Early detection of active Human Cytomegalovirus infection after living-donor liver transplantation

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Abstract

Human cytomegalovirus (HCMV) is a member of the beta herpes virinae. It is one of the most important virus in transplantation. It has direct and indirect impact on liver transplant recipient outcome. We aimed to diagnose early active HCMV infection in living donor liver transplant (LDLT) recipients. Also, to correlate the associated clinical and laboratory findings with HCMV infection. Here, we investigate 76 LDLT recipients for early detection of active HCMV infection in a period of 1-6 months after liver transplantation upon their suggested clinical data. These samples were collected in the period from 4/2013 to 12/ 2015 at Gastroenterology center, Mansoura University. They were 68 males and 8 females. HCMV infection diagnosed by ELISA (Ig M, Ig G) and real time PCR. Seventy-four patients were IgG seropositive recipient. Three patients (3.9%) were positive IgM. Ten samples from 76 patients (13.2%) were positive by real-time polymerase chain reaction (PCR). In this study, we concluded that, LDLT recipients are at high risk of HCMV infection (13.2%) and the most suitable method for HCMV detection was PCR.

Key words:

- HCMV: Human Cytomegalovirus
- LDLT: Living-donor liver transplantation
- PCR: Polymerase Chain Reaction.
- ELISA: Enzyme linked Immunosorbent Assay

Introduction 36 37

Opportunistic viral infections are common after liver transplantation. The herpes 38
virus infections are common after transplantation; Human cytomegalovirus (HCMV) 39
is most important virus of this group ⁽¹⁾. HCMV belongs to the beta herpes virus 40
family. Beta herpes viruses are closely related to each other with large genomic 41
overlapping ⁽²⁾. 42

The great seroprevalence and early acquisition of the virus have been associated 43
with lower socioeconomic circumstances, developing countries and over crowded 44
populations ⁽³⁾. HCMV infects 60%-100% of humans, with primary HCMV infection 45
occurring most commonly during the first two decades of life. In immunocompetent 46
individuals, the infected individuals are mostly asymptomatic or may present with a 47
benign febrile infectious mononucleosis-like illness ⁽⁴⁾. HCMV infection occur in the 48
majority of solid organ transplant patients, primarily in the first months when 49
immunosuppression is most intense, HCMV disease incidence ranges from 10% to 50
50% ⁽⁴⁾. 51

Liver transplant recipients are at a higher risk of HCMV infection, especially 52
those who did not have HCMV infection until they receive a latently infected organ 53
from a HCMV-seropositive donor (HCMV D+/R- mismatch). The risk of progression 54
into HCMV disease is magnified by the intense immunosuppression required to avoid 55
or to treat allograft rejection ⁽⁵⁾. The clinical impact of HCMV disease after liver 56
transplantation can be classified into: (1) an acute infection with clinical signs known 57
as direct effects (fever, mononucleosis, and invasive organ disease); and (2) a broad 58
range of immunomodulatory and vascular effects, referred to as indirect effects ⁽⁶⁾. 59
60

The aim of this study was to detect the incidence of HCMV infections in living- 61
donor liver transplantation recipients by ELISA and real time PCR. Also, to correlate 62
the laboratory findings with HCMV infections. 63

Materials and Methods 64 65

Subjects 66 67

This study was conducted on 76 living-donor liver transplant (LDLT) recipients 68
admitted to the Gastroenterology Surgery Center, Mansoura University in the period 69
from April 2013 to December 2015. They were 68 males and 8 females. Written 70

consent was obtained from patients. They were investigated for detection HCMV infections in a period up to 6 months after liver transplantation according to suspected clinical symptoms (e.g. fever, hepatitis, pneumonitis, urinary tract infections, gastroenteritis such as diarrhea and vomiting) (**Group I**). In addition, 10 living-donor liver transplant recipients of matched age and sex, who did not complain of any symptoms suggestive of HCMV, were included as a control group (**Group II**).

Methods

I. For the patients and control group, the followings were done:

- History was taken, thorough clinical examination, to detect suspected cases ,e.g. (fever , gastroenteritis as; diarrhea, vomiting, urinary tract infections, pneumonitis).
- Routine laboratory investigations as; Liver function tests (Bilirubin, Albumin, ALT, AST, ALP and GGT), kidney function tests (creatinine, urea and uric acid), C-reactive protein (CRP) were carried out using (Cobas Integra 800, Roche Diagnostics), and complete blood picture (CBC) was carried out using (Sysmex 1000).

