The Relationship Between HLA Typing and HCV Infection and Outcome of Renal Transplantation in HCV Positive Patients

Alaa Hadhoud[1], Azza M. Abdulaziz[1], Lubna Al Menawi[2], F.A.Shaheen[3], Aliah Abdulghaffar, Fahd Al Abas [5], Mohammed F. Al Mobrak[5],

The role of Human Leukocytic Antigen (HLA) antigens in susceptibility to Hepatitis C Virus (HCV) infection is still being debated. We analyzed HLA phenotype frequencies in two major ethnic groups, namely Egyptian and Saudi nationals. The Egyptian group included 110 patients of whom 55 were HCV positive and the other 55 HCV negative (control group). The Saudi group included 146 HCV positive patients and 122 HCV negative individuals (control group). The results for the Egyptian population revealed increased frequencies of some HLA phenotypes and decreased frequencies of others but without any statistically significant difference. In contrast, in the Saudi population, the HLA-A19 phenotype was significantly increased in HCV positive patients when compared with the control group while significantly decreased frequencies were found for HLA-B8, HLA-DRI and HLA DR3. Our data suggest that there was no significant association between HLA phenotypes and susceptibility to HCV infection among the Egyptian population while the overall data of the Saudi population seem to indicate that the expression of particular HLA alleles could be associated with susceptibility or resistance to the HCV infection. Further studies on larger numbers of patients are needed to support the role of the HLA system in HCV infection. A total of 108 HCV positive patients underwent renal transplantation at the Jeddah Kidney Center and the results were compared with 100 age and sex-matched controls. Graft survival at 36 months was 82% and 86% for HCV positive and control subjects respectively while patient survival was respectively 90% and 91%. Our data suggest that the outcome, at least in the short-time, of renal transplantation in HCV positive patients is very good.

Keywords: *Hepatitis C Virus; HLA; Egypt; Saudi Arabia; Renal Transplantation*

Dept. of [1]Microbiology, [2]Histopathology, [3]Nephrology, [4]Surgery, and [5]Internal Medicine, [1]Faculty of Medicine, Zagazig University, Egypt, [1-5] King Abdulaziz Hospital & Oncology Center, Saudi Arabia.

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***Address reprint requests to** : Faissal A.M. Shaheen, MD, Director General, Saudi Center for Organ Transplantation, P.O. Box 27049, Riyadh 11417, Kingdom of Saudi Arabia. **Fax**: 00966-1-4453934 ; **E-mail**: scot@naseej.com.sa*

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Hepatitis C Virus (HCV) is the major etiological agent of post-transfusion and sporadic non-A, non-B hepatitis worldwide [1-4]. At least 50-60% of people infected with HCV develop chronic hepatitis due to persistent HCV infection [3]. Approximately 40-50% of chronic hepatitis patients progress to liver cirrhosis caused by on going liver cell damage [4,5].

The prevalence of HCV infection differs from country to country and several reports have identified Egypt as a country with a relatively high prevalence [6-9]. Rural areas in Egypt have higher prevalence of HCV infection than the urban areas [10,11]. In Saudi Arabia, the prevalence of HCV infection in a population of intravenous drug users in Jeddah, was 74.6%, in drug dependent patients who did not use the intravenous route was 10.5% and in healthy Saudi males was 1.7% only [12].

The importance of genetic factors for HCV infection arises from the observation of different courses of infection despite the same source of infection [13]. Crucial genetic factors influencing the immune reaction are the human leucocyte antigens (HLA) encoded by the major histocompatibility complex (MHC) [14,15]. Associations between HLA determinants and susceptibility to HCV remain controversial; while some studies did not reveal any significant associations [16-19], others highlighted the relevance of the serologically determined HLA-DR5 antigen or the corresponding DNA determined HLA-DRB1 alleles in chronic hepatitis C [20,21]. Some authors have found other antigens such as DRB*1 09 [22,23], HLA-DR13 [24], DRB*104, DRB1*03 [25] or DRB1*1301, and DQA1*0103 associated with viral clearance of HCV infection [26].

In view of the high prevalence of HCV infection among dialysis patients, it is vital to know their outcome following renal transplantation. It is well known that viral proliferation increases following transplantation [27]. Also, there is an increased risk of liver disease following transplantation although it does not seem to reduce patient survival [28,29].

