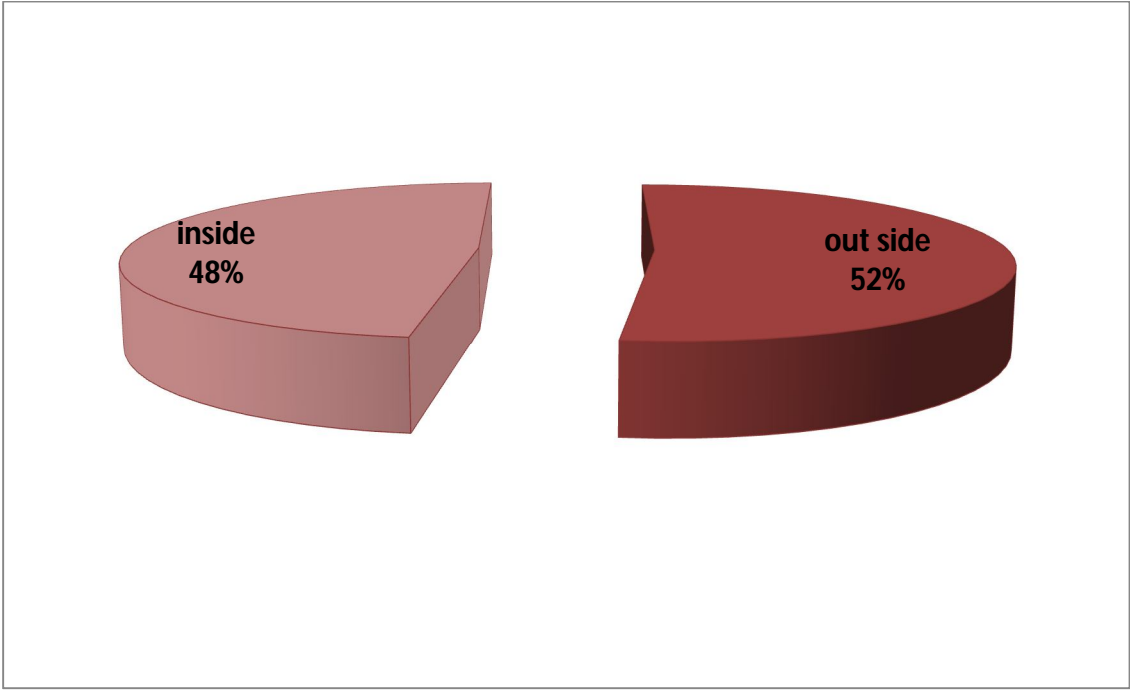


Figure (4.2): The distribution of transplantation place

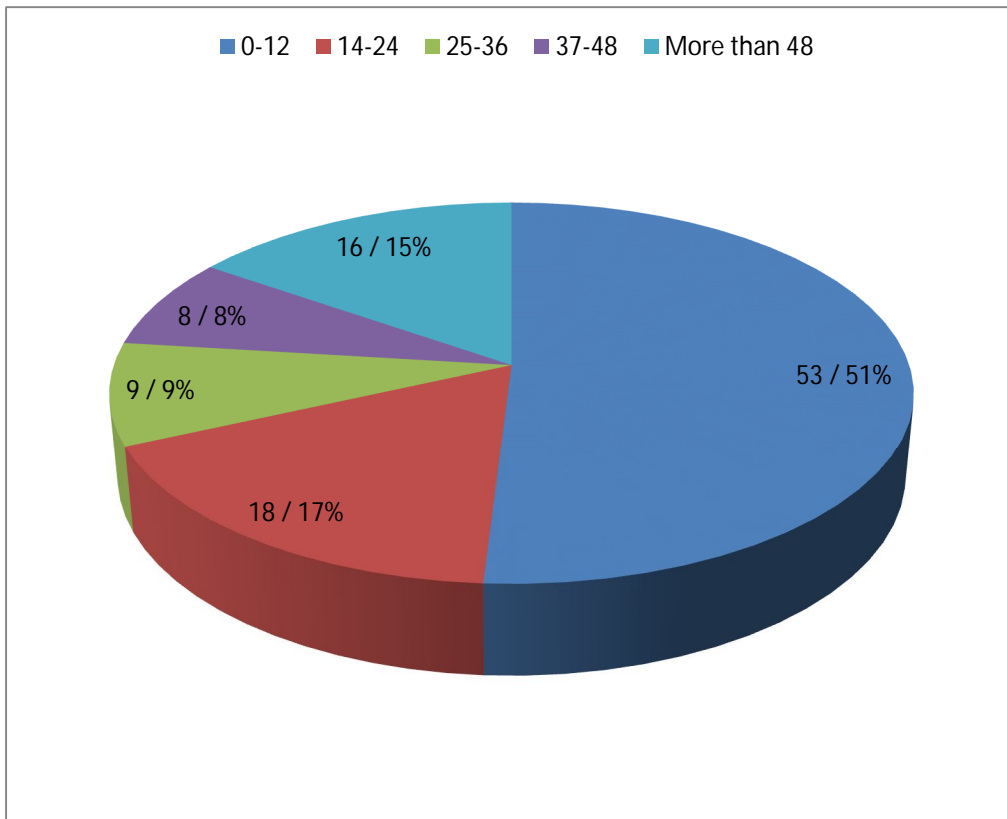


Figure (4.3): Post-renal transplantation time per month among the study group.

Table (4.2): Distribution of immunosuppressive drugs among renal transplant recipients

Immunosuppressive drug	Frequency	Percentage
Tacrolimus+ Prednisolone + Imuran	28	26.9%
Tacrolimus+ Prednisolone+ Cellcept	15	14.4%
Cyclosporine + Prednisolone + Cellcept	10	9.8%
Cyclosporine + Prednisolone+ Imuran	8	7.7%
Tracrolimus + Cyclosporine + Imuran	8	7.7%
Tracrolimus + Cyclosporine + Cellcept	7	6.7 %
Cyclosporine + Cellcept	5	4.8%
Tracrolimus + Cellcept	5	4.8%
Tracrolimus+ Prednisolone	5	4.8%5
Cyclosporine + Imuran	3	2.9%
Tracrolimus + Cyclosporine	3	2.9%
Cyclosporine + Cellcept + Imuran	2	1.9%
Tracrolimus + Imuran	2	1.9 %
Tracrolimus + Cellcept + Imuran	1	1%
Prednisolone + Imuran	1	1%
Cellcept	1	1%
Total	104	100 %

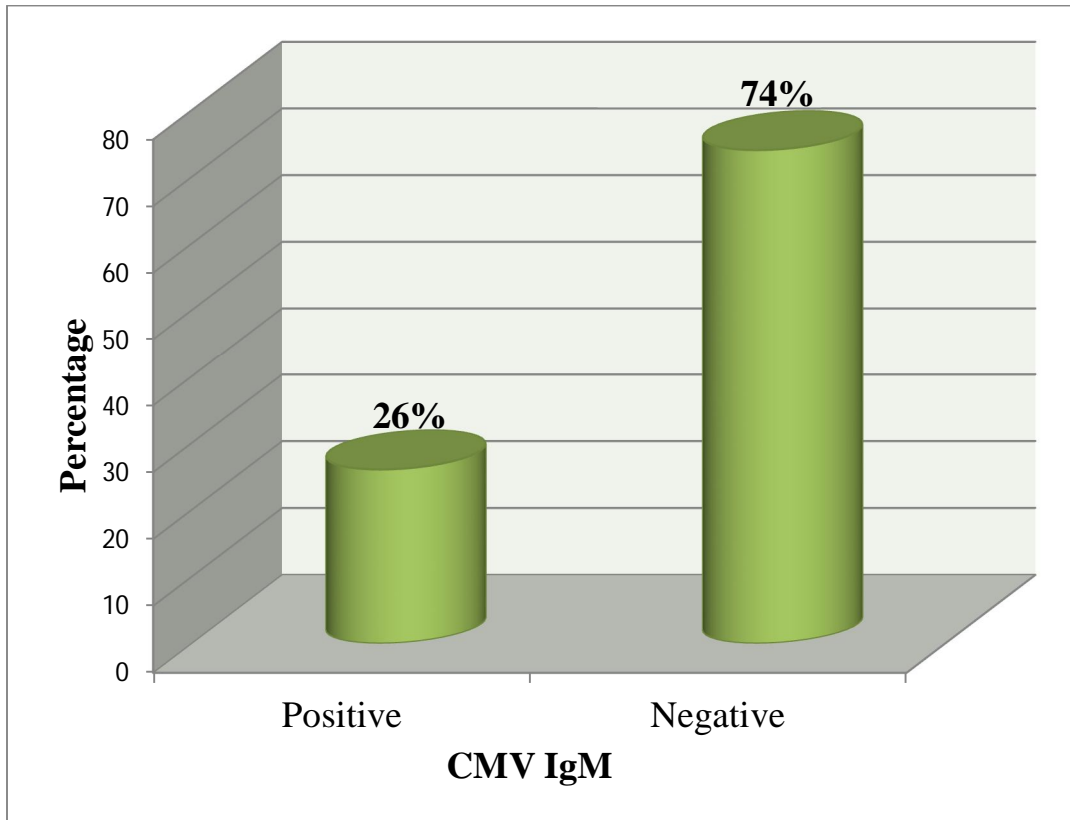


Figure (4.4): Frequency of CMV IgM in renal transplant recipients

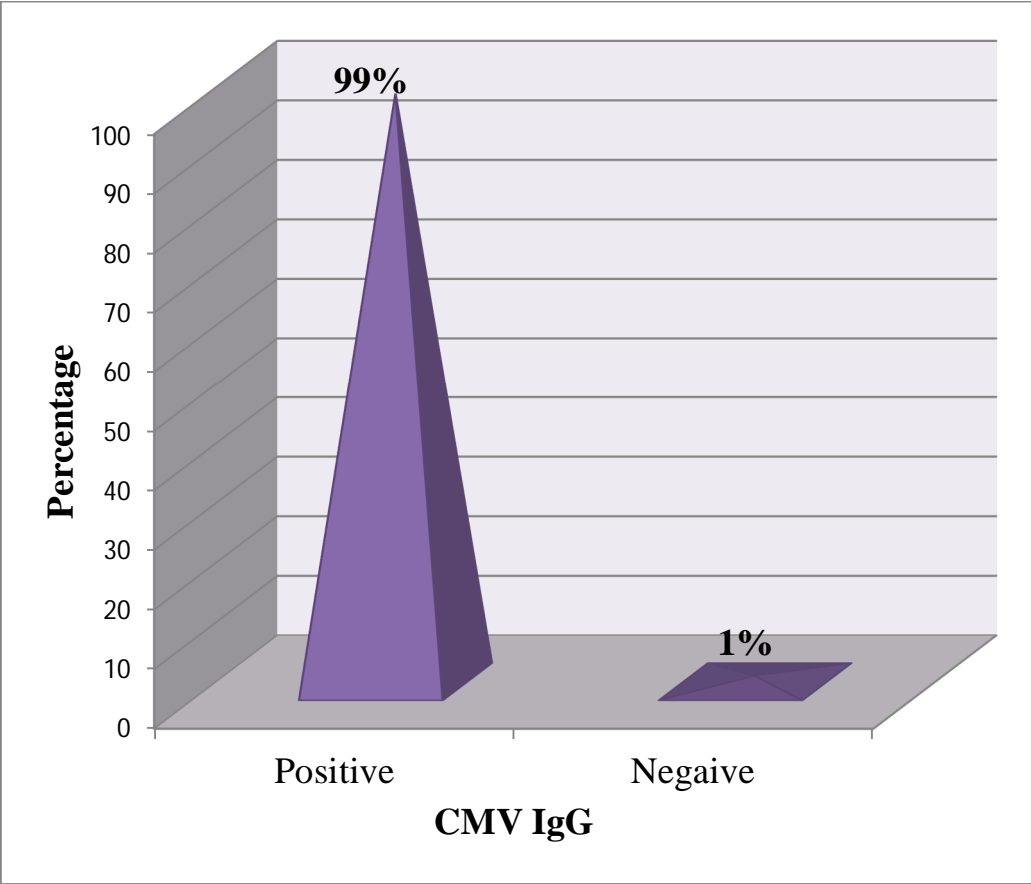
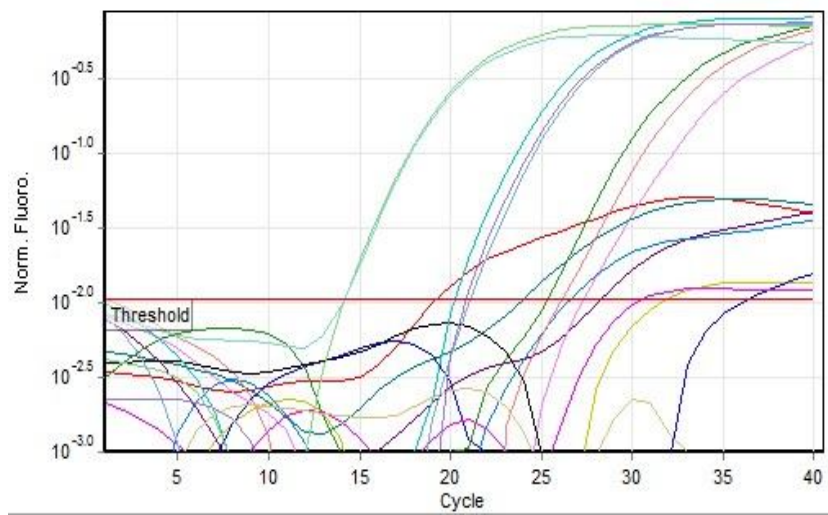


Figure (4.5): Frequency of CMV IgG in renal transplant recipients

Table (4.3): The relation between CMV IgM and IgG among renal transplant recipients

CMV IgM	CMV IgG		Total
	Positive %	Negative %	
Positive	27 (100%)	0 (0%)	27 (100%)
Negative	76 (98%)	1 (1.3%)	77 (100%)
Total	103 (99%)	1 (1%)	104 (100%)

(A)



(B)

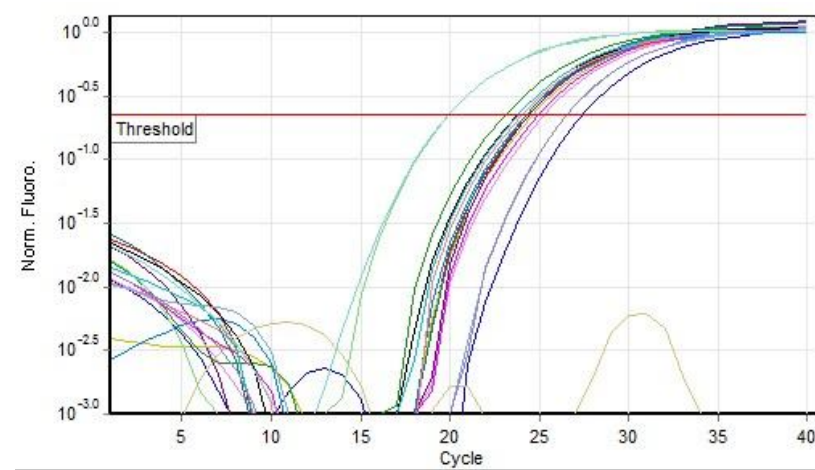
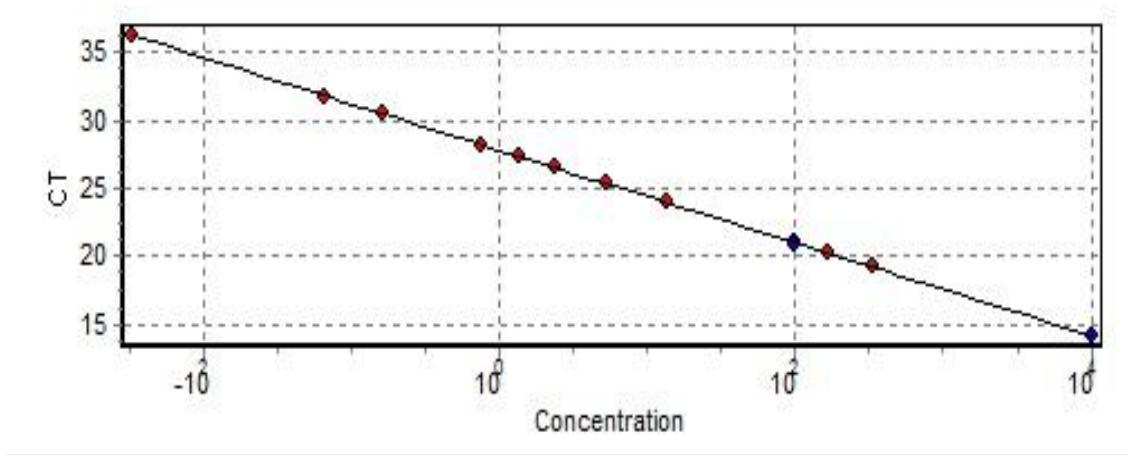


Figure (4.6): (A) Quantitation curve for cycling yellow (tested DNA); (B) for cycling orange (internal control).

