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ORIGINAL ARTICLE

Cytomegalovirus in Liver Transplant Patients

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ABSTRACT

Background: *Graft survival in liver transplant recipients is significantly lower in patients with a history of CMV infection compared to those without. In the absence of any preventive therapy 75% of recipients develop CMV infection post liver transplant.* ***Objectives:*** *This study detected the incidence of cytomegalovirus in liver transplant patients and evaluated post-transplant risk factors for HCMV and its complications.* ***Methodology:*** *A prospective study was conducted from the September 2018 till March 2020. Sixty subjects were involved; 30 patients were admitted for liver transplantation at the Gastroenterology Surgery Center (GISC), Mansoura university, and 30 donors. MELD score was calculated, blood samples were taken, CMV antibodies and CMV DNA were detected. Post transplantation follow up for 6 months and complications were reported.* ***Results:*** *HCMV viremia was detected in 46.6% recipients and in 10% donors by PCR. One recipient was positive for IgM and the rest were IgG positive and all donors were IgG positive. The most common reported complication after liver transplantation was bacterial infections (46.4%).* ***Conclusions:*** *Half of patients developed CMV infection after transplantation. The commonest risk factors for post-transplant CMV infection were seropositive donor or recipient >60 AU/mL, HCV patients, body mass index >25 and DM. Patients with positive HCMV infection had significantly higher MELD score than those reported negative HCMV.*

INTRODUCTION

Human Cytomegalovirus (CMV) is a double stranded DNA virus that belongs to the Herpesviridae family, subfamily Beta-herpesviridae, genus Cytomegalovirus [1]. Cytomegalovirus has the capacity to remain latent in lymphoid organs and myeloid cells. It can be transmitted by exposure to body fluids including blood and via transplantation of solid organs. Infection by this virus can cause many diseases as: pneumonia, retinitis, encephalitis, nephritis, hepatitis, myocarditis, and pancreatitis. In the United States the CMV infections has been reported to be around 70 % among high risk patients and a higher prevalence has been noted in developing countries[2]. In solid organ transplantation, the incidence of CMV infection within the first four months post-transplant is between 36-100% in which it can cause graft rejection or be a major cause of morbidity and mortality. This infection may occur due to transmission of the virus by the transplanted organ, primary infection, or reactivation of latent infection. The major risk factors are when the recipient is cytomegalovirus seronegative and the donor is seropositive[3]. Approximately 11% of all liver transplants performed are for acute liver failure. Infectious complications and Morbidity remain the most common causes of death and highlight the importance of intensive monitoring and early treatment of perioperative complication [4]. Detection of CMV IgM in recent infection and IgG for old one and Polymerase chain reaction (PCR) is rapid and sensitive method of CMV detection [5]. This study was carried out to detect the incidence of cytomegalovirus in liver transplant patients and to evaluate post-transplant reactivation risk factors and complications.

METHODOLOGY

Patients*:*

This prospective study was conducted from the beginning of September 2018 till March 2020. Sixty subjects were involved in this study; 30 patients were admitted for liver transplantation at the GISC, Mansoura University, and 30 donors. All the participants were adults and it was the first transplant for the recipient. The exclusion criteria were previous history of organ transplant and positive anti- EBV IgM. Follow up was done for six months after surgery to detect CMV reactivation and post transplantation complications. A consent was taken from each subject. Liver transplantation MELD score was calculated according to Malinchoc equation [6]. The study was approved by

institutional review board of Mansoura faculty of medicine, Mansoura University.

Blood samples were taken from patients under complete aseptic techniques.

Cytomegalovirus antibodies detection by ELISA:

Specific antibodies against CMV (IgM, and IgG) were detected by ELISA according to the manufacturer's instructions (IBL, American).