II. Detection of HCMV antibodies (IgM, IgG) by ELISA

II. a. Detection of HCMV IgM and IgG: (DRG International, Inc., USA)

Blood samples were collected in a sterile vaccutainers and the serum was separated and stored at -20 °C until processing.

II. b. Molecular detection of HCMV DNA by real-time PCR:

Blood samples were collected in a sterile vaccutainers and the serum was separated and stored at -20 °C until processing.

II. b. 1. DNA extraction:

HCMV DNA was extracted using QIAamp mini kit (Cat. No. 57704 QIAamp miniElute virus spin kit, QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden, Germany).

II. b. 2. HCMV DNA amplification and detection by real-time PCR.

The Artus CMV TM PCR Kit (QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden, Germany) was used for detection of CMV- DNA.

Principle:	104
HCMV detection by the polymerase chain reaction (PCR) is based on the	105
amplification of specific regions of HCMV genome. In real-time PCR, the	106
amplified product is detected via fluorescent dyes. These are usually linked to	107
oligonucleotide probes, which bind specifically to the amplified product.	108
Amplification and detection:	109
Amplification and detection performed using the STRATAGENE (Applied	110
Biosystems Inc., Foster City, CA)	111
Thermal profile	112
Initially pre-cycling denaturation and then Taq activation achieved at 95 °C for	113
10 minutes. Then 45 cycles of denaturation at 95 °C for 15 seconds, annealing	114
and extension at 55 °C for 1 minute (Table I).	115
The results were then quantified using HCMV DNA at predetermined	116
concentrations provided by the manufacturer or from standard curve (Figure	117
I).	118
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Statistical analysis 120

Data analyzed and processed with SPSS software (Statistical Product and 121
Service Solutions, version 18.0, SSPS Inc, Chicago, IL, USA). Qualitative data 122
expressed in frequency and percentage and analyzed with the chi-square test or the 123
Fisher exact test, when appropriate. Quantitative data expressed as the mean and 124
standard deviation and compared with the t test. The correlation between two 125
quantitative variables done with Pearson's correlation coefficient or Spearman's rank 126
correlation coefficient, when appropriate. In all tests, a P value of < 0.05 was 127
considered significant, and a P value of < .01 was highly significant. 128
129

Results 130

The age and sex distribution in the studied patients ranged from 29 to 63 years 131
old with a mean of 51.14 ± 7.116 . They were 68 males and 8 females. The mean age of 132
the control group (group II) was (49.90 ± 6.74) (**Table 2**). The mean time for 133
occurrence of active HCMV infections occurred during the second post-transplant 134
month (2.58 ± 5.347) (**Table 3**). 135

The clinical data of the studied patients. Fever was the most common presentation in all studied cases (69.7%) followed by hepatitis (56.6%), gastroenteritis (42.1%), pneumonitis (5.3%). There was a significant difference between patients versus control group regarding to gastroenteritis, hepatitis and fever ($p=0.011$, 0.001 and <0.001 respectively) (**Table 4**).

HCMV detection by different methods in the studied cases: out of 76 patients, 74 (97.45 %) were IgG seropositive, 3 (3.9%) were positive for IgM and 10 (13.2%) were positive by real-time PCR. They were compared to control subjects and there was no significant difference as regard IgG. On the other hand, there was significant difference as regards IgM and PCR (**Table 5**).

The sensitivity, specificity, positive and negative predictive values of ELISA IgM for HCMV in comparison to real-time PCR as the reference standard. Both techniques gave positive results in two cases and negative in 65 patients. The sensitivity, specificity, positive predictive value and negative predictive value of ELISA IgM for HCMV were (20.0%, 98.5%, 66.7% and 89.0% respectively). Prevalence of positive HCMV in studied group I= 13.2% (**Table 6**).

Clinical data of HCMV in studied group I ($n=76$). Gastroenteritis, hepatitis and fever showed significant differences between positive cases versus control subjects ($p=0.033$, 0.003 , 0.001 respectively). Pneumonitis did not differ significantly between positive cases and control subjects (**Table 7**).