(A)



(B)

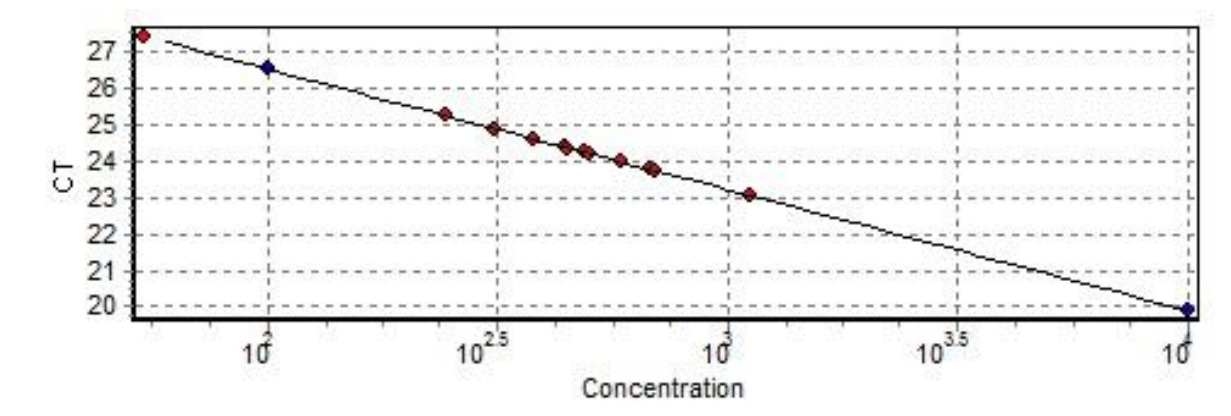


Figure (4.7): The standard curve (A): for cycling yellow (tested DNA), (B): for cycling orange (internal control)

$r = 0.99999$. $r^2 = 0.99999$. Efficiency = 1.00

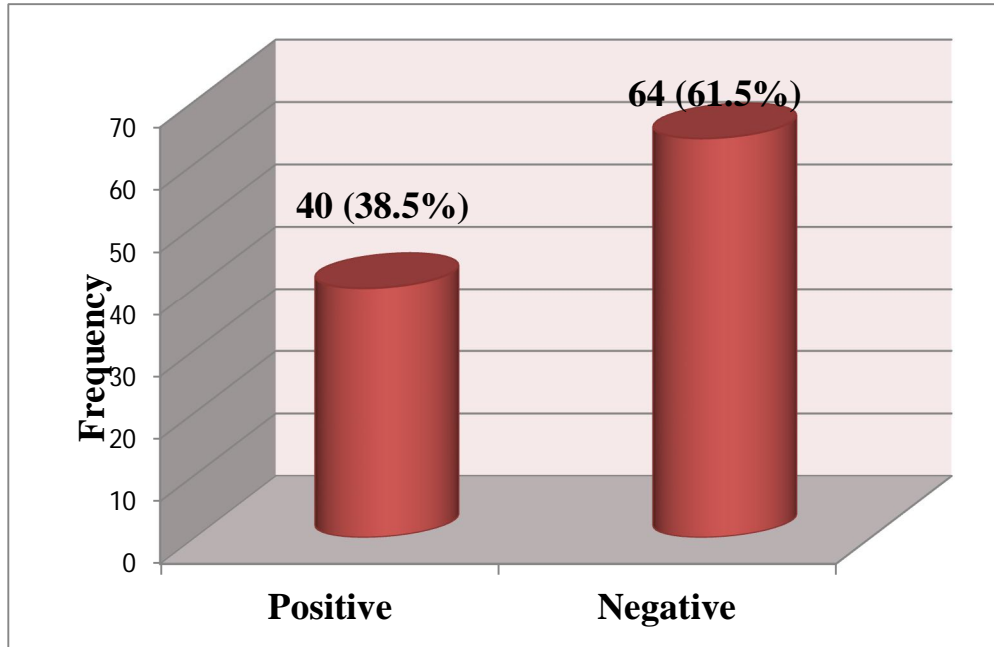


Figure (4.8): Frequency of positive CMV (viremia) in renal transplant recipients by qRT- PCR.

Table (4.4): The correlation between sex and CMV viremia.

Sex	Real time PCR		Total	<i>P. value</i>
	Positive (%)	Negative (%)		
Male	26 (25%)	46 (44.2%)	72 (69.2%)	0.546
Female	14 (13.5%)	18 (17.3%)	32 (30.8%)	
Total	40 (38.5%)	64 (61.5%)	104 (100%)	

Table (4.5): The association between positive CMV and Post - transplantation time / month.

Post-transplantation time / month	Real time PCR		Total	<i>P. value</i>
	Positive N (%)	Negative N (%)		
< than 1-12	24 (23.1%)	29 (27.9%)	53 (51%)	0.296
13-24	7 (6.7%)	11 (10.6%)	18 (17.3%)	
25-36	4 (3.8)	5(4.8%)	9 (8.7%)	
37-48	2 (1.9%)	6 (5.8%)	8 (7.7%)	
More than 48	3 (2.9%)	13 (12.5%)	16 (15.4%)	
Total	40 (38.5%)	64 (61.5%)	104 (100%)	

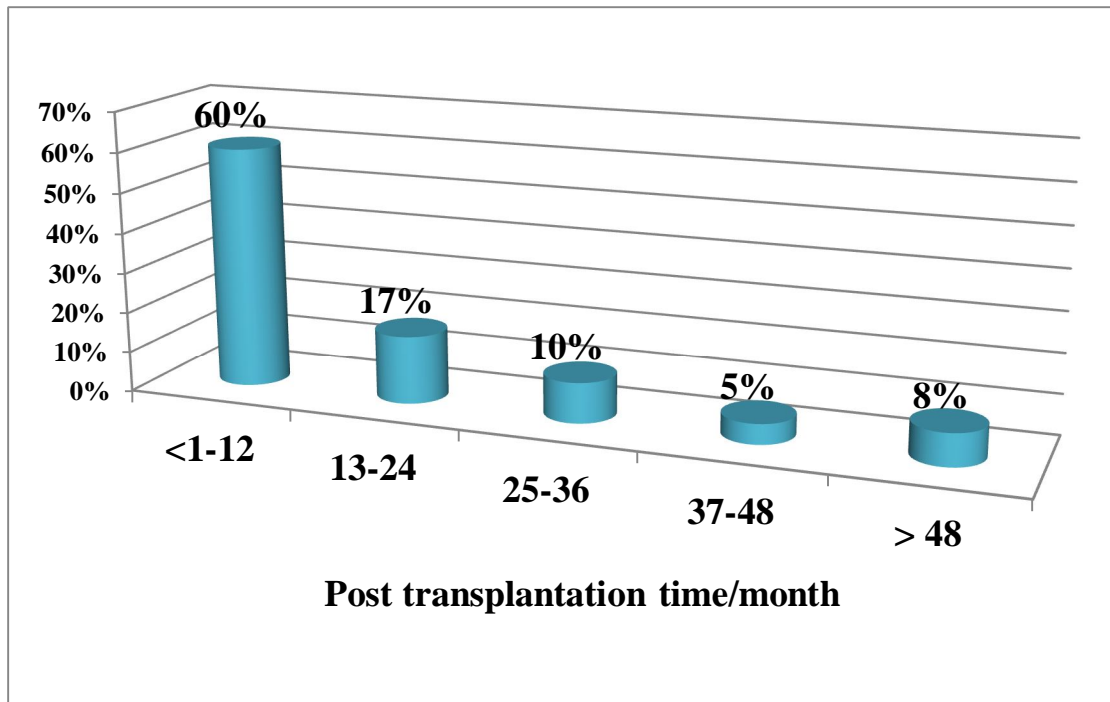


Figure (4.9): The relation between positive CMV viremia and post - transplantation time / month.

Table (4.6): The association between immunosuppressive drugs and positive CMV viremia.

Immunosuppressive Drugs	RT-PCR results		<i>P. value</i>
	positive CMV	Negative CMV	
Tracrolimus + Prednisolone+ Cellcept	10 (66.7%)	5(33.3%)	0.386
Tracrolimus + Prednisolone + Imuran	6 (66.7%)	22(72.6%)	
Tracrolimus + Cyclosporine + Cellcept	4(57.1%)	3(42.9%)	
Cyclosporine + Prednisolone+ Cellcept	4(40%)	6(60%)	
Tracrolimus + Cyclosporine + Imuran	3 (37.5%)	5(62.5%)	
Cyclosporine + Cellcept	3(60%)	2(40%)	
Tracrolimus + Prednisolone	3(60%)	2(40%)	
Cyclosporine + Prednisolone + Imuran	3 (37.5%)	5(62.5%)	
Cyclosporine + Imuran	1(33.3%)	2(66.7%)	
Tracrolimus + Cellcept	1(20%)	4(80%)	
Tracrolimus + Imuran	1(50%)	1(50%)	
Cyclosporine + Cellcept + Imuran	1(50%)	1(50%)	
Tracrolimus + Cyclosporine	0(0%)	3(100%)	
Cyclosporine + Imuran	1(33.3%)	2(66.7%)	
Tracrolimus + Cellcept+ Imuran	0(0%)	1(100%)	
Prednisolone + Imuran	0(0%)	1(100%)	
Cellcept	0(0%)	1(100%)	
Total	40(38.5%)	64(61.5%)	

Table (4.7): The association between immunosuppressive drugs and CMV infection and disease.

Immunosuppressive drugs	Viral load copies/ml		Total	P. value
	<1000	>1000		
Tracrolimus + Prednisolone+ Cellcept	2(18.2%)	9(81.8%)	11(100%)	0.05
Tracrolimus + Prednisolone + Imuran	2 (40%)	3 (60%)	5(100%)	
Tracrolimus + Cyclosporine + Cellcept	2(50%)	2(50%)	4(100%)	
Cyclosporine + Prednisolone+ Cellcept	2 (50%)	2 (50%)	4(100%)	
Tracrolimus + Cyclosporine + Imuran	1(33.3%)	2(66.7%)	3(100%)	
Cyclosporine + Cellcept	1(33.3%)	2(66.7%)	3 (100%)	
Tracrolimus + Prednisolone	2(66.7%)	1(33.3%)	3 (100%)	
Cyclosporine + Prednisolone + Imuran	2(100%)	0(0%)	2 (100%)	
Cyclosporine + Imuran	1 (50%)	1 (50%)	2 (100%)	
Tracrolimus + Cellcept	0 (0%)	1 (100%)	1 (100%)	
Tracrolimus + Imuran	0 (0%)	1 (100%)	1 (100%)	
Cyclosporine + Cellcept + Imuran	0 (0%)	1(100%)	1 (100%)	
Total	15(37.5%)	25(62.5%)	40 (100%)	

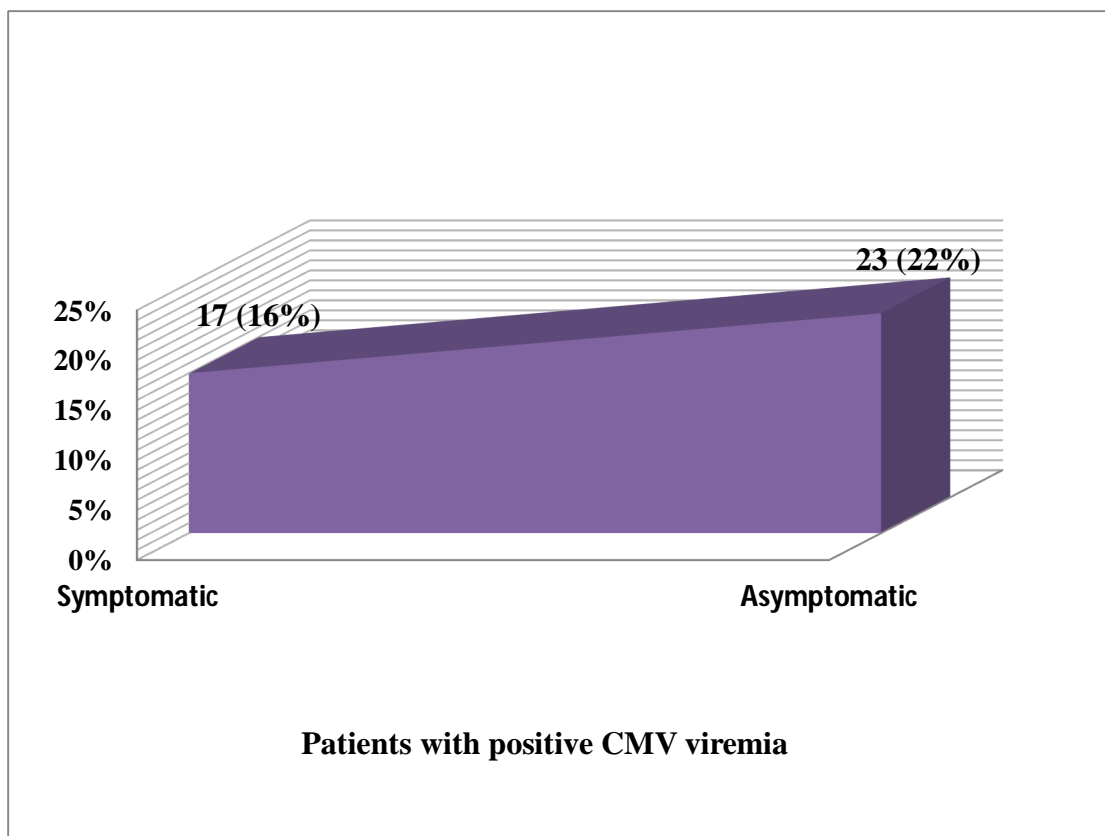


Figure (4.10): Frequency of CMV infection and CMV disease among renal transplant recipients

Table (4.8): The association between presence of symptoms and viral load copies/ml.

Presence of CMV Symptoms	Viral load copies/ml		Total	<i>P. value</i>
	<1000	>1000		
Yes	3(17.6%)	14 (82.4%)	17 (100 %)	0.000
No	22 (95.7%)	1 (4.3%)	23 (100 %)	
Total	25 (62.5%)	15(37.5%)	40 (100 %)	

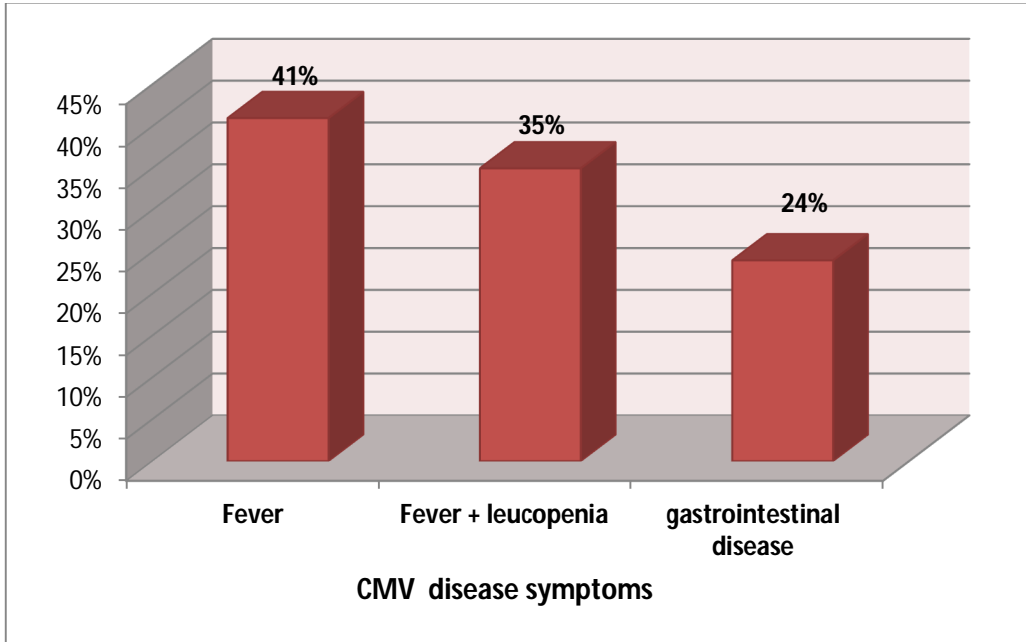


Figure (4.11): Distribution of CMV symptoms in recipients with CMV disease.