Cytomegalovirus DNA detection by PCR:

DNA was obtained from the samples by iNtRON Biotechnolgy G-spin Total DNA Extraction Kit, Biovision, cairo, according to the manufacturer's instructions. Extracted products were assayed for CMV DNA by using primer pair 5\-3\ (CCGCAACCTGGTGCCCATGG and CGTTTGGGTTGCGCAGCGGG) to amplify a target sequence of 139-bp within a gene code for the production of a late antigen gp64 specific to the CMV [7].

Statistical Analysis

Data were analyzed using SPSS, version 16. Independent t-test and chi-squared tests were used to detect significant differences (P<0.05).

RESULTS

Table 1 shows the frequency of the underlying aetiology requiring liver transplantation in studied patients.

Table 1: Frequency of the underlying liver disease in transplant patients

Underlying disease	Group I		Group II		Test of significance
	N=30	%	N=30	%	
HCC	3	10%	0	0.0%	FET P=0.237
HCV cirrhosis	23	76.7%	0	0.0%	χ^2=37.29 p<0.001*
HBV cirrhosis	2	6.7%	0	0.0%	FET P=0.492
Bud-chiari syndrome	1	3.3%	0	0.0%	FET P=1.0
Autoimmune	1	3.3%	0	0.0%	FET P=1.0

χ^2=Chi-Square test FET: Fischer exact test ; HCV: Hepatitis C virus.; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus.

All the differences were statistically significant when compares the recipient and donor in demographic and the laboratory data, as shown in Table 2.

Table 2: Comparison of demographic and laboratory data in studied subjects

Data		Gp I (Recipient) No.= 30	Gp II (donor) No.= 30	*P-value*
Hematolgic data	**Hemoglobin (g/dL) N=(13-18)**	12.23± 1.84	13.73 ± 1.48	0.001
	INR (U)	1.33 ± 0.39	1.01± 0.04	0.0002
	TLC (thousands/cmm) N=(4-11x1000)	6.46 ± 4.10	7.12 ± 1.96	0.5491
Liver function tests	**Serum albumin (g/dL) N=(3.4- 5.4)**	3.55 ± 0.83	4.31± 0.64	0.011
	Total bilirubin (mg/dL) N= (0.1- 1.1)	2.62 ± 1.95	0.53±0.18	< 0.0001
	Direct bilirubin (mg/dL) N= (0.1- 0.5)	1.71 ± 1.44	0.20 ± 0.14	< 0.0001
	ALT (U/ml) N= (0 -45)	45.53 ± 17.22	23.50± 6.13	< 0.0001
	AST (U/ml) N= (0-45)	56.67± 19.48	21.35 ± 2.16	< 0.0001
	GGT (U/l) N= (8-61)	48.73 ± 16.88	2.00 ± 0.00	< 0.0001
Renal function tests	**Creatine (mg/dl) N= (0.7-1.2)**	0.83 ± 0.18	0.70± 0.15	0.023
Chemical tests	**CRP (mg/L) N= (0-6)**	4.40±8.65	2.00± 0.00	0.2556
	F.B.G (mg/dL) N= (70-110)	126.70± 44.49	93.95±12.76	0.0003
Coinfection	**HCV positive**	23	0	-
	HBV positive	2	0	-
	HIV	0	0	-

N: Normal values
Serum creatine: 0.7- 1.2 mg/dl
Serum fasting blood glucose: 70 -110 mg /dl
TLC (Total leukocytic count): 4-11x109/l
Serum GGT (Gamma glutamyle transefrese): 8-61 U/L
HBV: Hepatitis B virus
HIV:Humman immunodeficiency virus
Serum albumin: 3.5 -.5 mg/dl
Total bilirubin: 0.1-1.1 mg/dl
Serum ALT (Alanine transaminase): 0-45 U/ml
Serum AST (Aspartate transaminase): 0-45 U/ml
CRP (C-reactive protein):0-6mg/L
HCV: Hepatitis C virus

All donors were IgG positive, only one recipient was positive for CMV-IgM, and 29 (96.67%) were CMV-IgG. The IgG titre was ≥60 AU/mL in 11 recipients and 2 donors. Cytomegalovirus Pp65 gene was detected in 14 blood samples from the recipient group while 3 blood samples showed this gene from the donors group as shown in Figure 1.

