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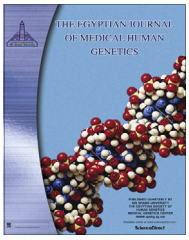

ORIGINAL ARTICLE

Human leukocyte antigen-A genotype as a predictor of cytomegalovirus-pp65 antigenemia and cytomegalovirus disease in solid-organ transplant recipients

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KEYWORDS

Cytomegalovirus;
CMVpp65 antigenemia;
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Transplantation

Abstract *Background:* Cytomegalovirus (CMV) infection is one of the most common and severe infections during the post-transplantation period. It threatens the survival of patients and the function of the transplanted organ.

Aim of the study: To screen for CMV infection among solid organ transplantation patients using monitoring of CMV phosphoprotein 65 (CMVpp65) antigenemia and to detect if CMV infection and disease were associated with certain human leukocyte antigen (HLA)-A locus genotypes among the studied group.

Subjects and methods: Thirty solid organ transplantation patients were included for post-transplantation follow up for symptoms and signs suggestive of CMV disease upto one year. Regular screening for CMV infection was done through CMVpp65 antigenemia detection by the immunofluorescence technique. In addition, HLA-A genotype was determined for all patients using the line probe assay.

Results: The present study showed that 9 out of 30 patients (30%) were positive for CMVpp65 antigenemia. The detected HLA-A alleles were HLA-A*01(no. = 16), HLA-A*02(no. = 11), HLA-A*11(no. = 5), HLA-A*19(no. = 1), HLA-A*24(no. = 4), HLA-A*29(no. = 1), HLA-A*30(no. = 16), HLA-A*92(no. = 4). Among the studied cases, 40% showed HLA-A*01–30 type. There was a significant difference ($P = 0.05$) among detected HLA types as regards CMVpp65 antigenemia, with HLA-A*02_11 representing 33.3% of CMVpp65 positive patients and HLA-A*01_30 representing 57.1% of CMVpp65 negative patients.

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Conclusion: Certain HLA alleles may have either a protective or predisposing role in CMV reactivation. Thus, HLA typing might be helpful in estimating the risk of CMV disease during the post-transplantation period and designing individualized therapy as regards the choice between preemptive and prophylactic CMV therapy.

1. Introduction

Cytomegalovirus (CMV) infection is a major cause of morbidity in recipients of solid organ and bone marrow transplants in spite of significant advances resulting from preemptive therapy and early diagnosis. Thus, it limits the effectiveness of organ transplantation as a procedure for the treatment of end-stage diseases [1].

The virus is generally known to cause both direct and indirect effects on transplant graft function. While direct effects of CMV are mostly visible, indirect effects are virtually not. It can result in a significant long-term complication, such as acute and chronic graft rejection, secondary opportunistic, or accelerated atherosclerosis that influences patients' and graft survival [2].

The immunosuppressive therapy decreases the function of cellular immunity, which is important in protection against viruses from Herpesviridae, Polyomaviridae and Papillomaviridae families. These viruses do not disappear after the acute primary infection, but latently remain in the infected organism for a long period. From time to time the latent viruses reactivate due to pro-inflammatory triggers. In the case of immunosuppression, the suppressed cellular immunity is not able to inactivate the hive of viruses and symptomatic secondary infection develops. The primary viral infection developed under immunosuppressed condition usually results in more severe symptoms. In most cases the source of the viruses is the graft-organ from a seropositive donor [3].

CMV is the most common virus pathogen in solid organ transplant recipients (kidney, heart, liver, lung and pancreas) during the first six months after transplantation. A solid organ recipient may be infected either by the exogenous virus or by reactivation of the latent virus if they were CMV positive pre-transplantation. Those at highest risk of symptomatic CMV disease are CMV sero-negative patients who receive organs from CMV seropositive donors, and CMV seropositive patients on heavily immunosuppressive regimens [4].