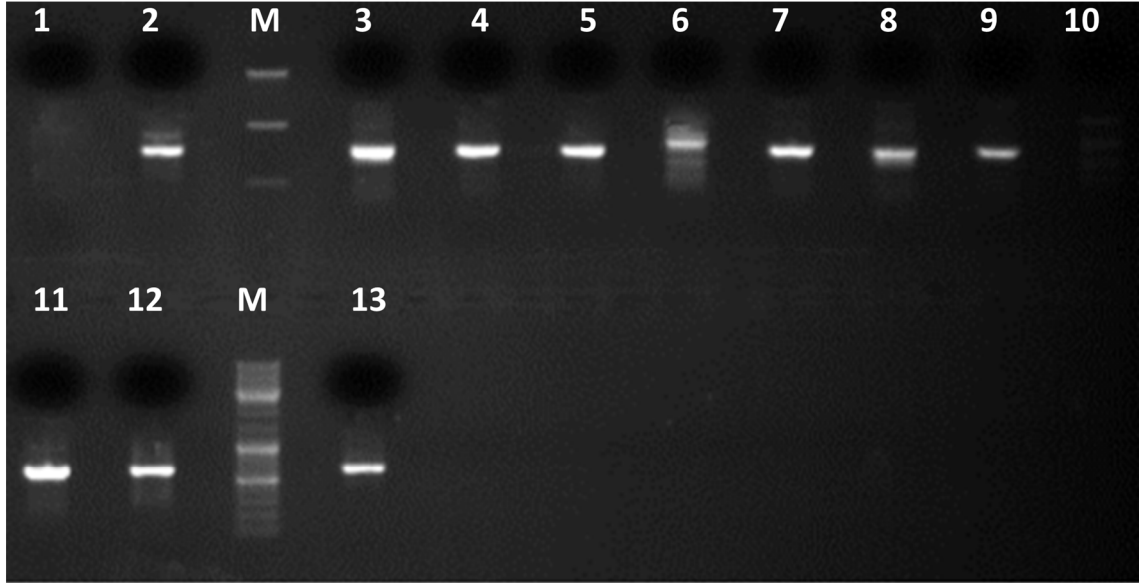


Figure (4.12): Gel electrophoresis of CMV UL55 gene PCR product (305 bp), M= Marker (50bp). Lane 1=Negative control, Lane 2, 3, 4, 5, 7, 8,9, 11, 12,13 tested samples.

Table (4.9): CMV isolates and its gB genotypes and accession number according to Genbank.

CMV Isolates	CMV Genotype	Accession Number
BankIt2021642 Isolate-24	gB-4	MF179785
BankIt2021642 Isolate-149	gB-4	MF179786
BankIt2021642 Isolate-230	gB-3	MF179787
BankIt2021642 Isolate-189	gB-3	MF179788
BankIt2021642 Isolate-164	gB-3	MF179789
BankIt2021642 Isolate-147	gB-3	MF179790
BankIt2021642 Isolate-135	gB-3	MF179791
BankIt2021642 Isolate-118	gB-3	MF179792
BankIt2021642 Isolate-10	gB-3	MF179793
BankIt2021642 Isolate-11	gB-3	MF179794

Table (4.10): Distribution of CMV gB genotypes among Sudanese renal teansplant recipients.

CMV gB genotype	Frequency	Percentage (%)
gB3	8	80%
gB4	2	20%
Total	10	100%

A

5'3' Frame 1

```
ttggaactggaacgttggccaatagctccgggtggaactccacgcgtagaaccaagaga
L E L E R L A N S S G V N S T R R T K R
agtacgggcaatacgaccaccctgtcgcttgaaagcgaatctgtacgaaatgtgctctac
S T G N T T T L S L E S E S V R N V L Y
gctcagctgcagttcacctatgatacgttgcgcagctacatcaatcgggctgtggcgag
A Q L Q F T Y D T L R S Y I N R A L A Q
atcgccgaggcctgggtgtggatcaacggcgaccctagaggcttcaaggaactcagc
I A E A W C V D Q R R T L E V F K E L S
aagatcaatccatcagccattctctcggccatctacaacaaaccgattgccgcggtttc
K I N P S A I L S A I Y N K P I A A R F
```

B

5'3' Frame 1

```
ttggaactggaaagttggccaatagctccgggtggaactccacgcgtagaaccaagaga
L E L E S L A N S S G V N S T R R T K R
agtacgggcaatacgaccaccctgtcgcttgaaagcgaatctgtacgaaatgtgctctac
S T G N T T T L S L E S E S V R N V L Y
gctcagctgcagttcacctacgatacgttgcgcagctacatcaatcgggctgtggcgag
A Q L Q F T Y D T L R S Y I N R A L A Q
atcgccgaggcctgggtgtggatcaacggcgaccctagaggcttcaaggaactcagc
I A E A W C V D Q R R T L E V F K E L S
aagatcaatccatcagccattctctcggccatctacaacaaaccgattgccgcggtttc
K I N P S A I L S A I Y N K P I A A R F
a
```

Figure (4.14): Normal codon and protein sequence of CMV wild type from Genbank (A) versus mutant codon and protein resulted (B) as indicated by blue color

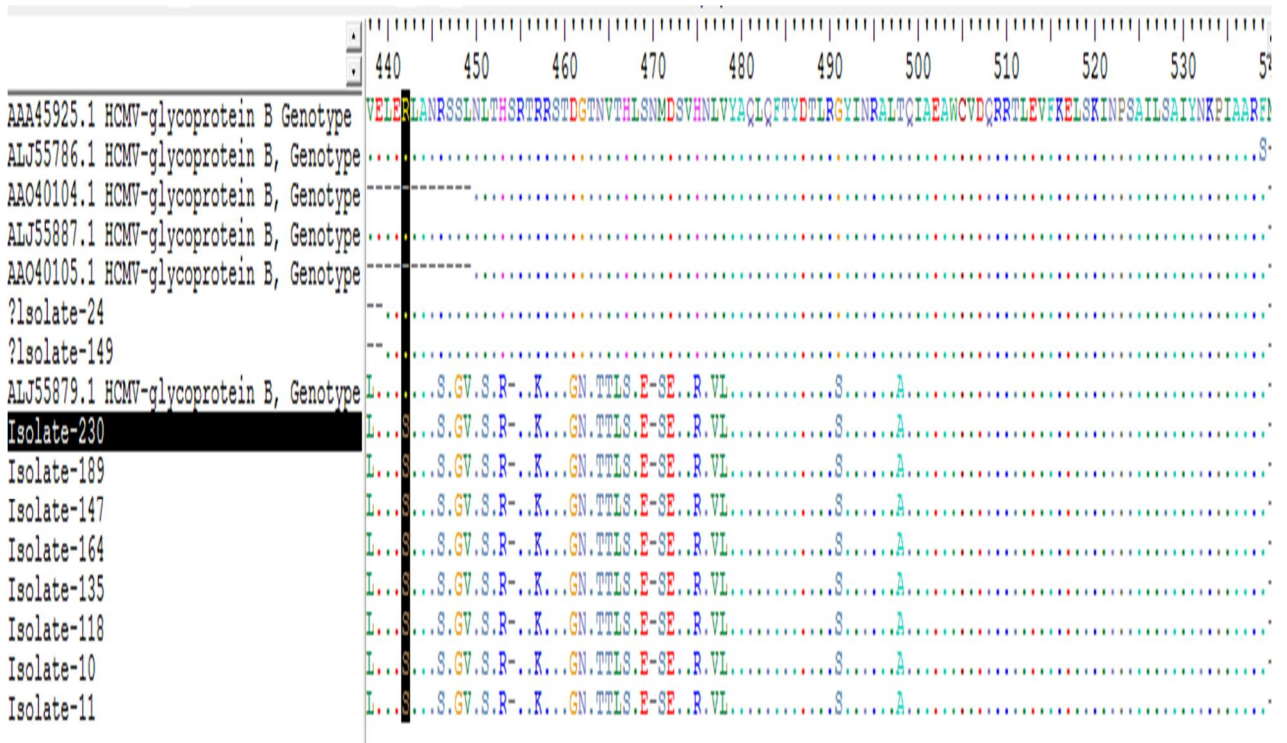


Figure (3.15): Amino acid multiple sequence alignment of Sudanese mutant gB gene compared to other gB genes from database. The transversion mutations in isolates 230, 189, 147, 164, 135, 118, 10, and 11. Substitution of the amino acid Arginine (R) to Serine(S) as indicated by black color. The alignment was performed using Bio-Edit



Figure (4.16): Tertiary protein structure of wild gB-3 gene of isolates 230 ,189, 147,164, 135, 118, 10 and 11. The predicted amino acid Arginine at position 85 from Genbank that predicted by Chimera software version 1.9.

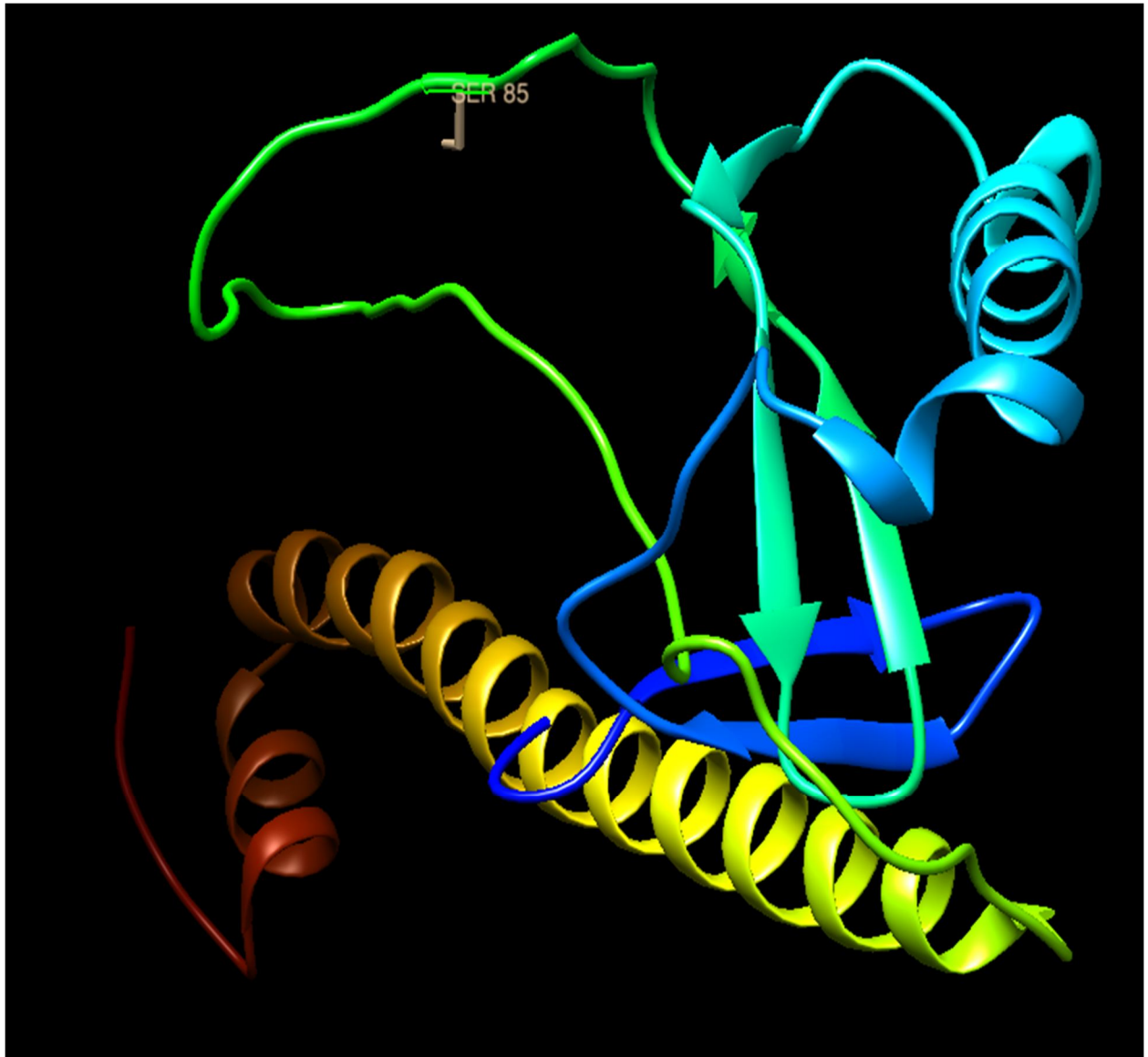


Figure (4.17): Tertiary protein Structure of Mutant gB-3 gene of isolates 230,189, 147,164, 135, 118, 10, and 11. The predicted amino acid Serine at positin 85 that drawn by Chimera software version 1.9.

Table (4.11): Predictor of protein stability

Characteristics	Value
Position of protein	85
Amino acid in wild type protein (WT)	R
New amino acid after mutation	S
Stability	Decrease
Reliability index (RI)	9
Temperature in Celsius degrees	25
Ph= $-\log(\text{H}^+)$	7

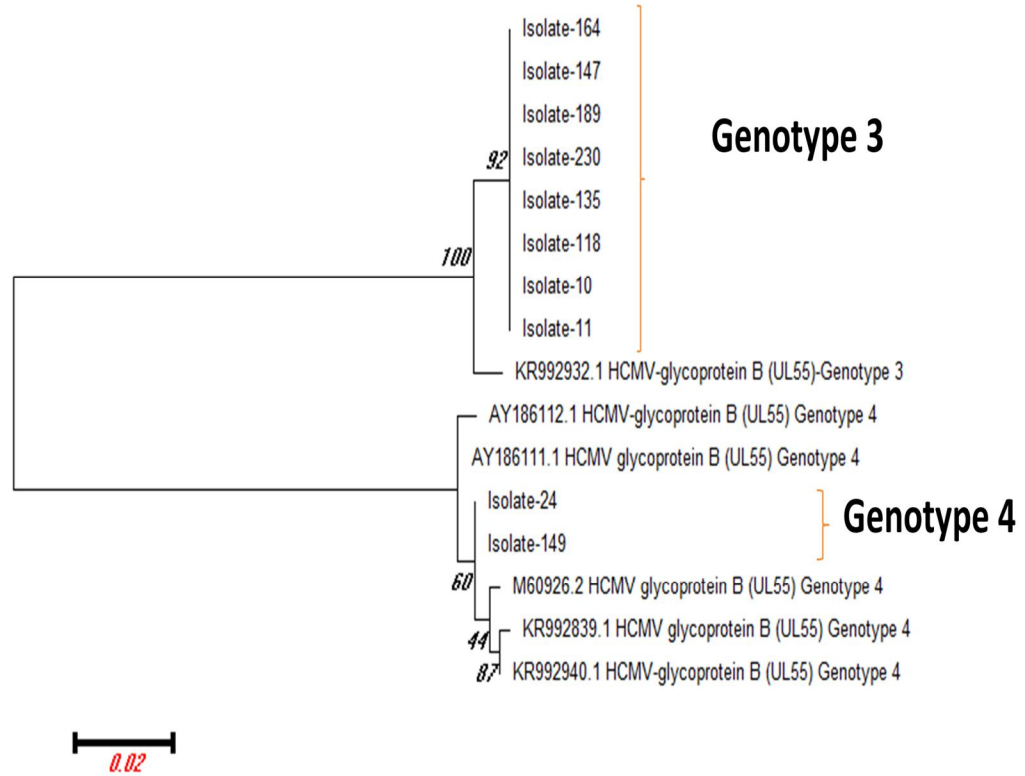


Figure (4.18): Phylogenetic tree based on gB gene sequences of 10 CMV isolates from renal transplant recipients. The phylogenetic tree analysis was constructed using the neighbor-joining method in MEGA 6.

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Cytomegalovirus infection is one of the most frequently encountered opportunistic viral pathogens in renal transplantation. CMV disease is a risk factor for acute allograft rejection in patients with kidney transplantation (Hasanzamani *et al.*, 2016). It is considered as a potential contributor to graft loss and a cause of severe mortality and morbidity. Without intervention and preventative therapy, symptomatic CMV infection can develop in 20% to 60% of kidney transplant recipients (Nafar *et al.*, 2014) of a mortality rate of 5% (Madi *et al.*, 2011a).

This study was designed to determine the frequency of CMV infection and its gB genotypes distribution among Sudanese renal transplant recipients.

The study population was 104 renal transplant recipients (69.2%) were males and (30.8%) were females. The study found that most of recipients were males with male/female ratio about 2:1. This finding in agreement with Hasanzamani *et al* (2016) in Iran, who reported that 41(62.1%) of population were males and 25(37.9%) were females. Similar results were observed by Khameneh *et al* (2013) in Iran, his finding showed that most of populations under study were males 22 (61.1%), and females were 14 (38.9%).