Clinical indications did not show significant differences between patients with positive HCMV infection versus those with negative HCMV infection and versus control subjects ($p>0.05$) (**Table 8**).

The relation between HCMV infection and laboratory data was increased serum bilirubin (total and direct) and GGT showed high significance differences between group I and control group (group II) ($p=0.002$, 0.001 , 0.009 respectively), increased ALP and glucose showed marginally significant differences ($p=0.093$, 0.071 respectively), increased CRP showed a significant difference ($p=0.038$). Mild reduction of hemoglobin concentration and mild elevation of INR showed marginally significance ($p=0.0503$, 0.066 respectively) (**Table 9**).

The relationship of post-transplant complications with active HCMV in-group I ($n=76$) versus control group. Recurrent HCV had higher incidence of HCMV infection (40.0% versus 12.1% in positive versus negative HCMV, $p=0.046$). Cases complicated with acute rejection had higher incidence of HCMV infection (50.0%

versus 24.2% % in positive versus negative HCMV), (p=0.128). No significant differences were found in recurrent HCC, biliary complications, other infections and pancytopenia (**Table 10**).

Discussion

The age of the studied patients ranged from 29 to 63 years with a mean 51.14±7.116 years. They were 68 (89.47 %) males and 8 (10.52%) females. We investigate 76 patients for HCMV antibodies (IgM and IgG) and real-time PCR assays. Out of 76 patients, 74 (97.36 %) patients were IgG seropositive and three patients (3.9%) were HCMV IgM seropositive. Real-time PCR was positive in 10 out of 76 patients (13.2%). The high prevalence of HCMV IgG antibodies in this study indicates that the incidence of HCMV infection is high in Egypt and this may be attributed to the poor socioeconomic status and hygienic practices.

Razonable and Pya, (2003) showed in their study that the incidence of HCMV infection ranges from 10% to 50% following solid organ transplantation ⁽⁴⁾. **Jain et al., (2005)** showed that, the incidence of HCMV infection was 14% after liver transplantation ⁽⁷⁾. In accordance **Wadhawan et al., (2012)** reported that HCMV reactivation was in 13% after LDLT, also, **Katsolis et al., (2013)** showed that HCMV infection was in 40% and disease was in 19% ^(8,9). In a study by **Garbinoa et al. (2005)**, they reported that infection episodes among the 78 out of 98 patients (80%), 44% were due to viral infections and HCMV infection was present in 29% of these viral infections ⁽¹⁰⁾. **Milan et al., (2013)** documented that 16 (32%) patients had HCMV infections after liver transplantation, including 13 (81%) with concomitant infections. Thirty-four patients (68%) did not have HCMV infections and eight of them (24%) had bacterial infection. There was a high correlation with bacterial infections among CMV-positive patients ⁽¹¹⁾.

We found two cases were positive by HCMV IgM and real-time PCR and 65 were negative by both techniques. The sensitivity, specificity, positive predictive value and negative predictive value of ELISA IgM for HCMV were (20.0%, 98.5%, 66.7% and 89.0%) respectively. The incidence of active HCMV infection by real-time PCR in studied group I= 13.2%.

Martín-Dávila et al.(2004) showed that The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of real-time PCR were 78%, 77%, 47%, 89% respectively ⁽¹²⁾. The study by **Schmidt et al., (1995)** for diagnosis of HCMV infection after liver transplantation, showed that in a total of 30 patients, fourteen patients (46%) were positive by PCR. The sensitivity and specificity of PCR was 100% and 76%, respectively. However, for the HCMV IgM, the sensitivity was only 66%, and the specificity was 84%.The demonstration of HCMV IgM antibodies is of little practical help as the antibody response develops too late in relation to infection ⁽¹³⁾. **Patel et al., (1994)** reported that the sensitivity of PCR in the diagnosis of symptomatic HCMV infection in liver transplant recipients was 100% ,the specificity was 57% and the positive predictive value was 50% ⁽¹⁴⁾.