CMV may manifest as a non-specific illness characterized by fever, mononucleosis, leucopenia and thrombocytopenia. It may also manifest as a variety of clinical syndromes including pneumonitis, hepatitis, encephalitis and focal gastrointestinal disease. In addition, CMV infection causes morbidity in organ recipients through indirect effects on their immune response, and is associated with increased risk of allograft injury and rejection, opportunistic infections and late onset malignancies such as Epstein Barr virus lympho-proliferative disease [5].

The occurrence of severe CMV infection in transplant patients has decreased thanks to modern diagnostics and effective drug-therapy. The sustained viral effects may lead to damage of parenchymal organs, especially of graft-organs. Thus, it is not enough to treat the CMV-disease, it is rather better to avoid the infection and the viral reactivation. When introducing the preventive therapy, it is recommended to determine the risk factors for CMV-infection and to choose the preventive procedures according to their effectiveness [6].

A 3-month prophylaxis against CMV infection with valaciclovir has been reported to be safe and effective in kidney transplant recipients. However, during prophylaxis, CMVpp65 antigenemia develops in some patients. This CMV infection may be either asymptomatic or mildly symptomatic (without fever). In kidney transplant recipients intensive monitoring of CMVpp65 antigenemia and early treatment of CMV infection, namely preemptive therapy, has been reported to be a safe alternative to prophylaxis [7].

In the high risk group, the average incidence of CMV disease was 20.5% for renal and liver transplant recipients receiving 90 days of valganciclovir prophylaxis during clinical trials. There appears to be very few viral or immunological markers that accurately predict the subset of patients who will develop CMV disease after prophylaxis [8].

Genetic variability influences susceptibility to several diseases. A widespread range of diseases have been linked with different human leukocyte antigen (HLA) genotypes, like ankylosing spondylitis, rheumatoid arthritis, celiac disease, insulin-dependent diabetes mellitus, multiple sclerosis, tuberculosis, etc. In the last two decades, findings concerning the association of CMV infection with HLA were reported. The authors investigated 15 types of HLA systems; 3 of them (HLA-Cw7, HLA-B16 and HLA-B55) were protective against CMV disease. The others (HLA-A2, HLA-A24, HLA-A32, HLA-B7 of donors, HLA-B52, HLA-Bw4, HLA-DR6, HLA-DR11, HLA-DR15) increased the risk of the development of CMV infection [9].

Major histocompatibility complex molecules are critical for antigen uptake, processing and presentation. The association of some HLA alleles with active CMV infection might be due to differential presentation of CMV peptides by HLA molecules or differential recognition by host CD8+ and CD4+ T lymphocytes. A Japanese study suggests that the deficient production of neutralizing antibodies against CMV in certain HLA types may lead to the increased susceptibility. Others suppose that distinct HLA types may enhance the production of TNF-α giving rise to CMV end-organ diseases [10].

The CMVpp65 antigenemia test is an immunofluorescence-based assay that utilizes an indirect immunofluorescence technique for identifying the CMVpp65 phosphoprotein of CMV in peripheral blood leukocytes. The CMVpp65 assay is widely used as the gold standard for monitoring CMV infections and the response of CMV positive patients to antiviral treatment [1].

To date, reports have suggested that the genetic mutation of hosts and HLA genotypes might play an important role in

the development of CMV infection. Also the establishment of sensitive measures for detecting CMV infection and HLA genotypes has significantly reduced the incidence of CMV infection and mortality of transplantation recipients [11].

2. Aim of the work

The present study aimed to screen for CMV infection among solid organ transplantation patients using monitoring of CMVpp65 antigenemia and to detect if CMV infection and disease were associated with certain HLA-A genotypes among the studied group.