In the present study 79 (76%) of population received organs from relative donors and only 25 (24%) were received organs from non-relative donors. This results is relatively differ from those obtained by several studies. Pourmand *et al* (2006) in Iran, 85% of his population received living non-relative organs, 8% living-relative organs and 7% cadaveric organs, Nafar *et al* (2014) in Iran found that most of his population received Living unrelative organs (71%), Living relative were (4%), and cadaveric were (25%). van Ree *et al.*, (2011) in Italy also found that (83%) of his population received cadaveric organs.

Most recipients in the present study received triple immunosuppressive therapy that make them more liable to CMV infection as reported by Nafar *et al* (2014) which indicate that high immunosuppressive regimen is associated with a higher

risk for CMV infection. Al-Alousy *et al* (2011) observed that the type, intensity of immunosuppressive therapy, and the level of immunosuppression act as a critical exogenous factor influencing the HCMV reactivation following transplantation such as cyclosporine.

The present results revealed high serofrequency of CMV IgG (99%), among this population which indicate that this population may experience CMV early in their life. This result in agreement with that obtained by Enan *et al* (2011)(100%), and Awad Alkareem *et al* (2013) (98%) in Sudan in the same population. Khairi *et al* (2013) also observed high seroprevalence of CMV in Sudan among pregnant women (97.5%). High prevalence of CMV in Sudan observed by Abd Alla *et al* (2015) among hemodialysis patients (95.7%), which indicate an earlier acquisition of the infection. The results in this study in agreement with Bates and Brantsaeter, (2016) who reported that seroprevalence of CMV IgG in Africa is close to 100% in most studies of adults, that could possibly attributed to lower socioeconomic status, broadly neglected diagnosis and treatment of CMV-related disease, and no randomized clinical trials of anti-CMV drugs have been conducted to date.

On the other hand the serofrequency of IgM antibodies among population was (26%), which indicates reactivation of CMV infection, this finding is relatively higher than those obtained by Enan *et al* (2011) and Awad Alkareem *et al* (2013) in Sudan, who reported that IgM was detected in (6%) of renal transplant recipients. In contrast, Khameneh *et al* (2013) in Iran obtained higher result of anti-CMV IgM seropositivity (37.5%) among transplant recipients. Lower finding was observed in another Iranian study of Tarabadi *et al* (2011), on renal transplant recipients, 16.1% were seropositive for anti-CMV IgM. Although serofrequency of IgM is high in this study in contrast to other previous studies in Sudan, but serological methods remain has a limited diagnostic value in the transplant patient groups. This might be to a time lag between active infection and IgM production and delayed seroconversion due to immunosuppressive therapy. Results can also be confounded by antibody based therapy (Heli, 2004), and blood transplantation (Ortiz and André, 2011).

The current study showed that CMV DNA (viremia) was detected in (38.5%) of renal transplant recipients using quantitative real time PCR. These results are relatively higher than those observed by William *et al* (2000) (22%), Madi *et al* (2007) in Kuwait (24%), Enan *et al* (2011) in Sudan (32.7%), and Lashini *et al* (2011) in Iran (25.9%). In addition, parallel finding to this results observed by Garrigue *et al* (2008) (36.6%) and Zhang *et al* (2012) in China (37.7%). In contrast, lower result was obtained by Cordero *et al* (2012) in Philippines (5.8%), Cupic *et al* (2012) in Serbia (12.5%), and Khalafkhany *et al* (2016) in Iran (15.9%). No antiviral prophylactic or preemptive therapy may explain the higher frequency of CMV among this study group.

It is of interest to observe that the average of CMV DNA viral load was 358×10^4 copies/ml (6.5 \log_{10}) with minimum viral load 62 copies /ml (1.8 \log_{10}) and maximum viral load 1.43×10^8 copies/ml (8.2 \log_{10}). The lack of screening in most patients probably explains the high viral loads at diagnosis and the large variation in viral loads.

In the present study (51%) of population had post-transplantation time from < than One - 12 months. This finding increases the possibility of primary CMV infection or reactivation. The frequency of CMV viremia from the total positive was higher in the first 12 month of transplantation (60%) compared with the later onset. Similar results were obtained by Khalafkhany *et al* (2016) in Iran who detected CMV viremia in 31.2% of 0-3 months, 30.7% of 4-6 months, 17.5% of 7-12 months, 10.2% of 13-24 months, and 6.4% of the recipients of more than 24 months post-transplantation.

Although Pereyra and Rubin, (2004), and Aguado *et al* (2012), reported that high doses of methyl prednisolone or agents such as ALG, ATG, mycophenolate mofetil (cellcept) and azathioprine (imuran) were associated with increased incidence of CMV viremia. The present results showed no significant difference ($P.value > 0.05$) between the types of immunosuppressive therapy and CMV viremia. On the other hand, there was significant difference ($P.value = 0.05$) between the types of immunosuppressive therapy and high viral loads which correlate with CMV disease, may be due to triple immunosuppressive therapy used . In addition to intensive immunosuppressive regimen,

such as using tacrolimus, and cyclosporine that associated with a higher risk of CMV infection and disease.

In this study higher viral load correlate precisely with development of CMV-related symptoms and viral loads were slightly lower with asymptomatic patients (high significant difference $P.value = 0.00$), in which (82.4%) of patients had clinical symptoms of CMV disease with viral loads >1000 copies/ml. Moreover (95.7%) of patients had no clinical symptoms of CMV disease with viral loads <1000 copies/ml confirming previous reports by Hadaya *et al.*, (2003), Knipe and Howley (2007), Madi *et al.*, (2007), Helanter *et al.* , (2010) and Rangbar-Kermani *et al.*, (2011)

Furthermore, the median level of viral load was higher in patients with symptomatic CMV disease than those without symptoms. The discrepancy only in three patients (17.6%) had clinical symptoms of CMV disease with viral load < 1000 copies/ml, and one patient (4.3%) had no obvious symptoms of CMV disease with viral load >1000 copies/ml. These discrepancy could be explained by several factors such as the source of donor kidney, nature of immunosuppressive, and genotypes of the virus.

Findings of the present study indicated that fever, fever leucopenia and gastrointestinal disease with abdominal pain and diarrhea were the most common presenting symptoms of CMV disease. This finding is similar to that obtained by Ardalan, (2012) who reported that most symptomatic CMV infections manifest as fever, fatigue, cytopenia and gastrointestinal tract is the most common site of tissue-invasive CMV infection. Another opinion was adopted by Helanter *et al.*, (2010), which found that most of population under study suffering from gastrointestinal symptoms, suggestive of gastrointestinal CMV disease. Lashini *et al.*, (2011) observed that fever, leucopenia, interstitial pneumonia and joint inflammation were the most prominent clinical symptoms of CMV disease.

The result of sequencing and genotyping of HCMV gB gene (UL 55) for 10 CMV isolate that were confirmed to be positive by PCR were taken from symptomatic transplant recipients revealed that gB3 (80%) was the most

frequent genotype among Sudanese renal transplant recipients whereas gB4 was (20%) and no mixed genotypes were observed to our knowledge. No data is available in Sudan on gB genotyping in renal transplant recipients nor immunocompetent host with CMV infection. The CMV sequences in this study considered the first Sudanese CMV sequences submitted and published in GenBank. These results in agreement with previous reports in Italy by Arista *et al.*, (2003) in which the predominant circulation of HCMV strains were gB type 2 and 3 among both the immunocompetent host with a primary HCMV and renal transplant recipients with or without HCMV disease. Rather similar results were obtained by de Vries *et al.*, (2011), in Netherland involved renal transplant recipients and congenitally infected newborns in which gB1 and gB3 being the most prevalent genotypes. Gandhoke *et al.*, (2012) in India found that gB 3 was the most prevalent genotype in symptomatic infants.

The results of this study differ from previous studies undertaken in other parts of the world. WOO *et al.*, (1997) in Hong Kong in which the most prevalent gB genotype in renal-transplant recipients was gB type 1, whether the patient manifested CMV disease or not. In contrast, Pasca *et al.*, (2003) in Kuwait reported that gB1 (27%) was the most frequent genotype followed by gB2 (25%), gB3 (19%), gB4 (1%) and mixed genotypes were (27%). Coaquette *et al.*, (2004) in France indicated that the distribution of gB genotypes among renal transplant was gB1 (28.9%) of patients; gB2 (19.6%); gB3 (23.7%) gB4 (2.0%); and mixed infection (25.8%). Dieamant *et al* (2009), reported different results, that gB1 and gB2 were the most frequent genotypes among Brazilian pediatric renal transplant patients. Differ results also was observed by Khalafkhany *et al.*, (2016) in Iran, which mentioned that gB1 (26.5%), gB2 (20.5%), gB3 (17.6%), and gB4 (5.9%) genotypes was detected. Mixed genotype infection was observed in 29.4% of the recipients.

The substantial differences in genotype frequencies in this study compared to previous reports and no gB1 and gB2 were detected might in part, be due to variation in the geographical distribution of the CMV genotypes.

In the current study, no mixed genotypes were observed this might be due, the fact that, limitation of the available technologies or PCR protocol, and or low number of individual clone is sequenced down to the level of 5%. In addition, mixed infections accounted for roughly one quarter to one-half of HCMV infections over a wide range of human populations (Renzette *et al.*, 2014).

The results of genotyping and sequencing in this study represent the first genetic characterization of HCMV mutations in Sudan. Transversion mutations in gB gene was identified in 8 of the Sudanese gB3 genotypes suggesting that a high gB3 gene nucleotide sequence variability correlated with an elevated amino acid substitution rate. This substitution mutation resulted in decrease of protein stability.

The obtained results of protein tertiary structure showed difference in size between wild type and mutant type. Wild type residue is bigger. This is probably altering or particularly increasing viral pathogenicity, as, gB gene is one of the most important envelope glycoproteins of HCMV, is implicated in virus entry, cell-to cell spread, and the fusion of infected cells (Jun *et al.*, 2012). The variability and mutations particularly in gB that arise can be advantageous to the virus resulting in increase in viral fitness and adaptation (Stangherlin *et al.*, 2017).

Findings of phylogenetics analysis in this study indicated that the HCMV was related to several strains worldwide that are far from Sudan (USA, Spain and Brazil). This is believed that their presence reflects the wider circulation of these strains in our geographical area and worldwide for both renal transplant recipients as well as immunocompetent with primary HCMV infection or disease.

5.2. Conclusion

This study concluded that qRT-PCR from plasma is a very useful and sensitive method, allowed an early diagnosis of CMV replication after transplantation and can help monitoring patients during CMV infection and disease.

CMV viremia viral loads were slightly lower in asymptomatic patients.

The present study documented the association of CMV disease with intensive immunosuppressive regimen, such as using triple therapy, tacrolimus, cyclosporine and as a higher risk factors for CMV infection and disease.

In this study, CMV gB3 is considered the most predominant glycoprotein B genotype in Sudanese renal transplant recipients.

The CMV sequences in this study considered the first Sudanese CMV sequences submitted and published in GenBank.

5.3. Recommendations

Early monitoring of CMV using sensitive method such as qRT-PCR that exactly detect viral replication can provide guiding information help the clinician to starting preemptive antiviral therapy that might have the advantages of reducing the occurrence of CMV disease

Additional research is suggested to investigating other distinct glycoprotein genotyping (gH, gO, gN) to detect the association of gB genotypes with specific clinical features that might eventually shed light on the relevance of gB to the development of CMV-associated disease.

Improved genotype-specific real-time-PCR-based assays or Ultra-deep pyrosequencing for gB genotyping, allowing detection and quantitation of distinct genotypes in mixed infections even less than 1%.

REFERENCES

- Abd Alla**, A. E., Altayeb, A. A., Alshareef, M. A., Elboni, M. S., Ali, S., Abosalif, K. O. (2015). Seroprevalence of Cytomegalovirus Antibodies among Hemodialysis Patients in Gezira State, Central Sudan. *WJPR*. **4** (7): 19-25.
- Afzali**, B., Lechler, R. and Lombardi, G. (2010). Graft Rejection: Immunological Suppression. In: Encyclopedia of life science (ELS). Jone Wiley and Sons, Ltd: Chichester www.els.net.
- Aguado**, J. M., Navarro, D., Juan, S. R. and Caston, J. J. (2012). Cytomegalovirus infection in solid organ transplantation. *Enferm Infecc Microbiol Clin*. **30** (Supl 2):57-62.
- Akhter**, K. and Bronze, M.S. (2015). Cytomegalovirus Workup. Copyright 1994-2016 by Web MD LLC.
- Al- Khaweledy**, A.J., Al- Ammar, M.H., Ziad, A.M. (2014). Cytomegalovirus Infections are the most Common Infection among Patients with Renal Failure at Al-Najaf Province. *Photon J Microbiol*. **107**: 200-206.
- Al-Alousy**, B. M ., Hasan Abdul-Razak ,S.H., Al-Ajeeli, K. S. and Al-Jashamy, K.A. (2011). Anti-HCMV IgG positivity rate among renal transplant recipients in Baghdad. *Saudi Kidney Dis Transplant*. **22**: 1269-1274.
- Al-Obaidi**, A. B., Abd, K H., Kadhim, H. S., Habib, M. A., Abdlameer A. S. and Shamran, H. A. (2015). BK polyomavirus and Cytomegalovirus Co-infections in renal transplant recipients: a single center study. *IJAR*. **3**(1): 856-864.
- Ardalan**, M. (2012). Rare Presentations of Cytomegalovirus Infection in Renal Allograft Recipients. *Nephro-Urol Mon*. **4**(2):431-6.
- Arista**, A., De Grazia, A., Giammanco, G.M., Di Carlo, P. and Iannitto, E. (2003). Human cytomegalovirus glycoprotein B genotypes in immunocompetent, immunocompromised, and congenitally infected Italian populations. *Arch Virol*. **148**: 547-554.

Atschul, S.F., Madden, T.L and Schaffer, A. A. (1997). Gapped BLAST and PSI-BLAST. A new generation of protein database search programmes. *Nucleic Acid Res.* **25**: 3389-3402.

Awadalkareem, A., Adam, M., Ahamed, I. F. and Khalafalla, A. I. (2013). Prevalence of IgG and IgM antibodies to human cytomegalovirus among Sudanese renal transplant recipients and haemodialysis patients. *Sudan Med Monit* .**8**(4):183-185.

Banaga, A. S., Mohammed, E. B., Siddig, R. M., Salama, D. E., Elbashir, S. B., Khojali, K. O., Babiker, R. A., Elmusharaf, K. and Homeida, M. M. (2015). Causes of end stage renal filature among haemodialysis patients in Khartoum State /Sudan. *BMC Res Notes*.**8**:502-508.

Barans. P., Blazquez-Gamero, D., Glaindo, A., Prieto, C., Olabarrieta, I., Guarado, I. and Folgueria, L. (2015). Cytomegalovirus genotype distribution among congenitally and postnatally infected patients; associated of particular glycoprotein (g) B and gN types with symptomatic disease. *Open Forum Infect Dis.* **2**(4). OFV151. www.ncbi.nlm.nih.gov.

Bates, M and Brantsaeter, A. B. (2016). Human cytomegalovirus (CMV) in Africa: a neglected but important pathogen. *J Virus Erad.* **2**: 136-142

Bernarde, K., Folkmane, I., Chapenko, S., Murovska, M. Rozentals, R. (2007). The Impact of Early Cytomegalovirus (CMV) Infection and CMV Disease on Long-Term Kidney Allograft Function and Late Complications after Kidney Transplantation: a Clinical Study. Scientific Paper University of Latvia .**12**:13-19.

Bhattarakosol, P. and Chantaraarphonkun, S. (2007). Prevalence of Human Cytomegalovirus (HCMV) GB Genotypes in Thai Patients. **38**(5): 835-840.

Bialas, K.M., Swamy, G. K. and Permar, S. R. (2015). Perinatal Cytomegalovirus Infections: Epidemiology, Prevention, and Treatment. *Neo Reviews*.**16** (4): 231-235.

BIO forum Europe. (2006). Monitoring of CMV Infections Using Real-Time PCR.

Bohl, D. L. Koch, M. J. Brennan, D. C. (2007). Viral Infections in Renal Transplantation: A Clue to Excessive Immunosuppression . *J Bras Nefrol.* **29**(3):185-190.

Brennan, D.C. (2001). Cytomegalovirus in renal transplantation. *J. Am. Soc. Nephrol.* **12**(4): 848-855.

British Transplantation Society Guidelines for the Prevention and Management of CMV Disease after Solid Organ Transplantation. (2011). 3^{ed} ed.

