Molecular Detection and Glycoprotein B (UL55) Genotyping of Cytomegalovirus among Sudanese Renal Transplant Recipients

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Abstract

Background Cytomegalovirus (CMV) is the most common opportunistic pathogen among solid organ transplant recipients' especially renal transplants with significant morbidity and mortality. This study was designed to detect CMV DNA and to determine the frequency of different glycoprotein B (UL55) genotypes among Sudanese renal transplant recipients. Methods One hundred and four renal transplant recipients were included in this study. A blood specimen was collected from each recipient. DNA was extracted from plasma using QIAamp DNA mini kit. CMV amplification and quantification (estimation of viral load) was performed using CMV Real – RT Quant kits. Genotyping of Human CMV gB was carried out by nested PCR and sequencing of the highly diverse region of glycoprotein B. Results Cytomegalovirus (CMV) DNA (viremia) was detected in 40/104 (38.5%) of renal transplant recipients. The average of CMV DNA viral load was 358 x104 copies/ml (6.5 log10) ranged from 62 copies/ml (1.8 log 10) to 1.43x108 copies/ml (9 log10). CMV viremia was detected in (60%) of recipients of less than 1-12 months, (17%) of 13-24, (10%) of 25-36, (5%) of 37- 48 and (8%) in more than 48 months post-transplantation with no significant association (P. value = 0.296) between CMV viremia and post renal transplantation time. The association between the type of immunosuppressive drugs and high viral loads (more than 1000 copies /ml) showed a significant difference (P. value =0.05). The correlation between CMV loads of more than 1000 copies/ml and the presence of symptoms of CMV disease were highly significant (P.value =0.00). Fever 7(41%), fever and leucopenia 6(35%) and gastrointestinal disease 4(24%) were the most common presenting symptoms of CMV disease. CMV-genotyping revealed 8 cases (80%) for gB3, and 2 cases (20%) for gB4 genotypes. The most frequent genotype among Sudanese renal transplant recipients was gB3 and no mixed genotypes were observed. Conclusions The frequency of CMV DNA is high among Sudanese renal transplant recipients. CMV viremia viral loads were slightly lower in asymptomatic patients. CMV gB3 is the most predominant glycoprotein B genotype in Sudanese renal transplant recipients.

Introduction

Human cytomegalovirus (CMV) is a ubiquitous double-stranded DNA belonging to the Herpesviridae family that establishes lifelong latency after primary infection and causes life-threatening disease in immunosuppressed patients [1]. It is the most important opportunistic viral pathogen after renal transplantation [2]. CMV replication in the graft after reactivation in the transplanted kidney or infection from the host [3], is a leading cause for allograft failure and mortality. Without prophylactic measures, 40%-100% of renal transplant recipients undergo CMV infection and about 67% develop CMV disease [2]. The effects of CMV infection in transplant recipients are classified to direct and indirect [4], which has been associated with active viral replication [5]. The major symptoms of direct effects are fever and neutropenia syndrome and end organ diseases such as pneumonia, enteritis, meningitis, and encephalitis. The release of cytokines, chemokines, and growth factors in response to viral infection of the body [6], which are immunomodulatory that intensify immunosuppression and increase the risk of other opportunistic infection considered as indirect effects [7]. The CMV disease in kidney and liver

transplant recipients is associated with an elevated rate of viral load and viral load peak [8]. CMV UL55 gene encodes for a glycoprotein B which involved in several essential steps in CMV virus pathogenesis including virus penetration into cells, cell-to-cell spread, and activation of the immune response *gB* [9] and is involved in the activation of innate immunity as the major antigen for the induction of neutralizing antibodies. *gB* antibodies have been of interest because of their therapeutic potential for neutralization [10]. Regarding UL55 polymorphism, CMV has been divided into 4 genotypes (*gB*1-4) [9]. A fifth *gB* genotype (*gB*5) was detected in several AIDS patients [11]. Furthermore detecting *gB* antigen in patients with HCMV infection may facilitate the monitoring of the infection [10]. Despite advances in viral diagnostic tools in the world, still, there is a difficulty in the diagnosis of CMV virus among transplants in Sudan because it depends on serological tests which has a limited diagnostic value, due to immunosuppressive therapy that causing delayed seroconversion of IgM. IgM level can remain undetectable (there is a time lag between primary infection and IgM antibody production) [12]. To our knowledge no published data about CMV genotyping. So this study focused on detecting CMV DNA by real- time PCR and the circulating gB genotypes of CMV among Sudanese renal transplant recipients

Methods

Study design, duration, and population

This is a descriptive cross-sectional study conducted in Kidney Transplanted Association Hospital and Ahmed Gassim Hospital in Khartoum State from June 2014 to June 2016. Renal transplant recipients, who agreed to participate in this study, adults, and children, of both sex with or without signs and symptoms of CMV infection were included in this study.