In this study, there was no significant differences between patients with positive HCMV infection versus those with negative HCMV infection as regards clinical data ($p>0.05$). Gastroenteritis (50%), hepatitis (70%) and fever (80%) showed significant differences between positive cases versus control subjects ($p=0.033$, 0.003 , 0.001 respectively). Pneumonitis (20%) did not differ significantly between positive cases and control subjects. The classic illness caused by HCMV after transplantation is manifested by fever, bone marrow suppression, and Invasive diseases, which had been traditionally categorized either as HCMV syndrome. The most common organ system involved during HCMV disease is the gastrointestinal tract (in the form of HCMV gastritis, esophagitis, enteritis, and colitis), accounting for over 70% of tissue invasive HCMV disease cases in solid organ transplant recipients ⁽¹⁵⁾. The clinical indications for liver transplantation did not show any significant differences between patients with positive HCMV infection versus those with negative infection or with control group ($p>0.05$).

The similarity of clinical indications for liver transplantation between the studied patients and control group might explain the insignificant difference between these groups. The incidence of recurrent HCV was higher in patients with active HCMV infection versus control 40.0% and 12.1% in negative HCMV ($p=0.046$) respectively. In addition, patients with active HCMV infection showed higher incidence of acute rejection compared to control group (50.0% and 24.2% %) respectively. On contrary, **Singh et al., (2005)** did not find an association between recurrent HCV and HCMV infection after liver transplantation. **Rosen et al. (1997)**

reported that the incidence of HCMV induced cirrhosis was 50% compared to 11% in non-infected patients^(16, 17). HCMV infection has been associated with an increased risk for severity of HCV recurrence and this might be related to immunosuppression induced by HCMV infection or immunosuppressive drugs⁽¹⁸⁾. *Singh et al., (2005)* showed that, recurrent HCV infection after LDLT occurred in 55.6% of the patients with HCMV infection and 49.8% of patients without HCMV infection⁽¹⁶⁾.

The HCMV positive cases in comparison with control group showed elevated serum bilirubin (total and direct) and serum GGT (p=0.002, 0.001, 0.009) respectively, and elevated serum ALP and hyperglycemia (p=0.093, 0.071) respectively. In addition, elevated level of CRP showed significant differences (p=0.038). Mild decrease in hemoglobin concentration and mild elevation of INR showed mild significant difference (p=0.0503, 0.066) respectively. In multivariable analysis, increased risk for HCMV infection was evident in patients with lower model for end-stage liver disease (MELD) score (P = 0.025), lower total bilirubin (P = 0.014), and longer operative time (P = 0.038)⁽⁹⁾.

Summary and conclusion

Out of 76 patients, 74 (97.36 %) patients were IgG seropositive and three patients (3.9%) were HCMV IgM seropositive. Real-time PCR was positive in 10 patients (13.2%). In this study, two cases were positive by HCMV IgM and real-time PCR and 65 were negative by both techniques. The sensitivity, specificity, positive predictive value and negative predictive value of ELISA IgM for HCMV were (20.0%, 98.5%, 66.7% and 89.0%) respectively. The incidence of active HCMV by real-time PCR in studied group I= 13.2%. Gastroenteritis (50%), hepatitis (70%) and fever (80%) showed significant differences between positive cases versus control subjects (p=0.033, 0.003, 0.001 respectively). The incidence of recurrent HCV was higher in patients with active HCMV infection versus control 40.0% and 12.1% in negative HCMV (p=0.046) respectively. In addition, patients with active HCMV infection showed higher incidence of acute rejection compared to control group (50.0% and 24.2% %) respectively. HCMV positive cases in comparison with control group showed elevated serum bilirubin (total and direct) and serum GGT (p=0.002, 0.001, 0.009) respectively, and elevated serum ALP and hyperglycemia (p=0.093, 0.071) respectively. According to laboratory investigations, elevated CRP showed significant

differences ($p=0.038$). Mild decrease in hemoglobin concentration and mild elevation of INR showed mild significant difference ($p=0.0503$, 0.066) respectively.