Britt, W. J. and Mach, M., (1996). Human cytomegalovirus glycoproteins. *Intervirology.* **39**(5-6): 401-412.

Brooks, G. F., Carroll, K. C., Butel, J. S. and Morse, S. A. (2007). Jawetz, Melnick, and Adelberg's Medical Microbiology, 24th ed. United States of America: McGraw-Hill Companies. ISBN-13: 978-0-07147666-9.

Bruggeman, C. A. (2000). Does Cytomegalovirus Play a Role Atherosclerosis. *Herpes.* **7**: 51-54.

Buonsenso, D., Serrantl, D., Gargiullo, L., Ceccarelli, M., Ranno, O. and Valentini, P. (2012). Congenital cytomegalovirus infection: Current strategies and future perspectives. *Eur Rev Med Pharmacol Sci.* **16**: 919-935.

Caliendo, A. M., George, K. S., Kao, S., Allegra, J., Tan, B. and LaFontaine, R. (2001). Comparative Quantitation of Cytomegalovirus (CMV) DNA in Solid Organ Transplant Recipients with CMV Infection by Using Two High-Throughput Automated Systems. *TRANSPLANT.* **39**(12):4472-4476.

Caltenco-Serrano, R., Sánchez-Huerta, José. Luis., Vargas-Jiménez ,R., Rodríguez-Suárez, R. S. and Gómez-Barreto, D. (2001). Cytomegalovirus infection in patients with solid-organ transplant. Recently reviewed immunologic response and pathogenicity mechanisms. *Rev Latinoam Microbiol.* **43**(4): 177-182.

Cannon, M. J., Terri, S. S. and Hyde, B. (2010). Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol.* **20**(4):202-213.

Capriotti, E., Fariselli, P., Rossi., I. and Casadio., R. (2008). A three state prediction of single point mutations on protein stability change. *BMC Bioinformatics.* **9**(2):S6

- Chakravarti, A., Kashyap, B., and Matlani, M. (2009).** Cytomegalovirus infection: An Indian perspective. *Indian J Med Microbiol* .**27**: 3-11.
- Chou, S. (1992).** Molecular epidemiology of envelope glycoprotein H of human cytomegalovirus. *J Infect Dis.* **166**(3): 604-607.
- Chou, S. (2014).** Comparative analysis of sequence variation in gp116 and gp55 component of glycoprotein B of human Cytomegalovirus virology **188**(1): 388-390.
- Chou, S. W. and Dennison, K. M. (1991).** Analysis of interstrain variation in cytomegalovirus glycoprotein B sequences encoding neutralization-related epitopes. *J Infect Dis.* **163**(6): 1229-1234.
- Coaquette, A., B, A., Dirand, C., Varin, A., Chen, W., and Herbein, G. (2004).** Mixed Cytomegalovirus Glycoprotein B Genotypes in Immunocompromised Patients. *ClinInfect Dis.***39**:155-161.
- Cobo, F. (2012).**Application of Molecular Diagnostic Techniques for Viral Testing. *O Virol J.* **6** (Suppl 1: M7):104-114.
- Collier, L. and Oxford, J. (2006).** The betaherpesviruses: cytomegalovirus and human herpesviruses 6 and 7 .Human Virology. 3rd ed. *New York*: Oxford University Press. pp: 149-152.
- Cordero, E., Casasola, C., Ecarma, R. and Danguilan, R. (2012).** Cytomegalovirus disease in kidney transplant recipients: incidence, clinical profile, and risk factor. *Transplant Proc.* **44**(3):694-700.
- Corte, F.D. M., Samot, J., Garrigue, I., Magnin, N., Reigadas, S. and Couzi, L., et al. (2010).** Variability and recombination of clinical human cytomegalovirus strains from transplantation recipients. *J Clinic Virol.* **47**(2):161-169.
- Costa, C. (2011).** Cytomegalovirus and Acute Rejection in Kidney Transplantation. *Nephro-Urol Mon.* **3**(4):237-239.
- Couzi, L., Levailant, Y., Jamai, A., Pitard, V., Lassalle, R. and Martin, K. et al. (2010).** Cytomegalovirus-induced gammadelta T cells associate with reduced cancer risk after kidney transplantation. *J. Am. Soc. Nephrol.* **21** (1): 181-188.

- Cukuranovic, J., Ugrenovic, S., Jovanovic, I., Visnjic, M. and Stefanovic, V. (2012).** Viral Infection in Renal Transplant Recipients. *Scientific World J.* **2012**: 1-18.
- Cunha, A. A., Aquino, V. H., Mariguela, V., Nogueira, M. L. and Figueiredo, L. T. M. (2011).** Evaluation of Glycoprotein B Genotypes and Load of CMV Infecting Blood Leukocytes on Prognosis of AIDS Patients. *Rev. Inst. Med. Trop. Sao Paulo.* **53(2)**:83-88.
- Cupic, M., Lazarevic, J., Pravica, V., Banko, A., Karalic, D., Naumovic, R. et al. (2012).** The prevalence of the most important viral infection in renal transplant recipients in Serbia. *Arch. Biol. Sci.* **64** (4): 1285-1296.
- Dal Monte, P., Pignatelli, S., Mach, M. and Landini, M. P. (2001).** The product of human cytomegalovirus UL73 is a new polymorphic structural glycoprotein (gpUL73). *J Hum Virol.* **4(1)**: 26-34.
- Dar, L. (2007).** Identifying human cytomegalovirus genotypes and defining their clinical significance. *Indian J Med Res.* **126**: 99-100.
- de Vries, J. J.C., Korver, A. M. H., Verkerk, P. H., Rusman, L., Claas, E. C. J., Loeber, J. G., Kroes, A. C. M. and Vossen, A.C.T.M. (2011).** Congenital Cytomegalovirus Infection in the Netherlands: Birth Prevalence and Risk Factors. *J Med Virol.* **83**:1777-1782.
- Deckers, M., Hofmann, J., Kreuzer, K., Reinhard, H., Edubio, A., Hengel, H. et al. (2009).** High genotypic diversity and a novel variant of human cytomegalovirus revealed by combined UL33/UL55 genotyping with broad-range PCR. *Virol. J.* **6**:210-221.
- Dieamant, D. D., Bonon, S. H. A. Prates, L. C., Belangelo, V. M. S., Pontes, E. R. and Costa, S. C. B. (2010).** Active human Cytomegalovirus infection and Glycoprotein B genotypes in Brazilian pediatric renal or hematopoietic stem cell transplantation patient. *Braz J Microbiol.* **41(1)**: 262-274. ISSN: 1678-4405.
- Dirk, R., Kuypers, J. and Vanrenterghem, Y. (1998).** Prophylaxis of cytomegalovirus infection in renal transplantation. *Nephrol Dial Transplant.* **13**: 3012-3016.

- Egli, A., Binggeli, S., Bodaghi, S., Dumoulin, A., Funk, G. A., Khanna, N., et al.** (2007). Cytomegalovirus and polyomavirus BK posttransplant. *Nephrol Dial Transplant.* **22** (Suppl 8): viii72–viii82.
- Emery, V. C., Asher, K. and Sanjuan, K. D.** (2012). Importance of the cytomegalovirus seropositive recipient as a contributor to disease burden after solid organ transplantation. *J Clin Virol.* **54**:125-129.
- Emery, V.C., Copea, A.V., Bowena, E. F., Gora, D. and Griffiths, P. D.**(1999).The Dynamics of Human Cytomegalovirus Replication in Vivo .*J E M.* **190**(2): 177-182.
- Enan, K.A., El-Hussein, A.M., El Eragi, A. and Elkhider, I.M.** (2009). Sero-reactivity to human cytomegalovirus in a cohort of Sudanese renal transplant patients. *Khartoum Medical J.* **2** (2): 197-199.
- Festary, A., Kourí, V., Correa, C. B., Verdasquera, D., Roig, T. and Couret. M. P.** (2015).Cytomegalovirus and Herpes Simplex Infections in Mothers and Newborns in a Havana Maternity Hospital .*MEDICC Review.* **17**(1):29-34.
- Figlerowicz, M., Modlinska -Cwalinska, A., Mania, A., Mazur-Melewska, K., Kemnitz, P., Jonczyk-Potoczna, K. and Sluzewski, W.** (2011). Human Cytomegalovirus Infection as A lifelong Health Problem. *J B C B B.* **92**(4): 352-357.
- Fowler, K. B., S. Stagno. and Pass, R.F.** (2003). Maternal immunity and prevention of congenital cytomegalovirus infection. *JAMA.* **289**(8): 1008-1011.
- Gandhoke, I., Hussain, S. A., Pasha, S.T., Chauhan, L.S., and Khare, S.** (2013). Glycoprotein B Genotyping in Congenital/perinatal Cytomegalovirus Infection in Symptomatic Infants. *Indian Pediatr.* **50**:663-667.
- Garrigue, I., Doussau, A., Asselineau, J., Bricout, H., Couzi, L., Rio, C., et al.** (2008). Prediction of Cytomegalovirus (CMV) Plasma Load from Evaluation of CMV Whole-Blood Load in Samples from Renal Transplant Recipients .*J. Clin. Microbiol.* **46**(2): 493-498.
- Görzer, I., Guelly, C., Trajanoski, S. and Puchhammer-Stöckl, E.** (2010). Deep Sequencing Reveals Highly Complex Dynamics of Human Cytomegalovirus Genotypes in Transplant Patients over Time. *J. Virol.* **84** (14):7195-7203.

- Greenwood, D., Barer, M., Slack, R. and Irving, W. (2011).** Herpesviruses. Medical Microbiology .A Guide to Microbial infection. 18th ed. Edinburgh: Churchill Livingstone Elsevier. pp: 437-441.
- Griffiths, P. D. and Lumley, S. (2015).** Cytomegalovirus Infections in Humans. Wiley Online Library.
- Griffiths, P., Baraniak, I. and Reeves, M. (2015).** The pathogenesis of human cytomegalovirus . *J. Pathol.* **235**(2): 288-297.
- Haaheim, L. R., Pattison, J. R. and Whitley, R. J. (2002).** A Practical Guide to Clinical Virology. 2nd ed. Chichester, West Sussex: John Wiley and Sons. pp:150-156.
- Habbal, W., Monem, F and Gartner, B.C. (2009).** Comparative evaluation of published cytomegalovirus primers for rapid real-time PCR: which are the most sensitive?. *J Med Microbiol.* **58**: 878-883.
- Hadaya, K., Wunderli, W., Deffernez, C., Martin, P. Y., Mentha, G., Binet, I., Perrin, L., Kaiser, I., (2003).** Monitoring of Cytomegalovirus infection in solid- organ transplant recipients by an ultrasensitive plasma PCR assay. *J Clin Micorbiol.* **41**(8):3757-3764.
- Hall, TA. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl.Acids.Symp.Ser.* **41**:95-98.
- Halling, V. W., Maine, G. T., Groettum, C. M., Wilson, J. A., Spesard, J., Brojanac, S., et al. (2001).** Clinical evaluation of a new recombinant antigen-based Cytomegalovirus immunoglobulin M immunoassay in liver transplant recipients. *TRANSPLANT.* **71**: 395-397.
- Halloran, P.F. (2004).**Drug Therapy: Immunosuppressive Drugs for Kidney Transplantation. *N Engl J Med.* **351**(26):2715-2729.
- Hariharan, S. (2007).** Update on Prevention, Detection, and Management of Viral infections in The Renal Transplant Recipients. *Adv Stud Med.* **7**(13):401-410.
- Hartmann, A., Sagedal, S. and Hjelmesaeth, J. (2006).** The natural course of cytomegalovirus infection and disease in renal transplant recipients. *TRANSPLANT.* **82** (2): S15-S17.

- Hartono, C., Muthukumar, T Suthanthiaran, M.** (2013). Immunosuppressive drug therapy. *Cold Spring Harb Perspect in Med.* **3**: a015487. www.perspectivesinmedicine.org.
- Harvey, R. A., Cornelissen, C. N. and Fisher, B. D.** (2013). Lippincott's Illustrated Reviews: Microbiology. 3rd ed. Philadelphia: Lippincott Williams and Wilkins. pp: 263-265.
- Hasanzamani, B., Hami, B., Zolfaghari, V., Torkamani, M., Sabagh, M. G. and Simab, A. S.**(2016). The effect of cytomegalovirus infection on acute rejection in kidney transplanted patients. *J Renal Inj Prev.* **5**(2): 85-88.
- Helanterä, I., Lautenschlager, I. and Koskinen, P.** (2009). Prospective follow-up of primary CMV infections after 6 months of valganciclovir prophylaxis in renal transplant recipients. *Nephrol Dial Transplant.* **24**(1):316-320.
- Helanterä, I., Kyllönen, L., Lautenschlager, I., Salmela, K. and Koskinen, P.** (2010). Primary CMV Infections are Common in Kidney Transplant Recipients after 6 Months Valganciclovir Prophylaxis. *Am. J. Transplant.* **10**(9): 2026-2032.
- Heli, P.** (2004). Quantitative PCR in the Diagnosis and Monitoring of Human Cytomegalovirus Infection in Organ Transplant Patients. Helsinki University Printing House. ISBN: 952-10-1883-6.
- Hobom, U., Brune, W., Messerle, M., Hahn, G. and Koszinowski, U.H** (2000). Fast screening procedures for random transposon libraries of cloned herpesvirus genomes: mutational analysis of human cytomegalovirus envelope glycoprotein genes. *J Virol.* **74**(17): 7720-7729.
- Hodson, E. M., Jones, C. A., Webster, A. C., Strippoli, G. F., Barclay, P. G., Kable, K., Vimalachandra, D. and Craig, J.C.** (2005). Antiviral medications to prevent Cytomegalovirus disease and early death in recipients of solid-organ transplants: a systematic review of randomised controlled trials. *Lancet.* **365** (9477): 2105-2115.
- Huang, C.C., Meng, E.C., Morris, J.H.** (2014). Enhancing UCSF Chimera through web. *Nucleic Acids Res.* **42**(Web Server issue): W478-484.

Huber, M. T. and T. Compton (1999). Intracellular formation and processing of the heterotrimeric gH-gL-gO (gCIII) glycoprotein envelope complex of human cytomegalovirus. *J Virol.* **73**(5): 3886-3892.

Husni, R. N., Gordon, S. M., Longworth, D. L., Arroliga, A., Stillwell, P. C. and Avery, R. K. (1998). Cytomegalovirus infection is a risk factor for invasive aspergillosis in lung transplant recipients. *Clin Infect Dis.* **26**(3):753-755. ISSN: 1058-4838.

Huurman, V.A.L. Kalpoe, J. S., van de Linde, P., Vaessen, N., Ringers, J. Kroes, A.C. M., Roep, B. O. and de Fijter, J. W. (2006). Choice of antibody immunotherapy influences cytomegalovirus viremia in simultaneous pancreas-kidney transplant recipients. *Diab Care.* **29**: 842-884.

Ishibashi, K. and Suzutani, T. (2012). Role of Cytomegalovirus Reinfection in Acute Rejection and CMV Disease after Renal Transplantation, Renal Transplantation - Updates and Advances. ISBN: 978-953-51-0173-4.

Issa, N., and Braun, W. E. (2013) . Renal Transplantation. Cleveland Clinic Foundation.

Johansson, I. (2014). Cytomegalovirus Infection in Heart and Lung Transplant Patients with focus on long-term-outcome .inger.johansson@infect.gu.se. ISBN 978-91-628-9157-2.

John, B., Alexandre, L. and Mark, B. (2002). Gene Mark S: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Res.* **29**:2607-2618.

Jun, F., Minhuan , L., Yadan , M., Yaping , H., Hanying , L., and Jianhua, H. *et al.* (2012). Development of two potential diagnostic monoclonal antibodies against human cytomegalovirus glycoprotein B. *Braz. J. Med. Biol.Res.* **45**: 583-590.

Kahan, B.D. (2000). Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomized multicentre study. *Lancet.* **356**:194-202.

Kalpoe, J. S., Kroes, A. C. M., de Jong, M. D. Schinkel, J., de Brouwer, C. S., Beersma, M. F. C. and Claas, E. C. J. (2004). Antigen Detection Criteria by Analysis of Correlation to Measurement and Definition of Treatment Cytomegalovirus Plasma DNA Load *J. Clin. Microbiol.* **42**(4):1498-1504.