Sampling technique

The study based on non-probability convenience sampling technique. Samples were taken from patients during their regular medical checkup.

Ethical consideration

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

Specimen collection

One-hundred and four (n=104) renal transplant recipients were selected for this study. Five ml of blood specimen were collected in EDTA container from each individual. Plasma was separated and stored at -20 C° until analyzed.

Quantitative Real -Time PCR for detection and viral load estimation

DNA was extracted from peripheral blood plasma according to the instruction of QIAamp DNA mini kit (Qiagen-Germany).CMV amplification and quantification (estimation of viral load) were done using hot start quantitative real- time PCR kits(CMV Real – RT Quant kits (Sacace- Italy). For reproducibility of qRT-PCR in measuring CMV viral load accurately in clinical specimens, intraassay variability was included using duplicates of CMV calibrator standard containing 10^2 and 10^4.

Glycoprotein B genotyping

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

Nested PCR for gB

Nested PCR glycoprotein B genotyping was performed using outer primer pairs *gB*-1, (5′CAAGARGTGAACATGTCCGA3′), *gB*-2 (5′GTCACGCAGCTGGCCAG3′)*gB*-3 , and inner primers pairs (5′TGGAACTGGAACGTTTGGC3′),*gB*-4 (5′GAAACGC G CGGCAATCGG3′),(Macrogen, Korea)[13].For outer nested PCR, the PCR mixture with a total reaction volume of 25 µl, containing 1 µl of both forward and reverse primers and 8 µl DNA, was subjected for amplification to an initial denaturation step at 95 ºC for 10 minutes. DNA was amplified for 35 cycles as followed: denaturation at 95 ºC for 1 minute, primer annealing at 55 ºC for 1 minute, followed by a step of elongation at 72 for ºC 1 minute, the final elongation was at 72 ºC for 7 minutes. For inner nested PCR the PCR mixture with a total reaction volume of 25 µl, containing 1 µl of both forward and reverse primers and 1µl DNA product was subjected for amplification to an initial denaturation step at 95 ºC for 10 minutes. DNA was amplified for 35 cycles as follow: Denaturation at 95 ºC for 30 seconds, primer annealing at 54 ºC for 45 seconds, followed by a step of elongation at 72 for ºC 30 seconds, the final elongation was at 72 ºC for 7 minutes[13]. The PCR products (520bp) for outer nested, (305bp) for inner nested were subjected to gel electrophoresis on 1.5% agarose. Gel results were photographed using a gel documentation system.

DNA sequencing

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

DNA Sequences similarity and alignment

Nucleotides sequences of both merged strands *gB* CMV genes were searched for similarity BLASTn [14] (http: //blast.ncbi.nlm.nih.gov/Blast.cgi). Then multiple sequence alignment of nucleotides and translated proteins was done by Bioedit software [15].

Mutant sequence analysis

The mutant nucleotides were confirmed by their reverse strands. I-mutant version 3[16], was used to study the stability of the mutant protein. Chimera software version 1.9 was used to predict the tertiary model of

protein [17].

Phylogenetic tree

Phylogenetic tree of CMV *gB* genes and their evolutionary relationship with well-characterized reference strains obtained from NCBI database was constructed by the neighbor-joining method with the bootstrap test of phylogeny in Molecular Evolutionary Genetics Analysis (MEGA) program, version 6 [18]. Bootstrap resembling strategy and reconstruction was carried out 1000 times to confirm the reliability of the phylogenetic tree.

Data analysis

Data were analyzed using statistical package for social science software (SPSS v.11.5). A *P.* value of 0.05 was considered significant for all statistical tests in the present study.