3. Patients and methods

- Patients: The study included 30 solid organ transplantation patients (6 liver transplantation and 24 renal transplantation patients) who underwent liver and renal transplantation at Ain Shams Center of Organ Transplantation (ASCOT) of Ain Shams specialized hospital, and provided a written consent to participate in the study during the period of December, 2012 till May, 2014.
- Methods: Patients were recruited one week post-transplantation and they were subjected to:

- Full history taking and meticulous clinical examination for any symptoms and signs suggestive of CMV disease as clinical involvement of organ dysfunction such as diarrhea, hepatitis, pancreatitis, retinitis, fever, and myocarditis. Clinical follow up was performed weekly during the first month, every other week during the next two months and monthly up to one year. Patients' serologic status was determined by detecting anti-CMV antibodies IgG (preoperative) and IgM (on clinical suspicion of CMV disease).
- Detection of HLA genotype of A locus using sequence specific polymerase chain reaction (PCR) and line probe assay (LIPA).
- Screening for CMV infection by monitoring of CMVpp65 antigenemia using the immunoflourescence technique. This was performed two weeks post-transplantation, three months post-transplantation, by the end of the follow up duration and on clinical suspicion of CMV disease.

3.1. Detection of HLA- A locus genotype

This was done using INNO-LiPA HLA-A Update Multiplex PCR kit (Innogenetics, Belgium) in combination with the INNO-LiPA HLA-A Update strips for the nucleic acid amplification of exon1, exon2, exon3 and exon4 of the HLA-A locus. LIPA is based on the reverse-hybridization principle. Amplified, biotinylated DNA material is chemically denatured, and the single strands are hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. This process is followed by a stringent wash step to remove any mismatched amplified material. Thereafter, streptavidin conjugated with alkaline phosphatase is added and bound to any biotinylated hybrid previously formed. Incubation with a substrate solution containing a chromogen results in a purple-brown precipitate. The reaction is stopped by a wash step, and the reactivity pattern of the probes is recorded [12].

In brief, whole genomic DNA was extracted using High Pure PCR Template Preparation kit (Roche, Germany) from 200 µl whole blood by adding 200 µl binding buffer and 40 µl proteinase K with incubation at 70 °C for 10 min, addition of 100 µl isopropanol in a High Pure filter tube with centrifugation for 1 min at 10,000 rpm, addition of 500 µl inhibitor removal buffer followed by two washing steps in 500 µl wash buffer and final elution at 70 °C in 200 µl elution buffer by centrifugation for 1 min at 10,000 rpm.

Amplification was performed in MicroAmp PCR tube, GeneAmp PCR system 9700 thermal cycler and LiPA-Taq. Five microliters of genomic DNA were added to 45 µl master mix (10 µl amplification buffer containing an excess of deoxynucleoside 5′-triphosphates (dNTPs), 10 µl HLA-A multiplex biotinylated primers solution and 1.25 µl thermostable LiPA-Taq DNA polymerase completed to 45 µl by autoclaved distilled water). Cycling conditions were programed as follows; initial denaturation at 96 °C for 5 min followed by 35 cycles of denaturation at 96 °C for 30 s, annealing at 64 °C for 50 s (5 cycles), annealing at 62 °C for 50 s (5 cycles), annealing at 60 °C for 50 s (10 cycles) and annealing at 55 °C for 50 s (15 cycles) followed by extension at 72 °C for 50 s for all cycles. Final elongation was done at 72 °C for 10 min.

The amplification products were subsequently hybridized using 2 typing strips (INNO-LiPA HLA-A Update Plus strips 1 and 2) on which 44 sequence-specific probes and 2 control lines are fixed. All reagents were brought to room temperature, then, 10 µl of the alkaline denaturation solution was incubated with 10 µl of the amplification product for 5 min at 25 °C in the developing trough. Two ml pre-warmed hybridization solution (saline sodium phosphate EDTA buffer containing 0.5% sodium dodecyl sulfate) were added to the denatured amplified product into each trough. Using a sterile forceps strips 1 and 2 were placed in the troughs (one strip per trough). The tray with the troughs was placed into a shaking water bath for 30 min at 56 °C.