References

- 1- **Zuckerman R and Wald A (2009):** Herpes simplex virus infections in solid organ transplant recipients. *Am J Transplant*; 4(Suppl 9): S104-S107.
- 2- **Efstathiou S, Gompels UA, Craxton MA, Honess RW, Ward K. (1988):** DNA homology between a novel human herpesvirus (HHV-6) and human cytomegalovirus. *Lancet*.; 1:63-64
- 3- **Ho M (1990):** Epidemiology of cytomegalovirus infections. *Rev. Infect. Dis.*; 12 (Suppl 7): S701–S710.
- 4- **Razonable RR and Paya CV (2003):** Herpesvirus infections in transplant recipients: current challenges in the clinical management of cytomegalovirus and Epstein-Barr virus infections. *Herpes*.; 10: 60-65.
- 5- **Dahiya D, Lee CF, Chan KM, Wu TJ, Chou HS, Cheng SS, Lee WC. (2011):** short-term preemptive treatment for cytomegalovirus infection in seropositive patients after liver transplantation. *J Hepatobiliary Pancreat Sci*.; 18: 32-38.
- 6- **Eid AJ, Arthurs SK, Deziel PJ, Wilhelm MP. (2010):** Clinical predictors of relapse after treatment of primary gastrointestinal cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant*.; 10: 157-161.
- 7- **Jain A, Orloff M, Kashyap R, Lansing K, Betts R, Mohanka R, Menegus M, Ryan C, Bozorgzadeh A. (2005):** Does valganciclovir hydrochloride (valcyte) provide effective prophylaxis against cytomegalovirus infection in liver transplant recipients? *Transplant Proc* 2005; 37: 3182-3186.
- 8- **Wadhawan M, Gupta S, Goyal N, Vasudevan R K, Makki K, Dawar R, Sardana R, Lal N and Kumar A. (2012):** Cytomegalovirus infection: its incidence and management in cytomegalovirus-seropositive living related liver transplant recipients: a single-center experience. *Liver Transpl*. 12:1448-1455.
- 9- **Katsolis JG, Bosch W, Heckman MG, Diehl NN, Shalev JA, Pungpapong S, Gonwa TA, Hellinger WC. (2013):** Evaluation of risk factors for cytomegalovirus

infection and disease occurring within 1 year of liver transplantation in high-	302
risk patients. <i>Transpl Infect Dis.</i> ;15(2):171-80.	303
10- Garbinoa J, Romandb J-A, Pittetc D, Giostrad E, Menthae G, Suter P.	304
(2005): Infection and rejection in liver transplant patients: a 10-year Swiss	305
single-centre experience. <i>SWISS MED WKLY</i> 2005;135:587–593.	306
11- Milan A, Sampaio AM, Guardia AC, .Pavan CR, Andrade PD, Bonon	307
SHA, Costa SCB, Ataíde EC, Boin IFSF, Stucchi RSB. (2013):	308
Identification of bacterial infections and clinical manifestation associated with	309
cytomegalovirus in liver transplantation patients. <i>Transplant Proc.</i> :	310
45(3):1130-1132.	311
12- Martín-Dávila P1, Fortún J, Gutiérrez C, Martí-Belda P, Candelas A, Honrubia	312
A, Barcena R, Martínez A, Puente A, de Vicente E, Moreno S. (2004): Analysis of	313
a quantitative PCR assay for CMV infection in liver transplant recipients: an	314
intent to find the optimal cut-off value. <i>J Clin Virol.</i> 33(2):138-44.	315
13- Schmidt CA, Oettle H, Peng R, Neuhaus P, Blumhardt G, Lohmann R, Wilborn F,	316
Osthoff K, Oertel J, Timm H, Schmidt CA. (1995): Comparison of polymerase	317
chain reaction from plasma and buffy coat with antigen detection and	318
occurrence of immunoglobulin M for the demonstration of cytomegalovirus	319
infection after liver transplantation. <i>Transplantation.</i> ; 59(8):1133-8.	320
14- Patel R, Smith TF, Espy M, Wiesner RH, Krom RA, Portela D, Paya CV.	321
(1994): Detection of Cytomegalovirus DNA in Sera of Liver Transplant	322
Recipients. <i>J Clin Microbiol.</i> ; 6: 1431–1434.	323
15- Fica A, Cervera C, Pérez N, Marcos MA, Ramírez J, Linares L, Soto G, Navasa	324
M, Cofan F, Ricart MA, Pérez-Villa F, Pumarola T, Moreno A. (2007):	325
Immunohistochemically proven cytomegalovirus end-organ disease in solid	326
organ transplant patients: clinical features and usefulness of conventional	327
diagnostic tests. <i>Transpl Infect Dis.</i> ; 9: 203-210.	328
16- Singh N, Wannstedt C, Keyes L, Wagener M M, Gayowski T, Cacciarelli TV.	329
(2005): Indirect outcomes associated with cytomegalovirus (opportunistic	330
infections, hepatitis C virus sequelae, and mortality) in liver-transplant	331
recipients with the use of preemptive therapy for 13 years. <i>Transplantation.</i> ;	332
79: 1428-1434.	333