Results

One hundred and four(n=104) renal transplant recipients participated in this study, their age ranged from 11 to 72 years and mean age of 37 years ±14.37 (SD). Males recipients were 72 (69.2%), while 32 (30.8%) were females. Fifty (48%) of renal transplant recipients had received their organs in localized hospitals, while 54 (52%) received their organs abroad. Most of the renal transplant recipients received organs form relative donors 79 (76%) and only 25 (24%) from non-relative donors. The mean total white blood cells count among renal transplant recipients was 7100 WBCs/cmm ±2586.669 (SD) with a minimum count of 3200 WBCs/cmm and maximum count of 18600 TWBCs/cmm. The mean post-transplantation time in renal transplant recipients was 54 months, ranging from < than one to 204 months. Less than one to 12 months 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 >48 months 16(15.4%) of recipients (Fig.1).

Fig. 1 Post-renal transplantation time per month among the study group

All plasma specimens (n=104) were investigated for the presence of CMV DNA and viral load. Based on the constructed standard curve the correlation coefficient was at least 0.999, the amplification efficiency was varies between 97% to 100% and coefficient of variation (CV %) was from 0.00% to 8.5% for tested DNA and internal control for all trails (Fig. 2).CMV DNA (viremia) was detected in 40/104 (38.5%) of renal transplant recipients, (Fig. 3).The average of CMV DNA viral load was 358 $x10^4$ copies/ml (6.5 $\log_{10}$) ranged from 62 copies/ml (1.8 $\log_{10}$) to viral load $1.43x10^8$ copies/ml (9 $\log_{10}$). 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, (Table. 1).

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

Fig. 2 The standard curve for CMV (tested DNA)

Fig. 3 Frequency of positive CMV (viremia) among renal transplant recipients by qRT- PCR

Results of table (2) showed statistical significant difference (*P*. value =0.05) between the type of received immunosuppressive drug and high viral loads (>1000 copies /ml). Recipients with positive CMV viremia showed CMV symptoms were 17/104 (16.3%), while 23/104 (22.1%) were positive CMV viremia and asymptomatic. Out of symptomatic positive CMV recipients 14/17(82.4%) with high viral loads (>1000 copies/ml) and 3/17(17.6%) with low viral loads (<1000 copies/ml). While 1/23(4.3%) asymptomatic recipient had high viral loads and 22/23 (95.7%) asymptomatic recipient with low viral loads. The results revealed that the correlation between CMV loads of >1000 copies/ml and the presence of symptoms of CMV disease was highly significant (*P.* value =0.000), (Table. 3). 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 (2.7to 9 $\log_{10}$). 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.

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 (Fig.4). The nucleotide sequences of 10 isolates and their accession numbers were deposited in the GenBank database. 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 were observed. BLAST nucleotide search showed that two isolates were 99% identity with CMV *gB* genotype 4 (Genbank accession number M60926.2) from the United State of America, Spain (KR992839.1, KR992940.1), Brazil (AY186111.1, AY186112.1). Eight isolates showed 100% identity with CMV *gB* genotype 3 (KR992932.1) from Spain (Fig. 5).

Fig. 4 Gel electrophoresis of CMV UL55 gene PCR product (305 bp). Key. M: Marker (50bp). Lane 1: negative control, Lane 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 are positive samples.

Multiple sequence alignment of obtained CMV *gB* sequences compared with reference sequences previously published in database exhibited transversions mutation in 8 isolates. In which C was replaced by A at position 253 from reference CMV *gB* 3 (KR992932.1) (Fig. 6). That resulted in substitution of the codon CGT Arginine (R), to AGT Serine (S) (Fig. 7). Substitution of the protein shown by tertiary protein structure of wild type (R), and mutant type (S) at position 85, (Fig. 8).This substitution resulted in a decrease of protein stability as indicated by I-mutant software. 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, (Fig. 9).

Fig. 5 Bio-Edit multiple sequence alignment of CMV gB gene compared to CMV reference strain from Genbank. The transversion mutations in 8 CMV isolates are indicated by the black arrow

Fig. 6 (A); Normal codon and protein sequence of CMV- gB wild type from Genbank versus, (B); mutant codon and protein resulted as indicated by the blue color

Fig. 7 Amino acid multiple sequence alignment of Sudanese mutant gB gene compared to other gB genes from database. Substitution of the amino acid Arginine (R) to Serine (S) as indicated by black color

Fig. 8 Tertiary protein structure of wild (Left) and mutant (right) gB-3 gene of isolates.The predicted amino acid Arginine at position 85 from Genbank that predicted by Chimera software version 1.9

Figure (9): 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.