After hybridization, the liquid was aspirated from the trough with a pipette. Stringent wash was done 3 times by adding 2 ml pre-warmed stringent wash solution with incubation for 3 min at 56 °C. Two ml of the conjugate working solution (Streptavidin labeled with alkaline phosphatase in Tris buffer) was added to each trough for 30 min while agitating the tray on a shaker followed by twice wash step then addition of 2 ml of the substrate working solution (5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt and nitroblue tetrazolium in dimethylformamide) with incubation for 30 min on a shaker. Stop step was done by washing the strips twice in 2 ml distilled water on a shaker for 3 min. Finally, the strips were removed by a forceps and left to dry before reading results using the reading card included in the kit (Fig. 1a and b).

3.2. Screening for CMV infection by monitoring of CMVpp65 antigenemia using immunofluorescence technique

The CMV antigenemia test was performed for the detection of CMVpp65 antigen in circulating peripheral blood leukocytes (PBL) within 6 h of sample collection. This was done according to Moses et al. [13]. Immunocytologic assay was performed by the use of CMV Brite™ Turbo Kit (monoclonal antibody (mouse, IgG1) against CMV lower matrix protein pp65) and fluorescein isothiocyanate (FITC)-conjugated sheep anti-

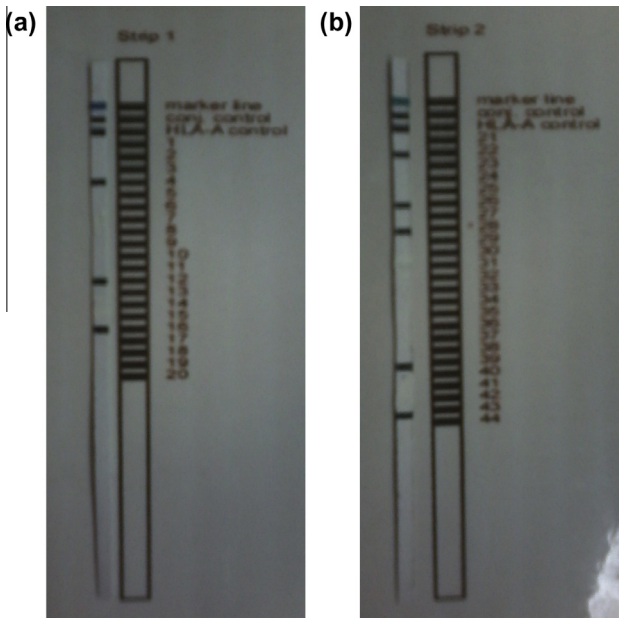

Figure 1 Developed INNO-LiPA HLA-A Update strip 1 (a) and strip 2 (b).

Figure 2 Immun-flourescence image of CMVpp65 antigen in PBL (as detected by fluorescent microscope during the present study).

mouse IgG antibody, supplied by IQ Products (Groningen, Netherlands).

Cold lysing steps were done by adding 2 ml venous blood to 30 ml erythrocyte lysis solution diluted 1:10 in distilled water for 5 min at 4 °C followed by centrifugation for 2 min at 2500 rpm. Pellet was re-suspended in 1 ml phosphate buffer saline (PBS) for cell count and cell number was adjusted to 2×10^6 cell/ml using a hemocytometer. Smears were prepared by adding 100 µl of cell suspension onto glass slides followed by centrifugation at 600 rpm for 4 min in cytospin. After overnight air drying slides were dipped for 5 min in 1:5 diluted fixative solution followed by washing in PBS then in 1:5 diluted permeabilization solution for 1 min at room temperature followed by washing in PBS.

After permeabilization, each slide was covered with 35 µl of anti-CMVpp65 monoclonal antibody and incubated for 20 min at 37 °C in a humid chamber. After washing three times in PBS, the slides were incubated with 35 µl of FITC conjugated anti-mouse immunoglobulin for 20 min at 37 °C in a humid chamber, followed by washing twice in fresh PBS. Mounting medium and micro cover slides were applied. Control slides provided with the kit contained a negative control spot and positive control spot. The positive control cells exhibited homogenous yellow green poly-lobate nuclear fluorescence staining while the negative control showed no yellow green staining. The number of cells with homogenous green poly-lobate nuclear fluorescence was scored under a fluorescent microscope at 400× magnification. A positive assay result was defined by the presence of at least 1 positively stained leukocyte on the slide, and the result was expressed as the number of CMVpp65-positive cells per 2×10^6 PBL (Fig. 2).