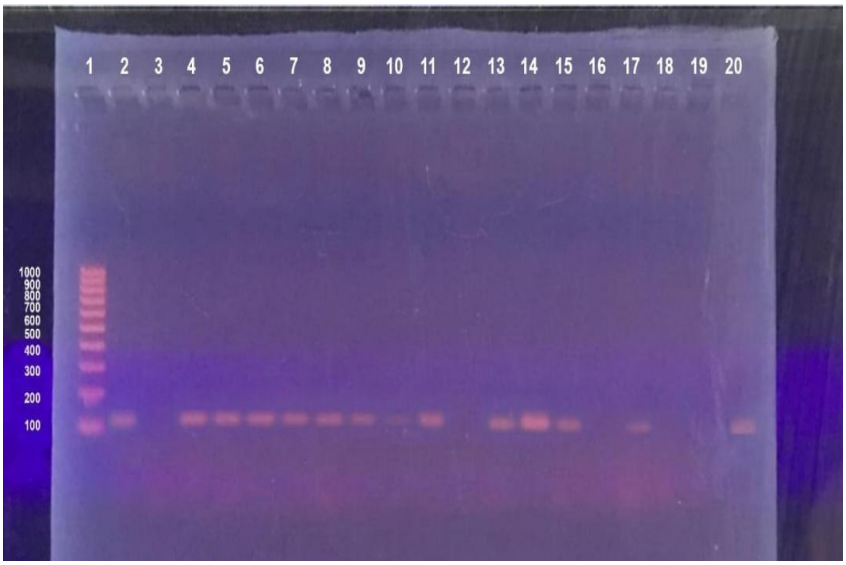

Fig. 1: Agarose gel electrophoresis of amplified CMV DNA from recipient samples Lane 1 shows 1000 bp DNA lonza ladder, and fourteen blood samples were positive for CMV and showed bands (Lanes 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 17, and 20).

Table 3 shows that all the differences were statistically non-significant except for the age, direct bilirubin, and CRP when compares CMV positive and CMV negative individuals in the demographic and the laboratory data. Dual HCV/CMV viral infection was statistically significant; and the OR was 202 (95 % CI: 10.76 to 3789; P = 0.0004). Triple HCV/HBV/CMV infection was statistically significant. Six patients were positive for HCV RNA in the CMV negative group. Table 4 shows the risk factors for positive CMV patients. There was a significant correlation in activation of HCV re-infection and bacterial infection with CMV positive patients as shown in table 5. The most common site of infection was chest infection followed as shown in table 6.

Table 3: Comparison between CMV positive and negative recipients in demographic and laboratory data

Point of comparison		**CMV POSITIVE No.= 14**	**CMV NEGATIVE No.= 16**	**P-value**
Demographic data	Age (year)	50±13.7	35.5±14.6	**0.001**
	Gender (M/F)	11/3	13/3	**0.120**
Hematological data	Hemoglobin (g/dL)	12.43±1.86	12.9±1.8	**0.370**
	INR (U)	1.27±0.43	1.21±0.29	**0.534**
	TLC (thousands/cmm)	7.1±4.72	6.66±2.2	**0.623**
Liver function tests	Serum albumin (g/dL)	3.81±0.7	3.89±0.9	**0.744**
	Total bilirubin (mg/dL)	1.5±0.8	3.02±2.2	**0.008**
	Direct bilirubin(mg/dL)	1.05±0.57	0.5±0.1	**< 0.0001**
	ALT (IU/l)	43.35±22.49	54.9±25	**0.103**
	AST (IU/l)	49.12±30.48	64.78±40	**0.152**
	GGT (IU/l)	40.37±53.8	24.56±37	**0.197**
Renal function test	Creatine (mg/dL)	0.78±0.21	0.75±0.14	**0.552**
Chemical tests	CRP (mg/L)	6.8±11.5	2.1±1.06	**0.009**
	F.BG. (mg/dL)	118±38.23	109±39.62	**0.427**
Score	MELD score	16.25 ±1.900	15.14 ±1.78	**0.042**
Virological assessment	HCV positive	12	6	**0.0004**
	HBV positive	2	0	**0.100**
	CMV/HCV HBV	**1**	**0**	**0.002**