Discussion

Cytomegalovirus infection is one of the most frequently encountered opportunistic viral pathogens in renal transplantation [19]. 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. Male/female ratio about 2:1. This finding in agreement with Khameneh et al [20] in Iran, males were (61.1%) and females were (38.9%), Hasanzamani et al [19] in Iran 41(62.1%) of his population were males and 25(37.9%) were females. Most recipients in the present study received triple immunosuppressive therapy that makes them more liable to CMV infection as reported by Nafar and his colleges [21] which indicate that high immunosuppressive regimen is associated with a higher risk for CMV infection. Al-Alousy et al [22] 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 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 Tong et al [23] (22%), Madiet al [24] in Kuwait (24%), Enan et al [25] in Sudan (32.7%), and Lashini et al [26] in Iran (25.9%). In addition, parallel finding to this results observed by Garrigue et al [27] (36.6%) and Zhang et al [28] in China (37.7%). In contrast, lower result was obtained by Cordero et al [29] in Philippines (5.8%), Cupic et al [30] in Serbia (12.5%),and Khalafkhany et al [31] 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 $x10^4$ copies/ml (6.5 $\log_{10}$) ranged from 62 copies /ml (1.8 log 10) to 1.43$x10^8$ 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 less than 0ne - 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 months of transplantation 24/40(60%) compared with the later onset. Similar results were observed by Khalafkhany and his colleges [31] in Iran who detected CMV viremia in 31.2% of 0-3 months, 30.7% of 4-6 months, and 17.5% of 7-12 months post-transplantation.

The present results showed a statistically 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 used immunosuppressive regimen that associated with a higher risk of CMV infection and disease.

In this study higher viral load correlate precisely with the 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. This results confirming previous reports by Hadaya her colleges [32], Knipe and Howley [33], Madi et al [24], Helanter et al [34] and Rangbar-Kermani et al [35]. Medium level of viral load was higher in patients with symptomatic CMV disease than asymptomatic. The discrepancies in three symptomatic patients (17.6%) with a viral load of < 1000 copies/ml, and one asymptomatic patient (4.3%) with a viral load of >1000 copies/ml. These discrepancies could be explained by several factors such as the source of the donor's kidney, nature of immunosuppressive, and genotypes of the virus.

Findings of this study indicated that fever, fever leucopenia and gastrointestinal disease with abdominal pain and diarrhea were the most common presenting symptoms of CMV disease. Similar results obtained by Ardalan, [36] 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.

The result of sequencing and genotyping of HCMV *gB* gene (UL 55) for 10 CMV isolate revealed that gB3 (80%) was the most frequent genotype among Sudanese renal transplant recipients whereas gB4 was (20%) and no mixed genotypes were observed. No publish data is available in Sudan on CMV *gB* genotyping neither in renal transplant recipients nor immunocompetent host with CMV infection. These results in agreement with previous reports in Italy by Aristaet al [37] in which the predominant circulation of HCMV strains were *gB* type 2 and 3. Rather similar results were obtained by De Vries et al [38], in Netherland involved renal transplant recipients and congenitally infected newborns in which *gB*1 and *gB*3 being the most prevalent genotypes. Gandhoke et al [12] 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. Pasca et al [39] in Kuwait reported that *gB*1 (27%) was the most frequent genotype followed by *gB*2 (25%), gB3 (19%), *gB*4 (1%) and mixed genotypes were (27%). Coaquette et al [40] in France indicated that *gB*1 were found in (28.9%) of patients; *gB*2 (19.6%); *gB*3 (23.7%) *gB*4 (2.0%); and mixed infection (25.8%). Dieamant et al [41], in which *gB*1 and *gB*2 were the most frequent genotypes among Brazilian pediatric renal transplant patients. Khalafkhany et al [31] in Iran, mentioned

that *gB*1 (26.5%), *gB*2 (20.5%), *gB*3 (17.6%), and *gB*4 (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 studies 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 to the low number of the 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 as mentioned by Renzette et al [42].

The results of genotyping and sequencing in this study represent the first genetic characterization of HCMV in Sudan. Transversion mutations in *gB* gene were identified in 8 of the Sudanese *gB* 3 genotypes leading to amino acid substitution which result in a decrease of protein stability. The obtained results of protein tertiary structure showed a 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 [43]. The variability and mutations, particularly in *gB* that arise can be advantageous to the virus resulting in an increase in viral fitness and adaptation [44].

Findings of phylogenetic 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. In this study only 10 isolates were subjected to sequencing due to financial limitation.