4. Definition of CMV infection and CMV disease

CMV infection was defined according to Ljungman et al. [14] as the detection of CMV pp65 antigen in blood leukocytes in the absence of clinical manifestations or organ function abnormalities. CMV disease was defined as the association of documented CMV infection with clinical symptoms, such as unexplained fever and leukopenia ($< 4 \times 10^9$/L in two consecutive samples) and/or thrombocytopenia ($< 150 \times 10^9$/L).

5. Ethical considerations

The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans and the study was approved by Ain Shams Center of Organ Transplantation ethics committee. The procedures of the study were discussed with participating patients who provided informed consent for the examination and tests included in the study.

6. Statistical methodology

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12) as follows:

- Description of quantitative variables as mean, SD and range.
- Description of qualitative variables as number and percentage.
- Chi-square test was used to compare qualitative variables between groups.
- Unpaired *t*-test was used to compare quantitative variables, in parametric data (SD < 50% mean).
- Mann–Whitney test was used instead of unpaired *t*-test in non parametric data (SD > 50%mean).

Table 1 Distribution of studied group as regard general data.

Variables	No	%
Gender		
Male	20	66.7%
Female	10	33.3%
Age (range, mean ± SD)		(18–56) 58.5 + 10 years

- One way ANOVA (analysis of variance) test was used to compare more than two groups as regards quantitative variable (LSD = least significant difference).
- Spearman correlation co-efficient test was used to rank variables versus each other positively or inversely.
- ROC curve (receiver operator characteristic curve was used to find out the best cut off value, and validity of certain variables).
- Sensitivity = true ve +/true +ve + false –ve= ability of the test to detect +ve cases
- Specificity = true −ve/true−ve + false +ve= ability of the test to exclude negative cases
- PPV(positive predictive value) = true+/true+ve + false +ve=% of true +ve cases to all positive

Table 2 Detected HLA-A alleles, CMVpp65 antigenemia and CMV disease among the studied group (no. = 30).

No	HLA-A	CMVpp65	CMV disease
1.	HLA-A*01_30	−VE	−VE
2.	HLA-A*02_92	−VE	−VE
3.	HLA-A*02_11	+VE	+VE
4.	HLA-A*01_30	−VE	−VE
5.	HLA-A*02_30	+VE	−VE
6.	HLA-A*01_30	−VE	−VE
7.	HLA-A*02_24	+VE	−VE
8.	HLA-A*01_30	−VE	−VE
9.	HLA-A*19_30	−VE	−VE
10.	HLA-A*01_24	−VE	−VE
11.	HLA-A*01_30	−VE	−VE
12.	HLA-A*02_19	+VE	−VE
13.	HLA-A*01_92	−VE	−VE
14.	HLA-A*01_30	−VE	−VE
15.	HLA-A*01_30	−VE	−VE
16.	HLA-A*02_11	+VE	+VE
17.	HLA-A*11_30	−VE	−VE
18.	HLA-A*19_24	−VE	−VE
19.	HLA-A*01_30	−VE	−VE
20.	HLA-A*02_29	+VE	+VE
21.	HLA-A*01_30	−VE	−VE
22.	HLA-A*01_92	−VE	−VE
23.	HLA-A*01_24	+VE	−VE
24.	HLA-A*02_11	+VE	−VE
25.	HLA-A*01_30	−VE	−VE
26.	HLA-A*02_30	−VE	−VE
27.	HLA-A*01_30	−VE	−VE
28.	HLA-A*02_11	−VE	−VE
29.	HLA-A*02_92	+VE	+VE
30.	HLA-A*01_30	−VE	−VE

Table 3 Detected HLA-A alleles and CMVpp65 antigenemia among studied group (no. = 30).