Table 4: Risk factors associated with CMV positive subjects by PCR

Comparison points	CMV+ve	CMV-ve	OR 95% CI	P-value
I-Recipient variables total (30)	No (14)	No (16)		
1-Age	50±13.7	35.5±14.6	3.11(1.60,6.03)	<0.001*
2-Male sex	11	12	1.03(0.79,1.34)	0.84
3-BMI (kg/m2)				
>25 kg/m2	6	10	4.7(0.983,23.682)	<0.03*
<25 kg/m2	8	6		
4-MELD score	16.25	15.14±1.78	1.00(0.96,1.03)	0.042*
5-CMV antibody titer	±1.900			
>60 AU/mL	8	3	5.78(1.118,29.847)	0.02*
<60 AU/mL	6	13		
6-Virus co-infection				
HCV± HBV/CMV	13	6	21.67(2.234,210.111)	0.004*
CMV only	1	10		
7- Use of corticosteroid	13	14	1.70(0.75,3.8)	0.20
8-Use o cytotoxic drug	10	12	1.11(0.94,1.311)	0.22
II-Donor variables total (30)	(3)	(27)		
1-Age	27.70±7.72	18±8.1	2.11(1.4,5.03)	<0.002*
2-Male sex	2	10	1.04(1.01,1.07)	0.001*
3-CMV serological-status				
>60 AU/mL	2	0	4.48(1.118,24.847)	<0.001*
<60 AU/mL	1	27		
III-Transplant variables				
1-living donor	14	16	0.081(0.44,1.48)	0.49
2-Blood transfusion				
<2L	3	9	0.45(0.104,1.946)	0.14
>2L	11	7		

Table (5): Post-liver transplant complications in CMV positive and negative recipients

Post-transplant complication	CMV +ve n=14		CMV -ve n=16		P-value
	N	%	N	%	
Rejection	0	0%	0	0.0%	…..
Hypertension	2	14.2%	2	12.5%	0.112
Activation of HCV infection	5	35.71%	1	6.25%	0.001*
DM	2	14.2 %	2	12.5%	0.112
Bacterial infection	9	64.28%	5	31.25%	0.031
Biliary complications	3	21.42 %	4	25%	0.211
Renal impairment	2	14.2 %	2	12.5%	0.121
Post-operative bleeding	3	21.4%	2	12.5%	0.112

Table (6): Post-transplant bacterial infections in CMV positive and negative recipients

Infection site	Bacterial isolates	Total bacterial infection (14)				P-value
		CMV +ve (9)		CMV –ve (5)		
		N	%	N	%	
Chest	Klebsiella pneumonia	3	43%	0	0%	<0.001*
	Staphylococcus aureus	2		0		
	E-coli	1		0		
Blood stream	Staphylococcus aureus	0	7.1%	2	4.28%	0.542
	E-coli	1		0		
Fecal infection	Salmonella	0	0%	2	21.5%	0.002*
	Shigella	0		1		
Nasal colonization	MARSA	2	14%	0	0.0%	0.033

There were statistically significant differences in direct bilirubin, total leukocytic count, and MELD score between pre and post-operative values as shown in table 7.