Conclusions

This study concluded that high frequency of CMV infection among Sudanese renal transplant recipients. CMV viral loads were slightly lower in asymptomatic patients. The present study documented the association of CMV disease with the intensive immunosuppressive regimen, such as triple therapy as higher risk factors for CMV infection and disease. In this study, CMV *gB*-3 is considered the most predominant glycoprotein B genotype in Sudanese renal transplant recipients.

We recommended early monitoring of CMV using sensitive method such as qRT-PCR that exactly detect viral replication and provide guiding information help the clinicians to starting preemptive antiviral therapy that might have the advantages of reducing the occurrence of CMV disease.

Abbreviations

CMV: Cytomegalovirus, DNA: Deoxyribonucleic acid, gB: glycoprotein B, qRT-PCR: quantitative Real -Time PCR.

Declarations

Additional files

Ethical approval and informed consent.

Consent for publication

Not applicable

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Availability of data and materials

The nucleotides sequences were submitted to Genbank with the following accession numbers: MF179785, MF179786, MF179787, MF179788, MF179789, MF179790, MF179791, MF179792, MF179793, MF179794.

Authors' contributions

HHA designed, conducted the study and drafted the paper. AAO supervised the study and coordinated the laboratory quality control, HNA performed sequencing and bioinformatics analysis and approved the manuscript critically for important intellectual contents. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table. 1 Association between positive CMV and Post - transplantation time / month

Post-transplantation time / month	Real time PCR		Total	*P.* value
	Positive N. (%)	**Negative N. (%)**		
Less 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%)	

Sig *P.* value < 0.05

Table. 2 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%)	

Sig *P.* value< 0.05

Table 3 Association between CMVsymptoms and viral load copies/ml

Presence of CMV Symptoms	Viral load copies/ml		Total	*P. value*
	<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 %)	

Sig *P.* value< 0.05

Figures

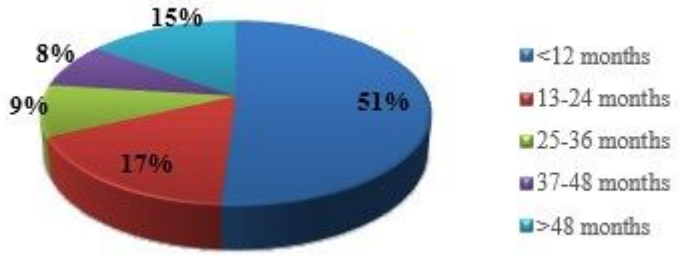
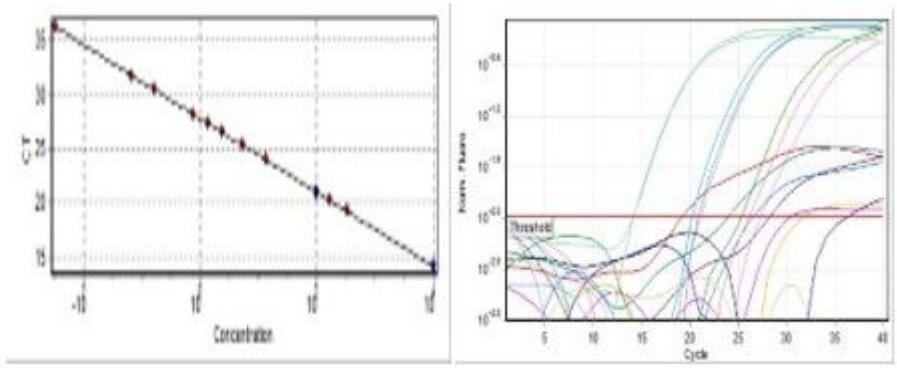


Figure 1

Post-renal transplantation time per month among the study group



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

Figure 2

The standard curve for CMV (tested DNA)