HLA-A	Number	CMVpp65 positive	CMVpp65 negative
HLA-A*01	16	1	15
HLA-A*02	11	8	3
HLA-A*11	5	3	2
HLA-A*19	1	1	–
HLA-A*24	4	2	2
HLA-A*29	1	1	–
HLA-A*30	16	1	15
HLA-A*92	4	1	3

Table 4 Distribution of the studied group as regards HLA types.

Variables	No	%
HLA-A*01_24	2	6.7
HLA-A*01_30	12	40.0
HLA-A*01_92	2	6.7
HLA-A*02_11	4	13.3
HLA-A*02_19	1	3.3
HLA-A*02_24	1	3.3
HLA-A*02_29	1	3.3
HLA-A*02_30	2	6.7
HLA-A*02_92	2	6.7
HLA-A*11_30	1	3.3
HLA-A*19_24	1	3.3
HLA-A*19_30	1	3.3
Total	30	100

- NPV(negative predictive value) = true−/true−ve + false −ve=% of the true −ve to all negative cases

P value > 0.05 insignificant
$P < 0.05$ significant
$P < 0.01$ highly significant

7. Results

Among the studied group, males were 66.7% of the studied cases with an average age of 58.5 years (Table 1). All patients (no. = 30) were seropositive (CMV IgG positive) with seronegative donors. Nine patients (30%) were positive for CMVpp65 antigenemia (Table 2). Among CMVpp65 antigenemia positive patients (no. = 9), 4 patients (44.4%) showed symptoms of CMV disease in the form of fever (4 patients), pneumonia (3 patients) and leukopenia (2 patients). Anti-CMV IgM was positive in 2 out of 9 CMVpp65 positive patients (22.2%). The number of CMVpp65-positive cells was between 1 and 5 cells per 2×10^6 PBL among the 9 CMVpp65 positive samples.

The detected HLA-A alleles were HLA-A*01(no. = 16), HLA-A*02(no. = 11), HLA-A*11(no. = 5), HLA-A*19(no. = 1), HLA-A*24(no. = 4), HLA-A*29(no. = 1), HLA-A*30 (no. = 16) and HLA-A*92(no. = 4) (Table 3). HLA-A*1–30 type represented 40% of the studied cases (Table 4).

There was a significant difference ($P = 0.05$) among the detected HLA types as regards CMVpp65 antigenemia, by using chi-square test, where the majority of CMVpp65 positive patients (33.3%) belonged to the HLA-A*02_11 type (compared to 11.1% among each of the other HLA types) and the majority of CMVpp65 negative patients (57.1%) belonged to the HLA-A*01_30 type (Table 5). Also, by using the chi-square test, there was a significant association between CMVpp65 antigenemia and CMV disease (Table 6). No statistically significant relation was found between HLA types versus CMV disease by using the chi-square test (Table 7).

8. Discussion

For patients who are immunosuppressed due to HIV infection or solid organ or hematopoietic cell transplantation, CMV is a potentially life-threatening complication. CMV has evolved

Table 5 Relation between HLA types versus CMVpp65 antigenemia.

Crosstab					
			CMVpp65		Total
			−ve	+ve	
HLA types	HLA-A01_24	Count	1	1	2
		% within CMVpp65	4.8%	11.1%	6.7%
	HLA-A01_30	Count	12	0	12
		% within CMVpp65	57.1%	0%	40.0%
	HLA-A01_92	Count	2	0	2
		% within CMVpp65	9.5%	0%	6.7%
	HLA-A02_11	Count	1	3	4
		% within CMVpp65	4.8%	33.3%	13.3%
	HLA-A02_19	Count	0	1	1
		% within CMVpp65	0%	11.1%	3.3%
	HLA-A02_24	Count	0	1	1
		% within CMVpp65	0%	11.1%	3.3%
	HLA-A02_29	Count	0	1	1
		% within CMVpp65	0%	11.1%	3.3%
	HLA-A02_30	Count	1	1	2
		% within CMVpp65	4.8%	11.1%	6.7%
	HLA-A02_92	Count	1	1	2
		% within CMVpp65	4.8%	11.1%	6.7%
	HLA-A11_30	Count	1	0	1
		% within CMVpp65	4.8%	0%	3.3%
	HLA-A19_24	Count	1	0	1
		% within CMVpp65	4.8%	0%	3.3%
	HLA-A19_30	Count	1	0	1
		% within CMVpp65	4.8%	0%	3.3%
Total		Count	21	9	30
		% within CMVpp65	100.0%	100.0%	100.0%
		$X^2 = 19$ $P = 0.05$(S)			