Table (7): Assessment of laboratory parameters in CMV +ve liver transplant patients

Laboratory parameters		N=14		P-value
		Pre-operative	Post-operative	
liver function tests	ALT	43.35±22.49	33.47 ± 18.20	0.19
	AST	49.12±30.48	34.10 ± 25.41	0.16
	Total bilirubin	1.5±0.8	0.87 ± 0.38	0.9
	Direct bilirubin	1.05±0.57	0.34 ± 0.24	0.0003*
Hematological	Hemoglobin	12.43±1.86	12.5±2	0.48
	INR	1.27±0.43	1±0.5	0.234
	TLC	7.1±4.72	4.8±2.8	0.003
renal function test	creatine	0.78±0.21	0.75±0.14	0.552
Chemical tests	F.B.G	118±38.23	110±24	0.24
	CRP	6.8±11.5	5±6.5	0.28
MELD	score	16.25±1.900	11.25±2.5	0.002

DISCUSSION

Cytomegalovirus continues to be the "troll" that so often interferes with the successful outcome of organ transplantation, not only causing significant morbidity and mortality from CMV disease itself, but also increasing the susceptibility of immunosuppressed organ transplant recipients to subsequent bacterial/fungal superinfections, as well as to graft rejection and decreased patient survival [8]. In this study, CMV infection was common in patients with a mean age 51.9 ± 19.7 who were going for liver transplantation, most of the recipients were rural residence 28 out of 30(93.3%) and 2 (6.7%) from urban as rural people are more common HCV infection end stage liver disease leading to liver transplantation and more exposure to CMV reactivation. Recipients were suffering from chronic depleting disease as diabetes mellitus 18 (60%) and hypertension 10 (33.3%) with p-value (<0.001, 0.028) respectively.

These data were parallel with Wai et al.[9], who reported that, the average age of liver transplant recipient 50.0 and 49± 2 years respectively. Blanco et al.[10], reported that 61 patient out of 115 going for liver transplantation were suffering from diabetes mellitus this is due to from impaired glucose metabolism or insulin resistance in a patient with poor liver function and Pisano et al.[11], found that arterial hypertension was uncontrolled (BP >140/90 mm Hg 158 (32%) in liver transplant recipients and controlled in 332 (68%) patient. Human cytomegalovirus is one of the most serious infections of human that results in development of liver cirrhosis. Transplantation is the choice for patients with end stage liver disease [12].

In Egypt, hepatitis C virus (HCV) prevalence is about 15% of the Egyptian population and remains the most common etiology of cirrhosis, HCC and indication for liver transplantation [13]. In our study, the main indication of liver transplantation in studied patients were HCV end stage liver cirrhosis found in 23 (76.7%) patients, hepatocellular carcinoma 3(10%), HBV liver cirrhosis 2 (6.7%), Budd-Chiari syndrome 1(3.3%) and autoimmune diseases 1 (3.3%) respectively. This in agreement with Albright et al.[14], reported that HCV associated liver disease accounted for 41.3% of all indications of liver transplantation, 6.5% HBV associated liver disease, and this may attributed to the locality. Simillary the Jabanese Liver Transplantation Society (2011), showed that HCV related disease is the main indication for adult recipients of living –donor liver transplantation by 32%.

On contrary Lee [15], found that HBV was the main indication of liver transplantation (81%) and HCV induced liver by 3%. Human cytomegalovirus IgM is detected in acute infection while IgG lasts for years persists in the host probably for life either in states of latency or low-level replication, with sporadic episodes of reactivation. Reactivation is detected with greater frequency in immunocompromised patients [16].

In this study, 29 (96.67%) patients were positive for IgG and 1 (3.3%) were positive for IgM, all donors were IgG positive out of the recipients there were 11 with IgG titre >60 AU/ml and 2 donors IgG titre >60 AU/ml. The high incidence of HCMV IgG antibodies in this study indicates that HCMV infection in Egypt is high and this may be due to low socio-economic and bad hygienic practices. This is in accordance with Tabll et al.[17], who detected higher CMV positivity 87% and 25% for IgG and IgM antibodies, respectively, among patients from Mansoura city. The level of CMV viremia plays a critical role in the pathogenesis of CMV disease as it is considered a major risk factor for the