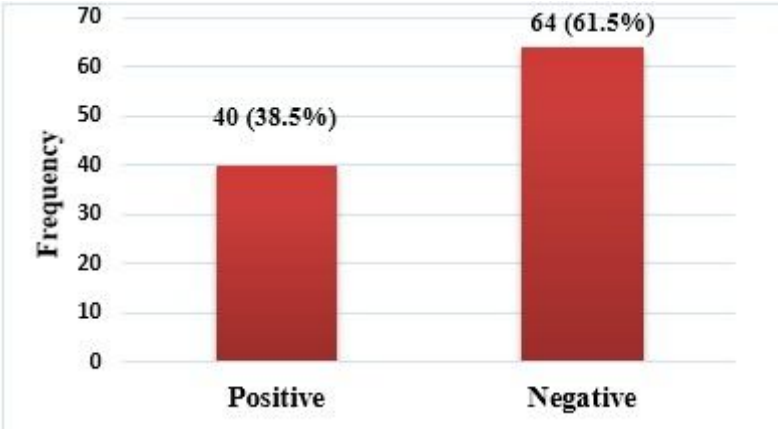


Figure 3

Frequency of positive CMV (viremia) among renal transplant recipients by qRT- PCR

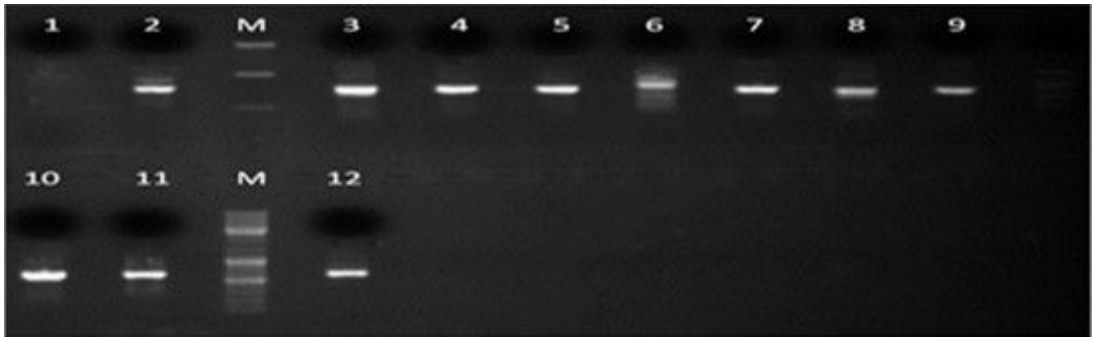


Figure 4

Gel electrophoresis of CMV UL55 gene PCR product (305 bp). Key. M: Marker (50bp). Lane 1: negative control, Lane 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 are positive samples.

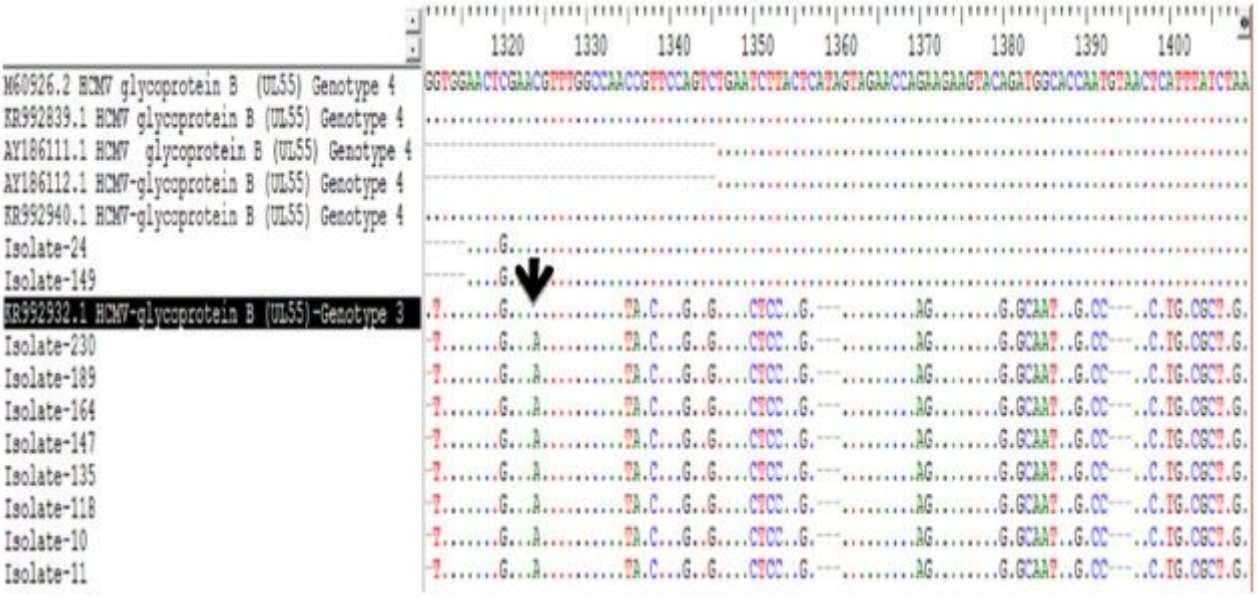


Figure 5

Bio-Edit multiple sequence alignment of CMV gB gene compared to CMV reference strain from Genbank. The transversion mutations in 8 CMV isolates are indicated by the black arrow