17- Rosen HR, Chou S, Corless CL, Gretch DR, Flora KD, Boudousquie A, Orloff	334
SL, Rabkin JM, Benner KG. (1997): Cytomegalovirus viremia: risk factor for	335
allograft cirrhosis after liver transplantation for hepatitis C. Transplantation.;	336
64(5):721-726.	337
18- Wiesner RH, Sorrell M and Villamil F (2003): Report of the first	338
International Liver Transplantation Society expert panel consensus conference	339
on liver transplantation and hepatitis C. Liver Transpl.; 9:S1-S9.	340
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Table I: Thermal profile of HCMV DNA real-time PCR ((Applied Biosystems Inc., Foster City, CA)

Steps	Taq Activation	PCR (45 cycles)	
	Hold	Denaturation	Annealing & Extension
Temperature	95°C	95°C	55°C
Duration	10:00 min	15 sec	01:00 min

Table (2): Age and sex distribution in all studied subjects.

		Group I (n=76)	Group II (n=10)	<i>p</i>
Age (years)		51.14±7.116	49.90±6.740	0.602
Sex	Males	68 (89.5%)	8 (80.0%)	0.328
	Females	8 (10.5%)	2 (20.0%)	

P: significance between total cases versus control.

Table (3): Time-related HCMV infection in living-donor liver transplanted patients.

	Group I (n=76)	Group II (n=10)
HCMV	2.58±5.347	1.6±0.699

Table (4): Clinical data of the studied patients.

Underlying liver disease	Group I (n=76)		Group II (n=10)		<i>P</i>
	No.	%	No.	%	
Gastroenteritis	32	42.1	0	0	0.011
Hepatitis	43	56.6	0	0	0.001
Fever	53	69.7	0	0	<0.001
Pneumonitis	4	5.3	0	0	1.000

P: significance between total cases versus control.

Table (5): HCMV detection rate by different methods in studied cases (n=76).

		Group I (n=76)		Group II (n=10)		<i>P</i>
		No	%	No	%	
CMV IgG (ELISA)	Positive	74	97.4	10	100.0	1.000
	Negative	2	2.6	0	0	
CMV IgM (ELISA)	Positive	3	3.9	0	0	1.000
	Negative	73	96.1	10	100.0	
CMV DNA Real time PCR	Positive	10	13.2	0	0	0.588
	Negative	66	86.8	10	100.0	

P: significance between total cases versus control.

Table (6): Sensitivity, specificity , positive and negative predictive values of ELISA IgM for detection of HCMV in comparison PCR.

Method of Detection		Negative HCMV (66)		Positive HCMV (10)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		No	%	No	%				
CMV IgM (ELISA)	Positive (n=3)	1	1.5	2	20.0	20.0	98.5	66.7	89.0
	Negative (n=73)	65	98.5	8	80.0				

PPV: Positive predictive value, NPV: Negative predictive value.

Table (7): Clinical data of HCMV infection in-group I (n=76).

Clinical presentations	Negative HCMV (66)		Positive HCMV (10)		Group II (n=10)		P
	No.	%	No.	%	No.	%	
Gastroenteritis	27	40.9	5	50.0	0	0	0.033
Hepatitis	36	54.5	7	70.0	0	0	0.003
Fever	45	68.2	8	80.0	0	0	0.001
Pneumonitis	2	3.0	2	20.0	0	0	0.474

P: significance between total cases versus control.

Table (8): Relationship between clinical indications for liver transplantation to HCMV infection in all patients (n=76) versus control (n=10).