- Kalpoe, J.S.** (2007). Quantum virology. Improved management of viral infections through quantitative measurements. ISBN: 978-90-6464-146-6.
- Karuthu, S.** and Blumberg, A. E. (2012). Common Infections in Kidney Transplant Recipients. *Clin J Am Soc Nephro.* **7** (12): 2058-2070.
- Kaye, J. F.,** Gompels, U. A. and Minson, A. C. (1992). Glycoprotein H of human cytomegalovirus (HCMV) forms a stable complex with the HCMV UL115 gene product. *J Gen Virol.* **73** (Pt 10): 2693-2698.
- KDIGO.** (2009). Special Issue: KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients. *Amer J Transplant.* **9**:1-55.
- Khairi, S. I.,** Intisar K. S., Enan K. H., Ishag M. Y., Baraa A. M. and Ali Y. H. H. (2013). Seroprevalence of cytomegalovirus infection among pregnant women at Omdurman Maternity Hospital, Sudan. . *J. Med. Lab. Diagn.* **4** (4):45-49.
- Khalafkhany, D.,** Makhdoomi, K. and Afshari, A.T. (2016). Prevalence and genotype distribution of cytomegalovirus UL55 sequence in renal transplant recipients in North West of Iran. *J Med Virol.* **88**(9):1622-1627.
- Khameneh, Z .R.,** Sepehrvand, N. and Aghazadeh, T. (2013). Cytomegalovirus Infection among Iranian Kidney Graft Recipients. *Transplant Proc,* **45**:178-181.
- Khoury, J.A.** and Brennan, D.C. (2005). Infection complications in kidney transplant recipients: review of the literature. *Saudi J Kidney Dis Transpl.* **16**: 453-497.
- Kim, J. M.** and Kim, S. J. (2011). CMV Infection in CMV Seropositive Kidney Transplant Recipients, After the Kidney Transplant - The Patients and Their Allograft. ISBN: 978-953-307-807-6.
- Kim, J.M.,** Kim, S.J., Joh, J.W., Kwon, C.H., Song, S. and Shin, M. (2011). Is cytomegalovirus infection dangerous in cytomegalovirus seropositive recipients after liver transplantation?. *Liver Transplant.* **17**(4): 446-455. ISSN: 1527-6473.
- Knipe, D. M.** and Howley, P. M. (2007). Cytomegaloviruses. Fields Virology. 5th ed. vol 2. Philadelphia: Lippincott Williams and Wilkins. pp: 2703-2705.

- Kotton, C. F. N.** (2013). CMV: Prevention, Diagnosis and Therapy. *Am.J.Transplant.* **13**:24-40.
- Kotton, C. N.** and Fishman, J. A. (2005). Viral Infection in the Renal Transplant Recipient. *J Am Soc Nephrol* .**16**: 1758-1774.
- Kotton, C.N., Kumar, D., Caliendo, A. M., sberg, A. A., Chou, S., Danziger-Isakov, L. and Humar, A.** (2013). Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation . *TRANSPLANT.* **96**(4): 1-28.
- Kotton, C.N., Kumar, D., Caliendo, A.M., Asberg, A., Chou, S., Snyderman, D.R., Allen, U. and Humar, A.** (2010). International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *TRANSPLANT.* **89** (7): 779-795.
- Kudesia, G. and Wreghitt, T.** (2009). Cytomegalovirus . Clinical and Diagnostic Virology. 1st ed. New York. Cambridge University Press. pp: 17- 20.
- Kumar, V. and Gaston, R. S.** (2011). Advances in Kidney Transplant Immunosuppression: Emerging Biologics. *Dial Transplant.***40** (1): 30-32.
- Lagasse, N., Dhooge, I. and Govaert, P.** (2000). Congenital CMV-infection and hearing loss. *Acta Otorhinolaryngol Belg.* **54**(4): 431-436.
- Lashini, H., Goodarzi, Z., Hosseini, M. J. and Saberfar, E.** (2011). Development of PCR assay for early detection of CMV infection in renal transplant recipients. *Nephro-Urol Mon.***2**:106-8.
- Lehner, R., Stamminger ,T. and Mach ,T.** (1991). Comparative sequence analysis of human cytomegalovirus strains. *J Clin Microbiol.* **29**(11): 2494-2502.
- Lepage, N., Leroyer, A., Cherot-Kornobis, N., Lartigau, I., Miczek, S. and Sobaszek, A.** (2011). Cytomegalovirus seroprevalence in exposed and unexposed populations of hospital employees. *Eur J Clin Microbiol Infect Dis.* **30**(1): 65-70.
- Levinson, W.** (2010). Enveloped DNA Viruses. Review of Medical Microbiology and Immunology. 11th ed. United States of America: The McGraw-Hill Companies. ISBN: 978-0-07-170028-3.

- Liang, C.**, Famure, O., Li, Y. Kim. J. (2013). Incidence and Risk Factors for Leukopenia in Kidney Transplant Recipients Receiving Valganciclovir for Cytomegalovirus Prophylaxis. Multi-Organ Transplant Program, Toronto General Hospital, University of Toronto, Toronto, O. Meeting: 2013 American Transplant Congress.
- Ljungman, P.**, Griffiths, P. and Paya, C. (2002). Definitions of Cytomegalovirus Infection and Disease in Transplant Recipients. *Am. J. Transplant.* **34**:1094-1097.
- Mach, M.**, Kropff, B., Dal Monte, P. and Britt, W. (2000). Complex formation by human cytomegalovirus glycoproteins M (gpUL100) and N (gpUL73). *J Virol.* **74** (24): 11881-11892.
- Madi, A.**, Al-Nakib, W., Mustafa, A., Saeed, T., Pacsa, A., and Nampoory, M.R.N. (2007). Detection and Monitoring of Cytomegalovirus Infection in Renal Transplant Patients by Quantitative Real-Time PCR. *Med Princ Pract.* **16**:268-273.
- Madi, N.**, Al-Nakib, W., and Pacsa, A. (2011a). Does Cytomegalovirus Develop Resistance following Antiviral Prophylaxis and Treatment in Renal Transplant Patients in Kuwait? *Adv Virol.* **2016**:1-6.
- Madi, N.**, Al-Nakib, W., Pacsa, A. and Saeed, A. (2011b). Cytomegalovirus Genotypes gB1 and gH1 Are the Most Predominant Genotypes among Renal Transplant Recipients in Kuwait. *Transplant.Proc.* **43**: 1634-1637.
- Manuel, O.**, A. Asberg. (2009). Impact of genetic polymorphisms in cytomegalovirus glycoprotein B on outcomes in solid-organ transplant recipients with cytomegalovirus disease. *Clin Infect Dis.* **49**(8): 1160-1166.
- Mattick, C.**, Dewin, D., Polley, S., Sevilla-Reyes, E., Pignatelli, S. and Rawlinson, W. (2004). Linkage of human cytomegalovirus glycoprotein gO variant groups identified from worldwide clinical isolates with gN genotypes, implications for disease associations and evidence for N-terminal sites of positive selection. *Virol. J.* **318**(2): 582-597.
- McKay, D.B.** and Steinberg, S.M. (2010). Kidney Transplantation: A Guide to the Care of Kidney Transplant Recipients. Springer New York Dordrecht London. ISBN: 987-1-4419-1689-1.

- McLaughlin**, K. and Wu, C. (2002). Cytomegalovirus sero-mismatching increases the risk of acute renal allograft rejection. *TRANSPLANT*. **74**(6): 813-816.
- Mengelle**, C., Sandres-Saune, K., Pasquier, C., Rostaing, L., Mansuy, J. M., Marty, M., *et al.* (2003). Automated extraction and quantitation of human cytomegalovirus DNA in whole blood by real-time PCR assay. *J Clin Microbiol*. **41**:3840-3845.
- Menghi**, V., Comai, G., Baraldi, O., D’Arcangelo, G. L., Lazzarotto, T. and Manna, G. L. (2016). Ganciclovir-Resistant Cytomegalovirus Infection in a Kidney Transplant Recipient Successfully Treated with Foscarnet and Everolimus. *Case Rep Nephrol*. **2016**:1-6.
- Meyer**, H., Sundqvist, V.A., Pereira, L., and Mach, M. (1992). Glycoprotein gp116 of human cytomegalovirus contains epitopes for strain-common and strain-specific antibodies. *J Gen Virol*. **73** (Pt 9): 2375-2383.
- Mocarski**, E. S., and Courcelle, C. T. Cytomegaloviruses and their replication. In Knipe, D. M., and Howley, P. M. (eds). (2001). *Fields virology*. Philadelphia: Lippincott, Williams and Wilkins. pp: 2629-2673.
- Moreira**, R.K., Norton, C., Rosado, F.N., Cohe, C. and Nolte, F. (2010). Histopathologically documented gastrointestinal cytomegalovirus infection in immunosuppressed patients: Clinicopathologic analysis with serum quantitative PCR correlation. *Int. J. Med. Med. Sci*. **2**(3): 49-59.
- Murray**, P. R., Baron, E. J., Jorgensen, J. H., Snnetary, M. L. and Pfaller, M. A. (2007). *Manual of Clinical Microbiology*. vol 2. 9th ed. Washington DC. ASM Press. pp:1549-1559.
- Murray**, P. R., Rosenthal, K. S. and Pfaller, M. A. (2013). *Medical Microbiology*. 7th ed. Philadelphia: Saunders. Elsevier. pp:477-481.
- Nafar**, M., Roshan, A., Pour-Reza-Gholi, F., Samadian, F., Ahmadpoor, P., Samavat, S and Amin, M. A. (2014). Prevalence and Risk Factors of Recurrent Cytomegalovirus Infection in Kidney Transplant Recipients . *Iran J Kidney Dis*. **8** (3): 231-235.
- Nankivell**, B.J. and Alexander, S.I. (2010). Rejection of the Kidney Allograft. *N Engl J Med*. **363** (15):1451-1462.

Nogueira, E., Tomiyama, H., and Granato, C. (2002). High degree of CMV glycoprotein B one variability analyzed by sequencing in renal transplantation recipients. Fernal University Sao Paulo Brazil .www.ncbi.nlm.nih.gov

Ohizumi, Y., Suzuki, H., Matsumoto, Y., Masuho, Y. and Numazaki, Y. (1992). Neutralizing mechanisms of two human monoclonal antibodies against human cytomegalovirus glycoprotein 130/55. *J Gen Virol.* **73** (Pt 10): 2705-2707.

Olyaei, A. B., Sabahi, F., Firouzan, A., Roustaei, M. H., Arzenani, M. K., Sarrami-Forooshani, R., Adeli, A. and Mahboudi. F. (2005). Pre-Symptomatic Human Cytomegalovirus Disease Diagnosis in Renal Transplant Recipients by the Virus DNA PCR. *Iranian J Publ Health.* **34** (3): 44-55.

Ortiz, J. and André, A. (2011). Infectious Complications in Kidney Transplantation. After the Kidney Transplant - The Patients and Their Allograft. Published by InTech pp: 3-10. ISBN 978-953-307-807-6.

Paradowska, E., ska, A. J., ska, M. S., Kasztelewicz, B., ska, B. Z., Wisniewska-Ligier, M. *et al.* (2014). Cytomegalovirus Glycoprotein H Genotype Distribution and the Relationship with Hearing Loss in Children. *J.Med.Viro.* **86**:1421-1427.

Pacsa , A. S., Essa , S., Voevodin , A., El-Shazly , A., Kazak ,H., Nampoory, M.R.N., Johnny , K.V., Said , T. and Al-Nakib, W. (2003). Correlation between CMV genotypes, multiple infections with herpesviruses (HHV-6, 7) and development of CMV disease in kidney recipients in Kuwait. *FEMS IMMUNOL MED MIC.* **35**:125-130.

Pascual, R. M., Bonventre, J. V. (1996). Acute renal failure. *NEJM.* **334**: 1448-1460.

Paterson, D. A., Dyer, A. P., Milne, R. S., Sevilla-Reyes, E. and Gompels, U.A. (2002). A role for human cytomegalovirus glycoprotein O (gO) in cell fusion and a new hypervariable locus. *Virol. J.* **293** (2): 281-294.

Paya, C.V. (2001). Prevention of cytomegalovirus disease in recipients of solid-organ transplants. *Clin. Infect. Dis.* **32** (4): 596-603.

Peleg, A. Y., Husain, S., Qureshi, Z. A., Silveira, F. P., Sarumi, M. and Shutt, K. A., *et al.* (2007). Risk factors, clinical characteristics, and outcome of Nocardia infection in

organ transplant recipients: a matched case-control study. *Clin Infect Dis.* **44** (10): 1307-1314. ISSN: 1537-6591.

Pereyra, F. and Rubin, R.H. (2004). Prevention and treatment of cytomegalovirus infection in solid organ transplant recipients. *Curr Opin Infect Dis.* **17**: 357-361.

Piedras, A.R., Arciniega, M.D. and Vazquez, J.R. (2013). Clinical pharmacology and therapeutic drug monitoring of immunosuppressive agents. In: Current issue and future direction in kidney transplantation. Chapter 15. ENTECH. <http://dx.doi.org/10.5772/54910>.

Pignatelli, S., Dal Monte, P. and Landini, M. P. (2001). gpUL73 (gN) genomic variants of human cytomegalovirus isolates are clustered into four distinct genotypes. *J Gen Virol.* **82** (Pt 11): 2777-2784.

Pignatelli, S., Dal Monte, P., Rossini, G., Lazzarotto, T., Gatto, M. R. and Landini, M. P. (2003a). Intrauterine cytomegalovirus infection and glycoprotein N (gN) genotypes. *J Clin Virol.* **28** (1): 38-43.

Pignatelli, S., Rossini, G., Dal Monte, P., Gatto, M.R. and Landini, M.P. (2003b). Human cytomegalovirus glycoprotein N genotypes in AIDS patients. *Aids.* **17**(5): 761-763.

Polz-Dacewicz, M., Foltyn, S., Macieląg, P. and Polz, D. (2013). Cytomegalovirus (CMV) - a new prospect for Prevention. *JPCCR.* **7**(2):118-123.

Pourmand, G., Pourmand, M., Salem, S., Mehrsai, A., Mahmoudi, M. T., Nikoobakht, M., Ebrahimi, R., Saraji, A., Moosavi, S. and Saboury. B. (2006). Posttransplant Infectious Complications: A Prospective Study on 142 Kidney Allograft Recipients. *Urology J.* **3**(1):23-31.

Preiksaitis, J.K. Brennan, D.C. Fishman, J. and Allen, U. (2005). Canadian society of transplantation consensus workshop on cytomegalovirus management in solid organ transplantation final report. *Am. J. Transplant.* **5**(2):218-227.

Puchhammer-Stöckl, E. Görzer, I. (2011). Detection of Mixed-genotype Populations of Human Cytomegalovirus. *Future Virol.* **6** (2):259-271.

- Ramanathan, V., Goral, S. and Helderman, J. H. (2001).** Renal transplantation. *Semin Nephrol.* **21**: 213-219.
- Rangbar-Kermani, R., Sharifi, Z., Mahmoodian-Shooshtari, M., Mousavi, K. (2011).** Quantitative Analysis of CMV-DNA Load in Renal Transplant Recipients Using Real-Time PCR. *Iran J Virol.* **5**(1): 28-30.
- Rasmussen, L., Geissler, A., Cowan, C., Chase, A. and Winters, M. (2002).** "The genes encoding the gCIII complex of human cytomegalovirus exist in highly diverse combinations in clinical isolates. *J Virol.* **76**(21): 10841-10848.
- Razonable, R.R., Rivero, A., Brown, R.A., Hart, G.D., Espy, M.J. and van Crujisen, H., et al. (2003).** Detection of simultaneous beta-herpesvirus infections in clinical syndromes due to defined cytomegalovirus infection. *Clin Transplant.* **17**(2): 114-120. ISSN: 0902-0063.
- Renzette, N., Gibson, L., Jensenand, J. D. and Kowalik, T. F. (2014).** Human cytomegalovirus intrahost evolution - a new avenue for understanding and controlling herpesvirus infections. *Curr Opin in Virol.* **8**:109-115.
- Requião-Moura, L. R., de Matos, A. C. and Pacheco-Silva, A. (2015).** Cytomegalovirus Infection in Renal Transplantation: Clinical Aspects, Management and The perspectives. *Einstein.* **13**(1):142-148.
- Reyes-Pérez, H., Sanchez-Huerta, J. L., Varela-Fascinetto, G., Romo-Vazquez, J. C., Morales-Sanchez, A., Panana, E. M. F., Parra-Ortega, I., Ramirez-Ramirez, G. and Lpoez- Marinez, A. (2016).** Correlation between viral load of cytomegalovirus and tacrolimus and sirolimus levels in transplanted pediatric patients. *Bol Med Hosp Infant Mex.* **73**(1):4-9.
- Rhoades, R. A. and Bell, D. R. (2009).** Medical physiology. Principles of Clinical Medicine. 3rd . Philadelphia: Lippincott William and Wilkins: 391-393.
- Rodrigo, E., Fernandez-Fresnedo, G., Valero, R., Ruiz, J.C., Pinera, C. and Palomar, R. (2006).** New-onset diabetes after kidney transplantation: risk factors. *Clin J Am Soc Nephrol.* **17** (No 12 Suppl 3): S291-295. ISSN: 1046-6673.