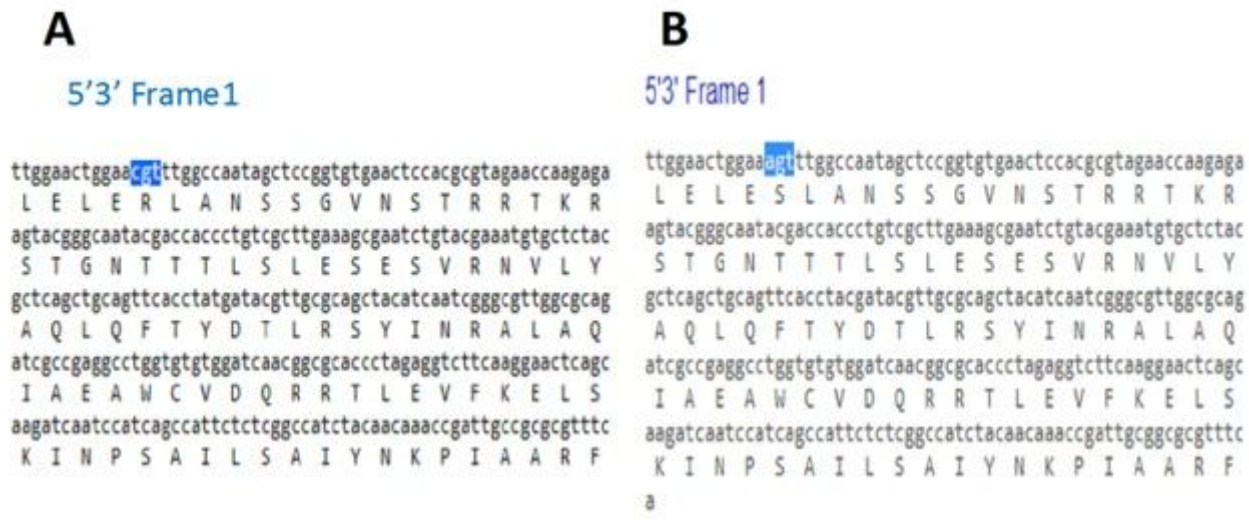


Figure 6

(A); Normal codon and protein sequence of CMV- gB wild type from Genbank versus, (B); mutant codon and protein resulted as indicated by the blue color

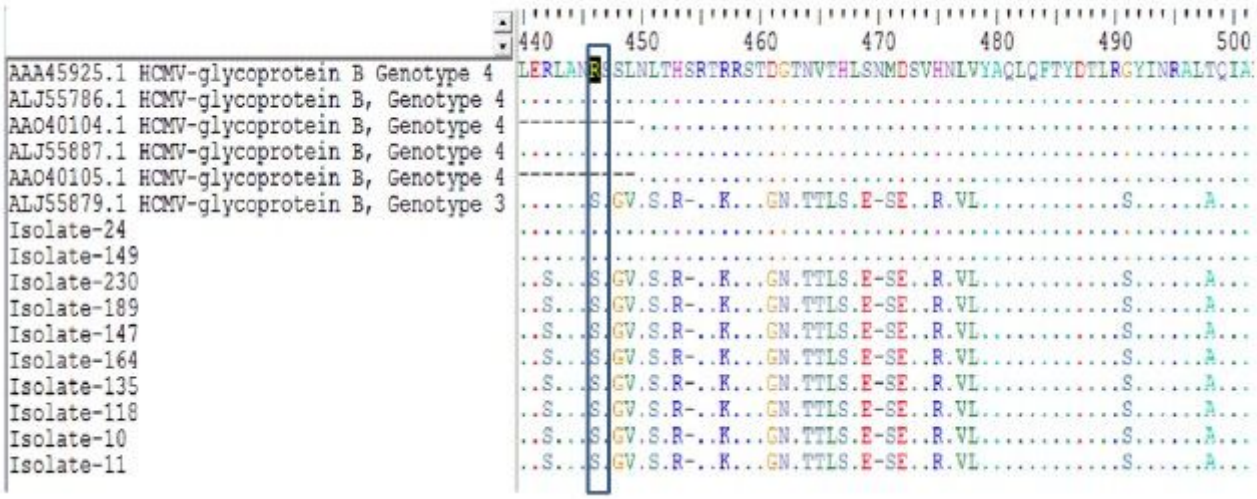


Figure 7

Amino acid multiple sequence alignment of Sudanese mutant gB gene compared to other gB genes from database. Substitution of the amino acid Arginine (R) to Serine (S) as indicated by black color