development of CMV disease. Polymerase chain reaction had high sensitivity and specificity for detection of CMV DNA in liver transplant patients [18]. In the current work, detection of Pp65gene was positive in 14 out of 30 (46.7%) blood samples from the recipient group and in 3 out of 30 (10%) in donors. this is like Hassan et al.[19], results who found that half of the liver transplant recipients had positive CMV by PCR with a significant relationship between the CMV viral load and the development of symptomatic CMV infection. In contrast Agha et al.[20], found that HCMV infection was about (11.7%) in transplant recipients by PCR. Antiviral prophylaxis by ganciclovir or valganciclovir is now adays widely established in patients with high-risk CMV immunoglobulin G donor (D)/recipient (R) sero-constellation (D+/R−) also anti-CMV Ig has for a long time been the corner stone in prophylaxis [21]. In the current study, HCMV infection was present in 60.8% of HCV patients and dual HCV/CMV and Triple HCV/HBV/CMV viral infection was statistically significant. Several studies showed support the same findings [22, 23].

However, it was documented that achieving sustained a virologic response to ribavirin plus pegylated interferon in chronic HCV patients could dramatically diminished during CMV infection [24]. In the present study, patients with CMV infection had significant difference in MELD score when compared with CMV negative individuals which were also reported by many studies which suggested increased the risk of patients for end-stage liver disease in CMV disease [25,26]. In our work, an assessment done to evaluate risk factors in liver transplant patients, showed that in out of 14CMV+ve recipients 8(57%) had CMV antibodies titre >60 AU/mL and 6 (43%) antibodies titre <60 AU/mL and there is a significant risk factor associated with virus co-infection and body mass index with p-value(0.004, <0.03) respectively. Bruminhent et al.[27], Found that anti-CMV antibody titre distribution was >60 AU/mL and <60 AU/mL in 136 patients (60.4%) and 89 (39.6%) respectively. The post transplantation complications occur both immediately post-transplantation and in the long-term. The main complications in the immediate postoperative period are related to dysfunction and rejection, the surgical technique, infections, and systemic problems. In the long term, the complications are typically a consequence of the prolonged immunosuppressive therapy, and include diabetes mellitus, systemic arterial hypertension and nephrotoxicity [28].

In the present study, in CMV positive patients there were many significant complications after transplantation. Such observation was in parallel with other [29, 30,31].

Infections usually occur 6-month after liver transplantation which may be related to the time of environmental exposure, combined viral infections, or late biliary complications, and these infections are common cause of high mortality rates [32]. It is estimated that up to 80% of liver transplant patients will develop at least one bacterial infection during the first year after transplantation, opportunistic infections are a leading cause of death during the first three years after transplantation[33].

In this study statistically there was a significant difference between pre and post-operative direct bilirubin, total leukocytic count and MELD score which indicate improved patient health after transplantation.

Lilford et al.[34], correlate analyses between transformed preoperative total bilirubin levels and postoperative rise in transaminases as a marker of ischemic reperfusion injury they showed significant negative coefficients for both ALT and AST, Rostved et al.[35], found that MELD score determined 14 days after liver transplantation is a strong predictor of survival or re-transplantation after liver transplantation.

CONCLUSION

The main indication of liver transplantation was HCV end stage liver cirrhosis (76.6%), hepatocellular carcinoma (10%), HBV liver cirrhosis (6.7%), Budd-Chiari syndrome (3.33%) and autoimmune liver disease (3.33%). The overall prevalence of CMV in liver transplant recipient was 23.3% and 5% in donors. CMV IgM was detected in 3.33% of the recipient group while CMV IgG was detected in 96.67% and all donors were IgG positive. The commonest risk factors for post-transplant CMV reactivation were seropositive donor or recipient >60 AU/mL, HCV patients, body mass index >25 and DM. Patients with positive HCMV had higher MELD score than HCMV negative patients. Gram negative bacterial infections were the commonest complication after transplantation and chest infection was the most common site.

Assignment Conflict: no significant competing financial, professional or personal interests that might have influenced the performance or presentation of the work described in the manuscript

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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