Ross, S.A., Novak, Z., Pati, Z and Boppana, S.B.(2011).Diagnosis of Cytomegalovirus Infections. *Infect Disord Drug Targets*. **11**(5): 466-474.

Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology, An Introduction To Infectious Diseases. 4th ed. United States of America. McGraw-Hill Companies. pp: 555-569.

Ryan, K. J., Ray, C. G., Ahmad, N., Drew, W. L. and Plorde, J. J. (2010). Herpesviruses. Sherris Medical Microbiology. 5th ed. United States of America. McGraw-Hill Companies. ISBN :978-0-07-160402-4.

Sagedal, S., Nordal, K. P., Hartmann, A., Degré, M., Holter, E., Foss, A. and Osnes, K., et al. (2000). A prospective study of the natural course of cytomegalovirus infection and disease in renal allograft recipients. *TRANSPLANT*. **70**(8): 1166-1174.

Sagedal, S., Nordal, P.K., Hartmann, A., Sund, S., Scott, H. and Degré, M., et al. (2002). The Impact of Cytomegalovirus Infection and Disease on Rejection Episodes in Renal Allograft Recipients. *Am. J. Transplant*. **2**: 850-856.

Sagedal, S., Rollag, H., and Hartmann, A. (2007). Cytomegalovirus infection in renal transplant recipients is associated with impaired survival irrespective of expected mortality risk. *Clin Transplant*. **21**(3): 309-313. ISSN: 0902- 0063.

Sanchez, J. L. and Storch, G.A. (2002). Multiplex, quantitative, real-time PCR assay for cytomegalovirus and human DNA. *J Clin Microbiol*. **40**: 2381-2386.

Scanlon, V.C. and Sanders, T. (2007). Essentials of anatomy and physiology. 5th ed. Philadelphia: F. A. Davis Company. pp: 420-424.

Schoppel, K., Hassfurth, K., Britt, W.J. and Mach, M. (1996). Antibodies specific for the antigenic domain 1 of glycoprotein B (gpUL55) of human cytomegalovirus bind to different substructures. *Viol.J*. **216**(1): 133-145.

Schulz, U., Solidoro, P., Müller, V., Szabo, A., Gottlieb, J., Wilkens, H and Enseleit, F. (2016). CMV Immunoglobulins for the Treatment of CMV Infections in Thoracic Transplant Recipients. *TRANSPLANT*. **100**(3): S5-S10.

- Shaikewitz**, S.T. and Chan, L. (1994). Chronic renal transplant rejection. *Am J Kidney Dis.* **23**:884.
- Sharif**, A. (2016). Renal Transplant. *Renal med.*
- Shimamura**, M., Mach, M. and Britt, W.J. (2006). Human cytomegalovirus infection elicits a glycoprotein M (gM)/gN-specific virus-neutralizing antibody response. *J Virol.* **80**(9): 4591-4600.
- Sia**, I.G., and Paya, C.V. (1998). Infectious complications following renal transplantation. *Surg Clin North Am.* **78**: 95-112.
- Sijmons**, S., Ranst, M. V. and Maes, P. (2014). Genomic and Functional Characteristics of Human Cytomegalovirus Revealed by Next-Generation Sequencing. *Viruses.* **6**: 1049-1072.
- Silva Junior**, H. T. (2015). Natural History of Cytomegalovirus (CMV) Infection and Disease among Renal Transplant Recipients. *ClinicalTrials.gov*
- Silva**, D. F. L., Arruda, L. M. F., Silva, N. F., Sagica, F. E. S., Moraes, M. M., JLSA, J. R., Santos, T. V. R. and Medeiros, R. S . (2015). Cytomegalovirus Infections in Patients with HIV/AIDS in a Unit of Health of the Amazonian Region, Belém, Pará, Brazil. *J Med Microb Diagn.* **4**(2):1-5.
- Sinclair**, J. and Sissons, P. (2006). Latency and reactivation of human cytomegalovirus. *J Genl Virol.* **87**(Pt 7):1763-1779.
- Sinclair**, J. H. and Reeves, M. B. (2013). Human Cytomegalovirus Manipulation of Manipulation of Latently Infected Cells .*Viruses.* **5** :2803-2824.
- Sing-Leung**, L. (2001). An Update on Immunosuppressive Medications in Transplantation. *Special Feature.* **6** (4):1-6.
- Smith**, J. M., Corey, L., Bittner, R., Finn, L. S., Healey, P. J., Davis, Connie. L. and McDonald, R. A. (2010). Subclinical Viremia Increases Risk for Chronic Allograft Injury in Pediatric Renal Transplantation. *J Am Soc Nephrol.***21**: 1579-1586.
- Smith**, T. F., Espy, M. J., Mandrekar, J., Jones, M. F., Cockerill, F. R. and Patel, R. (2007). Quantitative Real-Time Polymerase Chain Reaction for Evaluating DNAemia

due to Cytomegalovirus, Epstein-Barr Virus, and BK Virus in Solid-Organ Transplant Recipients. *Clin Infect Dis.* **45**:1056-1061.

South Australia Health. (2012). ABN: 97 643 356 590.

Sowmya, P., Dhanya, V., Madhavan, H. N. and Therese, K.L. (2007). Comparative efficacy of PCR-based restriction fragment length polymorphism (RFLP) and multiplex PCR for glycoprotein B (gB) genotyping of human cytomegalovirus. *Indian J Med Res.* **126**: 122-127.

Spaete, R. R., Perot, K., Scott, P. I., Nelson, J. A., Stinski, M. F. and Pachl, C. (1993). Coexpression of truncated human cytomegalovirus gH with the UL115 gene product or the truncated human fibroblast growth factor receptor results in transport of gH to the cell surface. *Viol. J.* **193**(2): 853-561.

Stangherlin, L. M., dePoula, F. N., Icimoto, M. Y., Ruuiz, L.G.P., Noqueita, M. L., Braz, A. S. K., Juliano, L. and da Saliva, M. C. C. (2017). Positively selected sites at HCMV gB Furin processing region their effects in cleavage efficiency. *Front Microbiol.* **8**:1-10

Taherkhani, R., Farshadpour, F., Makvandi, M., Hamidifard, M., Esmailizadeh, M., Ahmadi, B. and Heidari, H. (2015). Determination of Cytomegalovirus Prevalence and Glycoprotein B Genotypes Among Ulcerative Colitis Patients in Ahvaz, Iran. *Jundishapur J Microbiol.* **8** (2): e17458.

Tamura, K., Stecher, G., Peterson, D., Filipki, A, and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Bio and Evol.* **30**: 2725-2729.

Tarabadi, F. A., Ghaledi, J., Shayegan, M. and Babaei, G. R.(2005). Comparison of prevalence of anti-CMV antibodies (IgM and IgG) and CMV Ag in renal transplant recipients. *Blood (Khoon).* **2**:145.

The American College of Obstetricians and Gynecologists. (2015). Cytomegalovirus, parvovirus B19, varicella zoster, and toxoplasmosis in pregnancy. *Obstet Gynecol.* **125**:1510-1525.

Urban, M., Klein, M., Britt, W. J., Hassfurth, E. and Mach, M. (1996). Glycoprotein H of human cytomegalovirus is a major antigen for the neutralizing humoral immune response. *J Gen Virol.* **77** (Pt 7): 1537-1547.

van Ree, R. M., de Vries, A. J. P., Zelle, M. D., de Vries, L. V., Oterdoom, L. H., Gans, R. O. B., Schouten, J. P., Lems, S. P. M., van Son, J. W. and Bakker, S. J. L. (2011). Latent cytomegalovirus infection is an independent risk factor for late graft failure in renal transplant recipients. *Med Sci Monit.* **17**(11): 609-617.

Watson, C. J., Dark, J. H. (2012). Organ transplantation: historical perspective and current practice. *Br J Anaesth.* **108** (Suppl 1): i29.

Weikert, B. C. and Blumberg, E. A. (2008). Viral infection after renal transplantation: surveillance and management. *Clin J Am Soc Nephrol.* **3**(2): S76-S86.

William, T. C. Y., Cuevas, L. E., Helen, W. and Ali, B. (2000). Prediction and Diagnosis of Cytomegalovirus Disease in Renal Transplant Recipients Using Qualitative and Quantitative Polymerase Chain Reaction. *TRANSPLANT.* **69**(5): 985-991.

Woo, P. C. Y., Chi-Yuen, L. O., Lo, S. K. F., Siau, H., Peiris, J. S. M., Wong, S. S. Y. (1997). Distinct Genotypic Distributions of Cytomegalovirus (CMV) Envelope Glycoprotein in Bone Marrow and Renal Transplant Recipients with CMV Disease. *Clin. Diagn. Lab. Immunol.* **4**(5):515-518.

Yan, H., Koyano, S., Inami, Y., Yamamoto, Y., Suzutani, T., Mizuguchi, M., Ushijima, H., Kurane, I. and Inoue, N. (2008). Genetic linkage among human cytomegalovirus glycoprotein N (gN) and gO genes, with evidence for recombination from congenitally and post-natally infected Japanese infants. *J Gen Virol.* **89**:2275-2279.

Yilmaz, S. and Hayry, P. (1993). The impact of acute episodes of rejection on the generation of chronic rejection in rat renal allografts. *TRANSPLANT.* **56**:1153-1156.

Yilmaz, S., Koskinen, P. K., Kallio, E., Bruggean, C. A., Hayry, P. J. and Lemstrom, K. B. (1996). Cytomegalovirus infection-enhanced chronic kidney allograft rejection is linked with intercellular adhesion molecule-1 expression. *Kidney Int.* **50**: 526-537.

Zhang, C. B., Lai, H. Y., Xu, H. T., Wang, D. G. and Xiao, F. (2012). Clinical application of real time-polymerase chain reaction in determining cytomegalovirus viral DNA load in renal transplant recipients. *Chin Med J (Engl)*. **125** (19):3575-3577.

Zhang, S., Zhou, Y., Li, L. and Hu. Y. (2010). Monitoring human cytomegalovirus infection with nested PCR: comparison of positive rates in plasma and leukocytes and with quantitative PCR. *Viol. J.* **7** (73): 1-7.

Zuckerman, A. J., Banatvala, J. E., Schoub, B. D., Griffiths P. D. and Mortimer, P. (2009). Principles and Practice of Clinical Virology. 6th ed. Chichester. West Sussex: John Wiley and Sons. ISBN: 978-0-470-51799-4:160-190.

APPENDICES

Appendix (I):

CMV Real – RT Quant Reagents (Sacace- Italy)

PCR – mix -1-FRT CMV, PCR – mix -2-FRT, Polymerase (TaqF), RNA – buffer, DNA calibrator (QS1), DNA calibrator (QS2). Negative Control (C-), Positive Control DNA CMV/human DNA. Internal Control (IC).

Appendix (II):


















Results of controls DNA samples together with the Internal Control.

Control	Stag of control	Ct in channel				Interpretation
		HEX/JOE/Yellow /Cy3		ROX/ Orange/ Texas Red		
		Qualitative format		Quantitative format		
NCE	DNA extraction amplification	Negative	Negative	Positive (<boundary value)	Positive (<boundary value)	OK
PCE	DNA extraction amplification	Positive (<boundary value)	Ct value is in the range indicated in data card	Positive (<boundary value)	Positive (<boundary value)	OK
NCA	Amplification	Negative	Negative	Negative	Negative	OK
QS1 and QS2	Amplification	-	Ct value and calculated, concentration determined	-	Ct value and calculated, concentration determined	OK

If the controls are out of the expected range (results and control), all of the specimens and controls from that run must be processed beginning from the sample preparation step.


















Appendix (III):

The results of tested samples, negative control, positive control, negative control of amplification and quality control standards (calibrators) for cycling yellow(Tested DNA).

No.	Color	Name	Type	Ct	Given Conc (copies/ml)	Calc Conc (copies/ml)	% Var
1		s161	Unknown	19.18		336.26693428523	
2		s149	Unknown	31.78		6.60613594414525	
3		s147	Unknown	36.22		3.27574876664754	
4		s118	Unknown	28.22		0.738612150894464	
5		s116	Unknown	27.30		1.37588671456156	
6		s54	Unknown	26.52		2.33428554535428	
7		ss46	Unknown	23.91		13.6319561437354	
8		s19	Unknown				
9		s18	Unknown	25.31		5.27181006630924	
10		s8	Unknown	30.47		0.160804875669612	
11		NCE	Negative Control				
12		PCE	Positive Control	20.23		164.938801551174	
13		NCA	NTC				
14		QS1	Standard	14.18	10000	9879.85694237687	1.2%
15		QS1	Standard	14.15	10000	10121.6040458114	1.2%
16		QS2	Standard	21.09	100	92.1837600006739	7.8%
17		QS2	Standard	20.85	100	108.478977207337	8.5%

Appendix (IV):

The results of tested samples, negative control, positive control, negative control of amplification and quality control standards (calibrators) for cycling orange (internal control).

No.	Color	Name	Type	Ct	Given Conc (copies/ml)	Calc Conc (copies/ml)	% Var
1		s161	Unknown	24.35		451.198860583959	
2		s149	Unknown	24.60		380.713628004375	
3		s147	Unknown	27.41		53.9819233949	
4		s118	Unknown	24.38		442.691811948081	
5		s116	Unknown	25.23		244.72128442064	
6		s54	Unknown	24.21		498.565495190861	
7		ss46	Unknown	24.23		491.447820215091	
8		s19	Unknown	23.97		589.648579329013	
9		s18	Unknown	23.05		1117.13386741044	
10		s8	Unknown	24.88		313.345881474178	
11		NCE	Negative Control	23.73		696.86685375983	
12		PCE	Positive Control	23.76		683.445040549418	
13		NCA	NTC				
14		QS1	Standard	19.90	10000	9977.52547248266	0.2%
15		QS1	Standard	19.89	10000	10022.5251517316	0.2%
16		QS2	Standard	26.54	100	98.80462543322	1.2%
17		QS2	Standard	26.50	100	101.20983664635	1.2%

Appendix (V):

10 X TBE Buffer

Tris EDTA 48.4 gram

Boric acid 55.9 gram

EDTA 7.44 gram

D.W 500 ml

Appendix (VI):

1 X TBE Buffer

1 ml of 10 X TBE Buffer + 9 ml of D. W.