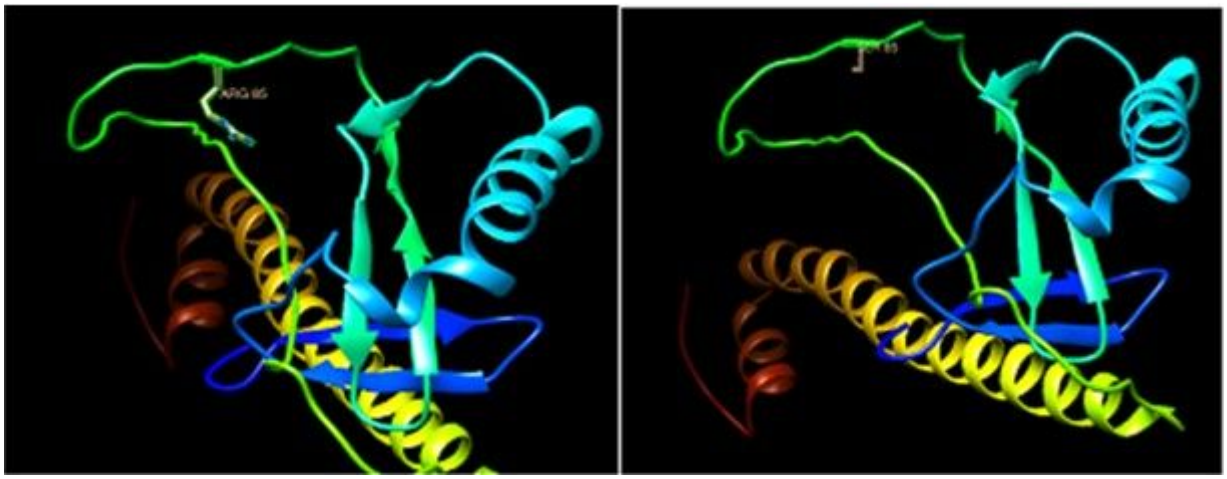


Figure 8

Tertiary protein structure of wild (Left) and mutant (right) gB-3 gene of isolates.The predicted amino acid Arginine at position 85 from Genbank that predicted by Chimera software version 1.9

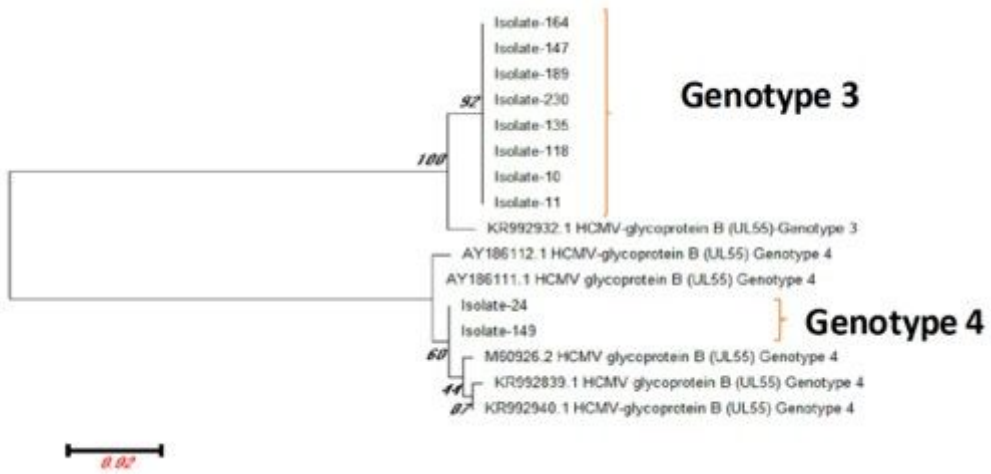


Figure 9

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.