Table 6 Relation between CMVpp65 antigenemia versus CMV disease.

			CMV disease		Total
			−ve	+ve	
CMVpp65	−ve	Count	21	0	21
		% within CMV disease	80.8%	0%	70.0%
	+ve	Count	5	4	9
		% within CMV disease	19.2%	100.0%	30.0%
Total		Count	26	4	30
		% within CMV disease	100.0%	100.0%	100.0%
		$X^2 = 10.7$ $P = 0.002$ (S)			

escape mechanisms to evade both the innate natural killer (NK) cells and adaptive (CD8+ T cells) immune responses. Down-regulation of class I HLA expression and interference with antigen presentation by virus-encoded genes diminishes T-cell recognition but renders infected cells susceptible to NK-cell lysis [15].

In immune-compromised patients, such as allogeneic bone marrow transplant recipients, CMV infection can be a significant complication. In these patients the recovery of CMV-specific CD8+ cells response has been correlated with an improved outcome from CMV disease. These observations suggest an important role for the cellular immune response in the control of CMV infection, and have encouraged studies to identify the target antigens recognized by CMV-specific CD8+ cells. One of the predominant viral antigens recognized by CMV-specific CD8+ cells is the lower matrix 65-kDa phosphoprotein (pp65) [16].

Risk factors for CMV reactivation include pre-transplantation donor and recipient CMV serologic status and post-transplantation development of acute graft-vs-host disease (aGvHD). HLA allele type is an additional factor in CMV infection [17].

The present study aimed to screen for CMV infection among solid organ transplantation patients using monitoring of CMVpp65 antigenemia and to detect if CMV infection and disease were associated with certain HLA-A genotypes among the studied group. The study included 30 solid organ transplantation patients including 6 liver transplantation and 24 renal transplantation patients. Patients were subjected to full history taking and meticulous clinical examination for any symptoms and signs suggestive of CMV disease as clinical involvement of organ dysfunctions such as diarrhea, with hepatitis, pancreatitis, retinitis, fever, and myocarditis, determining of patients' serologic status by detection of anti-CMV

Table 7 Relation between HLA types versus CMV disease.

Crosstab					
			CMV disease		Total
			−ve	+ve	
HLA types	HLA-A01_24	Count	2	0	2
		% within CMV disease	7.7%	0%	6.7%
	HLA-A01_30	Count	12	0	12
		% within CMV disease	46.2%	0%	40.0%
	HLA-A01_92	Count	2	0	2
		% within CMV disease	7.7%	0%	6.7%
	HLA-A02_11	Count	2	2	4
		% within CMV disease	7.7%	50.0%	13.3%
	HLA-A02_19	Count	1	0	1
		% within CMV disease	3.8%	0%	3.3%
	HLA-A02_24	Count	1	0	1
		% within CMV disease	3.8%	0%	3.3%
	HLA-A02_29	Count	0	1	1
		% within CMV disease	.0%	25.0%	3.3%
	HLA-A02_30	Count	2	0	2
		% within CMV disease	7.7%	0%	6.7%
	HLA-A02_92	Count	1	1	2
		% within CMV disease	3.8%	25.0%	6.7%
	HLA-A11_30	Count	1	0	1
		% within CMV disease	3.8%	0%	3.3%
	HLA-A19_24	Count	1	0	1
		% within CMV disease	3.8%	0%	3.3%
	HLA-A19_30	Count	1	0	1
		% within CMV disease	3.8%	0%	3.3%
Total		Count	26	4	30
		% within CMV disease	100.0%	100.0%	100.0%
		$X^2 = 17$ $P = 0.1$ (NS)			

antibodies IgG and IgM, detection of HLA genotype of A locus, using sequence specific PCR and LIPA, and screening for CMV infection by monitoring of pp65 antigenemia using the immunofluorescence technique.