Appendix (VII):

Sequencing of comparing CMV reference GenBank Genotype.

```
>M60926.2 HCMV glycoprotein B (UL55) Genotype 4
ATGGAATCCAGGATCTGGTGCCGGTAGCTGCGTTAACTTGTGTATCGTCTGCTGGGTGCTGCGGTTTCCTTCTTACTTCTCGTGAACCTTACTACTATAATGG
AAACCATACTTCTCATAACGACGCTGCTCAAACCCGGTCAATCTCCTCTCAACCGGTAACGCTTCTGAAAGCCGTCAGTCATAGAGCCAACGAGACTATCTACAAC
ACCACCCTCAAGTACGGAGATGTGGTGGGGTTAACACCACCAAGTACCCCTAATCGTGTGTGTTCTATGGCTCAGGTACGGATCTTATTTCGCTTTGACCGCAAC
TCGCTGCACCCCGATGAAGCCTATCAATGAAGACTTGGACGAGGGTATCATGGTGGTCTACAACCGCAACATCGTCCGACACCTTAAAGGTACGGGTCTACC
AGAAGGTTTTGACGTTTCGTCGTAGCTACGCCTACATTCACACCCTTATCTGCTGGGAGCAATACGGAAATACGTGGCCGCTCCTATGTGGGAGATTCATCATAT
CAACAGCCACAGTCAAGTGTACAGTTCCTACAGCCGGTATAGCAGGACCGGTTTTCGTGGCTTATCATAGGGACAGCTATGAAAAACAAACCATGCAATTAAT
GCCCAGCATTTTCCAACCCACAGTACCCGTTACGTGACGGTCAAGGATCAGTGGCACAGCCGCGCAGCACCTGGCTCTATCGTGAGACCTGTAATCTGAA
TTGATATGGTACACAACTACGCGGCTCCAAATTCCTTATCATATTTTCGCCACTTACGGGTGACGTTGACATTTCTCCTTCTACAACGGAACCAATC
GCAATGCCAGTACTTTGGAGAAAAACGCGGCAAGTTTTTTCATTTTCCGAACTACACTATCGTCTCTGACTTTGGAAGACCGAATTCGCGTTAGAGACCCACAG
GTTGGTGGCTTTTCTGAACGTGCGGACTCGGTGATTTCTGGGATATACAGGACGAAAAAGAAATGTCACTTGTCAACTACTTTCTGGGAAGCTCGGAACGCACC
ATTCGTTCCGAAGCCGAGGAGTCAATACATTTCTTTCGCAAAAATGACCGCTACTTTCCTGTCTAAGAAAGCAAGAGGTGAACATGTCGGATTCGCGCTGGACT
CGGTACGTGATGAGGCTCTAAATAAGTTACAGCAGATTTTAAATGCTTACATAACAGACATATGAAAAGTACGGAAACGTGTCCTGCTTCCGAACTACCGGCG
GACTAGTAGTGTTCGGCAAGGTAACAAGCAAAAATCTCTGGTGGAACTCGAACGTTGGCCAAACCGTTCAGTCTGAACTTACTACTAGTAGAACCAGAAGAA
GTACAGATGGCACCATGTAATCTATTAATCTAATATGGACTCGGTACACAATCTGGTCTACGCCAGCTGCAGTTCACCTACGACACGTTGCGCGGTACATCAA
CCGGGCGTTGACACAACTCGCAGAACGCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACATAGCAAGATCAACCCCGTCAAGTATTTCTCGGC
CATCTACAACAAACCGATTGCGCGCGTTTATGTTGATGCTTGGGTGAGTCCGTTGGCCAGCTGCGTGACCATTAACCAAAACAGCGTCAAGGTGCTGCGTGATA
TGTGAAGGAATCGCCAGGACGCTGCTACTACGACACAGTGGTCACTTTAAATTCGCCAACAGCTCGTATGTCAGTACGGTCAACTGGGTGAGGATAACGAAAT
CCTGTGGGCAACACCGACTGAGGAATGTCAGCTTCCAGCTCAAGATCTTCACTGCGCGGCAACTCGGCTACGAGTACGTGGACTACCTTCTCAACGCAT
GATTGACCTACGACATCTCCACTGTCGACAGCATGATCGCCCTAGACATCGACCCGCTGGAAAAACCCGACTTACGGGTACTGGAACTTACTCGCAGAAAAGA
ACTGCGTTCCAGCAACGTTTTGATCTCGAGGAGATCAATGCGCGAGTCAATTCGTATAAGCAGCGGGTAAAGTACGTGGAGGACAAGGTAGTCGACCCGTTGCC
CCCTACCTCAAGGCTTGGACGACCTATGAGCGGCTGGGCGCGCGGGAAAGCCGTTGGCGTAGCCAATGGGGCCGTTGGGTGGCGCGGTGGCTCCGTTGG
TCGAAAGGCGTTGCCACCTTCTCAAAAACCCCTTTGGAGCCTTACCATCATCTCTGTCGCCATAGCCCGTCAATATCATATTTATTGATCTATACTCGACAGCGG
CGTCTGCAATGACGCGCTGACAGAACCTTTCCCTATCTGGTGTCCGCGGACGGGACCACCGTACGCTCGGCAACACCAAGACACGCTGTTACAGGCTCCG
CCTTCTACGAGGAAAGTGTATAAATCTGGTCGCAAAAGACCGGACCACCGTCTGATGATCAACCGGCGCTCCGCTTACACCAACGAGCAGGCTTAC
CAGATGCTTCTGGCCCTGGTCCGCTTGGACGACAGCAGCGAGCGCAGCAGAACGGTACAGATTTCTTGGACGGACAGACTGGCACGCAAGGACAAGGACAGAA
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>KR992839.1 HCMV glycoprotein B (UL55) Genotype 4
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ACCCTAGAGGCTTCAAGGAACCTAGCAAGATCAACCCGTCAGCTATTTCTCGGCCATCTACAACAAACCGATTGCCGCGCGTTTC

>AY186111.1 HCMV glycoprotein B (UL55) Genotype 4
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TCACCTACGACACGTTGCGCGGTACATCAACCGGGCGTTGACACAAAATCGCAGAAGCCTGGTGTGGATCAACGGCGCACCCCTAGAGGCTTCAAGGAACCTA
GCAAGATCAACCCGTCAGTATTTCTCGGCCATCTACAACAAACCGATTGCCGCGCGTTTCA

>KR992940.1 HCMV-glycoprotein B (UL55) Genotype 4
GCAACTTTCCTGTCTAAGAAGCAAGAGGTGAACATGTCGCCGATTCGCGCTGGACTGCGTACGTGATGAGGCTCTAAAATAAGTTACAGCAGATTTTTAATGCTTCA
ACAATCAGACATATGAAAAGTACGGAACGCTGTCGCTTTCGAAACTACCGCGGACTAGTAGTGTCTGGCAAGGTATCAAGCAAAAATCTCTGGTGGAACTC
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ACCCTAGAGGCTTCAAGGAACCTAGCAAGATCAACCCGTCAGCTATTTCTCGGCCATCTACAACAAACCGATTGCCGCGCGTTTC

>AY186112.1 HCMV-glycoprotein B (UL55) Genotype 4
TGAATCTTACTCATAGTAGAACCAGAAGAAGTACAGATGGCACCATGTAACCTATTTATCTAATATGGATTCCGTTACACAATCTGGTCTACGCCAGCTGCAGT
TCACCTACGACACGTTGCGCGGTACATCAACCGGGCGTTGACACAAAATCGCAGAAGCCTGGTGTGGATCAACGGCGCACCCCTAGAGGCTTCAAGGAACCTA
GCAAGATCAACCCGTCAGTATTTCTCGGCCATCTACAACAAACCGATTGCCGCGCGTTTCA

>KR992932.1 HCMV-glycoprotein B (UL55)-Genotype 3
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AGGCTCTAAAATAAGCTACAACAGATCTTCAACGCCTCGTACAATCAACATACGAAAAGTACGGTAAACGCTGTCGGTTTTTGAAACCAAGCGCGCTTGGTGGTGT
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ACCACCTGTCGCTTGAAGCGAACTGTACGAAATGTCTTACGCTCAGCTGCAGTTCACCTATGATACGTTGCGCAGCTACATCAATCGGGCGTTGGCGCAG
ATCGCCGAGGCTGGTGTGGATCAACGGCGCACCCAGAGGCTTCAAGGAACCTAGCAAGATCAATCCATCAGCCATTTCTCGGCCATCTACAACAAACCG
ATTGCCGCGCGTTTC
```

Appendix (VIII):

Nucleotide sequencing of CMV isolates gB gene from (1306-1611) .

>Isolate-24

AACTGGAACGTTTGGCCAACCGTTCAGTCTGAATCTTACTCATAGTAGAACAGAGAAGTACAGATGGCACC AATGTA AACTCATTAT
CTAATATGGACTCGGTACACAATCTGGTCTACGCCAGCTGCAGTTCACCTACGACACGTTGCGCGGCTACATCAACCGGCGTTGACAC
AAATCGCAGAAGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTTAGCAAGATCAACCCGTCAGCTATTCTCTCGG
CCATCTACAACAACCGATTGCCGCGCTTTC

>Isolate-149

AACTGGAACGTTTGGCCAACCGTTCAGTCTGAATCTTACTCATAGTAGAACAGAGAAGTACAGATGGCACC AATGTA AACTCATTAT
CTAATATGGACTCGGTACACAATCTGGTCTACGCCAGCTGCAGTTCACCTACGACACGTTGCGCGGCTACATCAACCGGCGTTGACAC
AAATCGCAGAAGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTTAGCAAGATCAACCCGTCAGCTATTCTCTCGG
CCATCTACAACAACCGATTGCCGCGCTTTC

>Isolate-230

TTGGAAGTGGAAAGTTTGGCCAATAGCTCCGGTGTGAACTCCACGCgTAGAACCAAGAGAAGTACGGGCAATACGACCACCCTGTCGCTT
GAAAGCGAATCTGTACGAAATGTGCTCTACGCTCAGCTGCAGTTCACCTACGATACGTTGCGCAGCTACATCAATCGGGCGTTGGCGCAG
ATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
ATCTACAACAACCGATTgCGGCGCTTTC

>Isolate-189

TTGGAAGTGGAAAGTTTGGCCAATAGCTCCGGTGTGAACTCCACGCgTAGAACCAAGAGAAGTACGGGCAATACGACCACCCTGTCGCTT
GAAAGCGAATCTGTACGAAATGTGCTCTACGCTCAGCTGCAGTTCACCTACGATACGTTGCGCAGCTACATCAATCGGGCGTTGGCGCAG
ATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
ATCTACAACAACCGATTgCGGCGCTTTC

>Isolate-164

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GATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
CATCTACAACAACCGATTgCGGCGCTTTC

>Isolate-147

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ATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
ATCTACAACAACCGATTgCGGCGCTTTC

>Isolate-135

TTGGAAGTGGAAAGTTTGGCCAATAGCTCCGGTGTGAACTCCACGCgTAGAACCAAGAGAAGTACGGGCAATACGACCACCCTGTCGCTT
GAAAGCGAATCTGTACGAAATGTGCTCTACGCTCAGCTGCAGTTCACCTACGATACGTTGCGCAGCTACATCAATCGGGCGTTGGCGCAG
ATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
ATCTACAACAACCGATTgCGGCGCTTTC

>Isolate-118

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ATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
ATCTACAACAACCGATTgCGGCGCTTTC

>Isolate-10

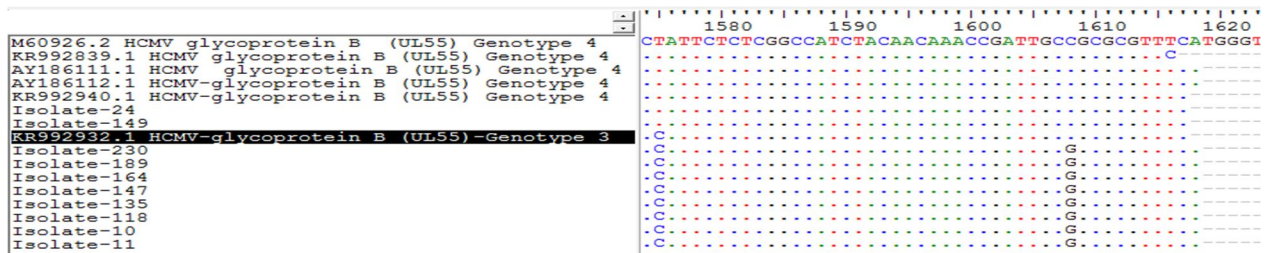
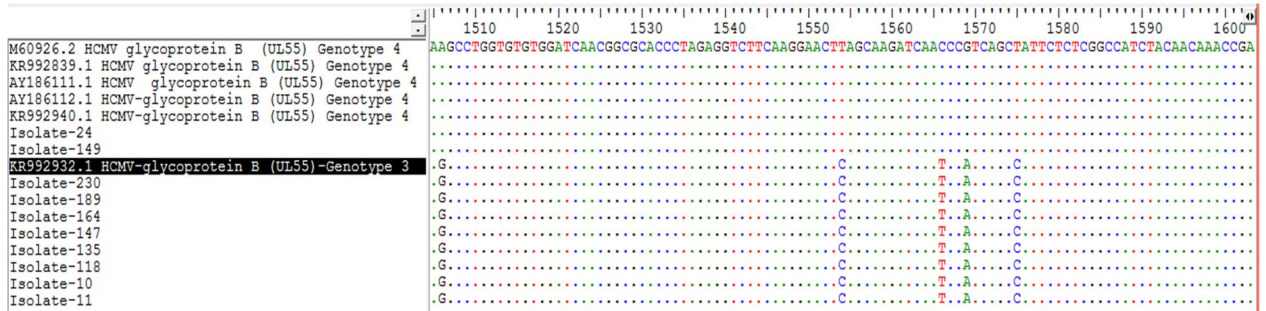
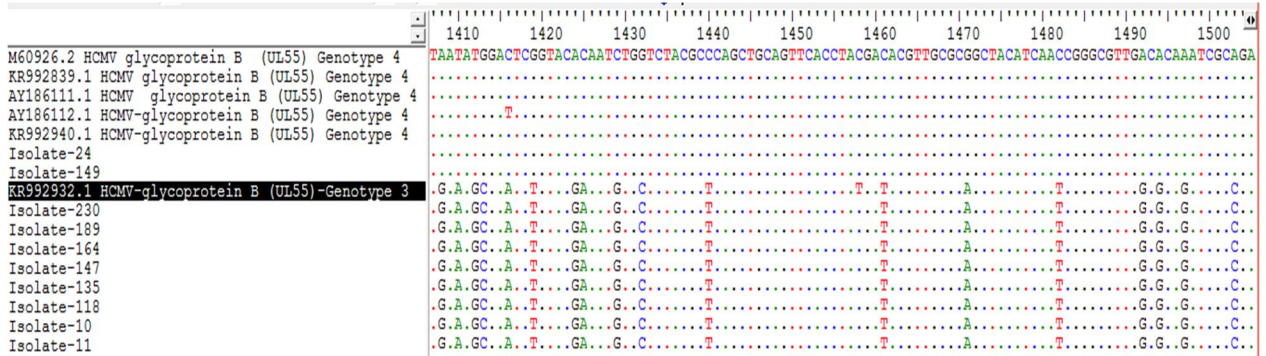
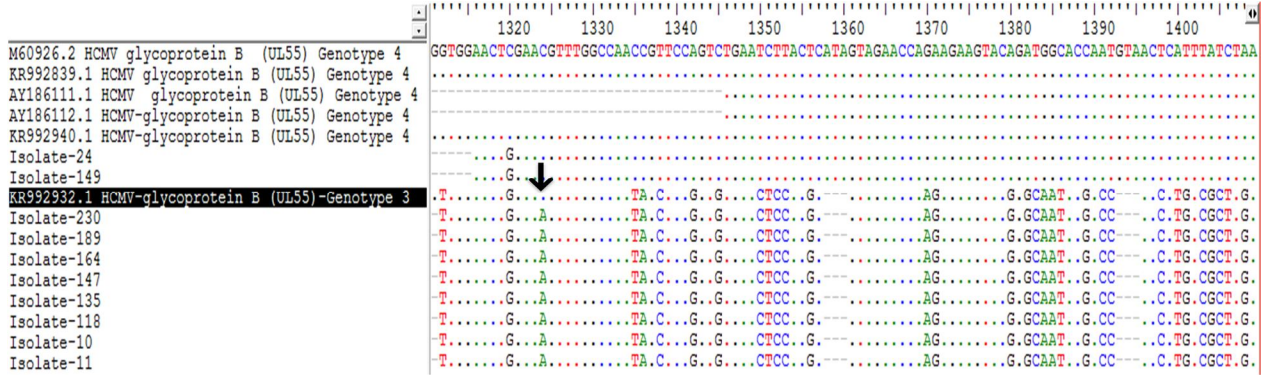
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ATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
ATCTACAACAACCGATTgCGGCGCTTTC

>Isolate-11

TTGGAAGTGGAAAGTTTGGCCAATAGCTCCGGTGTGAACTCCACGCgTAGAACCAAGAGAAGTACGGGCAATACGACCACCCTGTCGCTT
GAAAGCGAATCTGTACGAAATGTGCTCTACGCTCAGCTGCAGTTCACCTACGATACGTTGCGCAGCTACATCAATCGGGCGTTGGCGCAG
ATCGCCGAGGCCTGGTGTGGATCAACGGCGCACCTAGAGGTCTTCAAGGAACTCAGCAAGATCAATCCATCAGCCATTCTCTCGGC
ATCTACAACAACCGATTgCGGCGCTTTC

Appendix (IX):

BioEdit multiple sequence alignment of CMV gB gene compared to other CMV gB Gene from Genbank. The transversion mutations in isolates 230,189 147,164, 135, 118, 10 and 11 are indicated by black arrow.



Appendix (X):

**Sudan University of Science and Technology
College of Graduate Studies
Microbiology Program
Questionnaire**

Serological Detection and Molecular Characterization of CMV and its Glycoprotein B
(UL55) among Sudanese renal transplant recipients

1. Name-----
2. Age-----
3. Sex-----
4. Date of collection-----
5. Date of transplantation-----
6. Place of transplantation:

Ubsina Ahmed Gassim Outside Other

7. Symptoms if found-----

8. Donor relationship:

Relative Non- relative

9. Received treatment

Tacrolimus. Cyclosporine Sirolimus. Cellcept
Myfortic. Imuran

10. Immuno-status of donor and recipient for CMV

Donor----- Recipient-----

Results:

- 1- WBCS
- 2- Platelets count
- 3- IgM.....
- 4- IgG.....
- 5- CMV viremia.....
6. Viral load copies/ml.....
- 7-gB genotype