Indications	Negative HCMV (66)		Positive HCMV (10)		Group III (n=10)		P
	No.	%	No.	%	No.	%	
HCV liver cirrhosis	44	66.7	6	60.0	7	70.0	1.000
HCC	15	22.7	3	30.0	3	30.0	0.693
HBV liver cirrhosis	1	1.5	0	0	0	0	1.000
HCV and HBV liver cirrhosis	1	1.5	0	0	0	0	1.000
Cryptogenic liver cirrhosis	2	3.0	0	0	0	0	1.000
Budd-Chiari syndrome	2	3.0	1	10.0	0	0	1.000
Autoimmune liver cirrhosis	1	1.5	0	0	0	0	1.000

HCV: Hepatitis C virus.

HCC: Hepatocellular carcinoma.

HBV: Hepatitis B virus

P: significance between positive cases versus control.

Table (9): Comparison between HCMV infection and other laboratory data in group I (n=76) versus control (group II: n=10).

Laboratory data		Negative HCMV (n=66)		Positive HCMV (n=10)		Group II (n=10)		<i>P</i>	<i>P*</i>
		Mean	SD	Mean	SD	Mean	SD		
Albumin (g/dl)		3.55	0.49	3.66	0.62	3.83	0.440	0.512	0.490
Bilirubin	Total (mg/dl)	2.93	5.92	2.19	1.02	0.99	0.179	0.697	0.002
	Direct (mg/dl)	1.85	4.91	1.31	0.80	0.15	0.053	0.729	0.001
ALT (U/ml)		50.26	94.79	28.20	11.72	26.90	6.707	0.467	0.764
AST (U/ml)		78.21	149.81	34.80	13.43	29.30	8.042	0.365	0.281
GGT (U/L)		152.48	138.61	111.30	81.38	25.20	5.266	0.364	0.009
ALP (KAU)		11.33	9.18	8.70	2.54	11.50	4.301	0.373	0.093
Creatinine (mg / dl)		1.24	0.69	1.06	0.62	0.91	0.145	0.450	0.464
F.B.Glucose (mg / dl)		138.83	61.09	140.00	64.52	100.70	6.056	0.956	0.071
CRP (mg / L)		41.95	53.92	38.40	45.56	3.50	1.080	0.844	0.038
TLC (X10 ⁹ /l)		8.25	7.07	6.60	3.15	6.740	2.202	0.474	0.912
Hb (g/dl)		10.29	2.02	10.13	1.13	11.270	1.295	0.720	0.0503
Platelets (X10 ⁹ /l)		157.50	88.48	204.80	116.24	165.00	68.323	0.135	0.363
INR		1.28	0.63	1.31	0.38	1.060	0.070	0.897	0.066

P: significance between total cases versus control.

P*: significance between positive cases versus control.

Table 10:

Relationship of post-transplant complications to active HCMV in group I (n=76).

Complications		Negative HCMV (66)		Positive HCMV (10)		Group II (n=10)		P	P*
		No	%	No	%	No	%		
Acute rejection	Early	16	24.2	5	50.0	0	0	0.128	0.033
	Late	5	7.6	0	0	0	0	0.368	-
Recurrent HCV		8	12.1	4	40.0	0	0	0.046	0.087
Recurrent HCC		4	6.1	0	0	0	0	0.424	-
Biliary complications		12	18.2	1	10.0	0	0	0.522	1.000
Portal vein stenosis		7	10.6	0	0	0	0	0.584	-
Renal impairment, toxicity and failure		11	16.7	0	0	0	0	0.341	-
DM		5	7.6	0	0	0	0	0.368	-
HTN		6	9.1	0	0	0	0	0.320	-
Pneumonia and pleural effusion		4	6.1	0	0	0	0	0.424	-
Other infections		8	12.1	1	10.0	0	0	0.847	1.000
Pancytopenia		2	3	1	10.0	0	0	0.349	1.000

HCV: Hepatitis C virus.

HCC: Hepatocellular carcinoma.

DM: Diabetes Mellitus

HTN: Hypertension

P: significance between positive cases versus control.

p*: significance between positive cases versus negative cases.

Figure I: Standard curve of quantitation of HCMV by real-time PCR