Results of the present study showed that all patients (no. = 30) were seropositive (CMV IgG positive) with seronegative donors. Nine patients (30%) were positive for CMVpp65 antigenemia. Anti-CMV IgM was positive in 2 out of 9 CMVpp65 positive patients (22.2%). Among CMVpp65 antigenemia positive patients (no. = 9), 4 patients (44.4%) showed symptoms of CMV disease in the form of fever, pneumonia and leukopenia. The number of CMVpp65-positive cells was between 1 and 5 cells per 2×10^6 PBL among the 9 CMVpp65 positive samples. The detected HLA-A alleles were HLA-A*01(no. = 16), HLA-A*02(no. = 11), HLA-A*11(no. = 5), HLA-A*19(no. = 1), HLA-A*24(no. = 4), HLA-A*29(no. = 1), HLA-A*30(no. = 16) and HLA-A*92(no. = 4). HLA-A*1–30 type represented 40% of the studied cases. There was a significant difference among detected HLA types as regards CMVpp65 antigenemia, where the majority of CMVpp65 positive patients (33.3%) belonged to the HLA-A*02_11 type (compared to 11.1% among each of the other HLA types) and the majority of CMVpp65 negative patients (57.1%) belonged to the HLA-A*01_30 type Also, there was a significant association between CMVpp65 antigenemia and CMV disease. However, no statistically significant relation was found between HLA types versus CMV disease.

Similarly in the study of Fan et al. [11] liver transplantation recipients were serum CMV IgG positive (100%). Thirty-three recipients (84.6%) were CMV antigenic positive with average of 7.2 ± 4.2 positive leukocytes per 50,000 leukocytes on. Thirteen patients developed CMV pneumonia, with CMV antigenic positive (100%). They concluded that some HLA alleles were associated with the occurrence and extent of CMV antigenemia. However, their results demonstrated that HLA-A2 was the higher frequency allele for patients with antigenemia ($P < 0.05$). In the lower antigenemia group, HLA-A11 was higher in frequency than others ($P < 0.05$). Besides, none of the patients carrying HLA-B16 allele developed clinical symptoms of CMV infection ($P < 0.05$). They failed to detect anti-CMV IgM among patients with CMV disease. It was explained by the immunosuppression condition of recipients.

The study of Kekik et al. [17] included 108 patients who received an allogeneic stem cell graft from an HLA-identical sibling between 1993 and 2004. All recipients and donors were typed for HLA-A, HLA-B, and HLA-DR alleles using serologic or molecular methods. In contrary to the results of the present study, seropositive patients with post-transplantation CMV infection demonstrated a higher incidence of HLA-A30, HLA-B40, and HLA-DRB1*15 compared with those without CMV infection. This difference may be attributed to difference in sample size.

Also Varga [6] reported that genetic variability influences susceptibility to infectious diseases and HLA-molecules are critical for viral antigen up-taking, processing and presenting.

They studied 129 of high-risk patients and suggested that recipients positive for HLA-DQ3 are more susceptible to CMV-infection than a comparable group of patients negative for this HLA-type.

Thus, the variability of HLA alleles might modulate immune response to CMV infection. HLA examination before transplantation should be made for prevention and treatment of CMV infection after operation [11].

9. Conclusions and recommendations

Certain HLA alleles may have either a protective or a predisposing role in CMV reactivation. Thus, HLA typing might be helpful in estimating the risk of CMV disease during the post-transplantation period and designing individualized therapy as regards the choice between preemptive and prophylactic CMV therapy. Further studies including other HLA loci genotypes are recommended to clarify the role of other HLA types in the susceptibility of solid-organ transplantation patients to CMV infection.

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