The aim of the current study is to investigate a possible relationship between susceptibility to H C Virus infection and human leukocyte antigen alleles among Egyptian and Saudi populations and to assess the outcome of renal transplantation in HCV positive patients.

Materials and Methods

This study was conducted between July 1999 to June 2001 in the microbiology division of the King Abdulaziz Hospital & Oncology Center, and the Jeddah Kidney Center, Jeddah, Kingdom of Saudi Arabia.

Patients: The patients were divided into two major groups. The first group was 110 Egyptian people who live in Saudi Arabia and the second was 268 Saudi people. None of the patients carried hepatitis B surface antigen (HbsAg) in the serum or had antibody to HIV to exclude predisposing factor for HCV infection. Each group was divided into two sub-groups, the first sub-group was positive for HCV (55 Egyptian patients and 148 Saudi patients) and the second sub-group was negative for HCV (55 Egyptian people and 123 Saudi people selected from blood and organ donors) as controls.

Blood samples were withdrawn in two sterile vacutainers (Becton Dickinson). One was without anticoagulant and the separated serum was used for detection of HbsAg, HIVAb, and HCVAb, while the second container with acid citrate dextrose was used for HLA typing.

HBV was excluded by the detection of HbsAg using enzyme linked immunosorbent assay (ELISA) technique (DiaSorin, Italy), and HIV excluded by the use of ELISA technique (Murex Diagnostics, England) for the detection of anti-

bodies to HIV types 1 and 2 according to the manufacturer's instructions.

Detection of HCV infection: Serum samples were initially screened for antibodies to HCV by fourth generation ELISA (Innotest HCVAbIV, Innogenetics, Belgium) and positive results were confirmed by a four antigen recombinant immunoblot assay (RIBA, Chiron RIBA HCV, ortho diagnostics).

HLA typing: We did serological HLA typing of class I and II antigens for all patients and controls using a standard complement-dependent micro droplet lymphocyte cytotoxicity test (Terasaki-NIH-Standard method, One Lamada) [30] and a panel of highly selected allo-antisera were used to identify HLA-A, B, DR and DQ alleles expressed on peripheral blood lymphocytes. All samples were tested for the 47 HLA antigens, according to the 1991 WHO Nomenclature Committee for Factors of HLA systems [31]. HLA-C antigens were not considered in this study because of the unreliability of the serological assignments for this locus.

Statistical Analysis: All serological data of patients were analyzed using the computer program data base (version 4), calculating phenotype frequencies. The P-values provided by the chi-squared test were corrected by multiplying them by the number of comparisons made i.e., at least the number of alleles tested. A value of $p<0.05$ was considered to be statistically significant.

A total of 108 HCV positive patients who underwent renal transplantation were studied along with 100 age and sex-matched HCV negative recipients. Patients were followed-up for 36 months and on each visit, graft function and liver function tests were performed. A total of 25 patients underwent liver biopsies during follow-up of which 15 were routine and 10 biopsies were performed for persistent elevation of liver enzymes.

Results

HLA Typing and HCV Prevalence

Egyptian Population: We analyzed data of HLA typing of 55 Egyptian patients positive for HCV and 55 negative for HCV (control group). In this analysis, we found increased frequencies of some HLA phenotypes and decreased frequencies of other but without statistical significance Also, in some HLA phenotypes, the results of both patients and control groups were the same (Table 1 and 2).

Saudi Population: Comparing the phenotype frequencies of HLA class I (Table 3) and class II alleles (Table 4), in 146 HCV positive patients with 122 HCV negative persons (control), we found increased frequencies of HLA-A19 (53.4% versus 31.1%) with significant P-value (0.01). Also, increased frequencies were found but without significant P-value of a few other alleles. In addition, decreased frequencies of HLA-B8 (P=0.04), HLA-DR1 (P=0.04) and HLA-DR3 (P=0.02) were found in HCV positive patients. Similarly, decreased frequencies, but without statistically significant difference, were found for some other alleles as well (Tables 3 and 4).

Renal transplantation and HCV

During the follow-up period, elevated liver enzymes were found in 29.6% of HCV positive patients and 16% of control subjects. Twenty five HCV positive patients underwent liver biopsy (23 showed mild hepatitis, 2 showed ful minent hepatitis) while 6 controls on biopsy showed: mild hepatitis in three, drug induced hepatitis in 2 and

Table 1. HLA class I frequencies of serologically typed of Egyptian population with negative HCV (control group) and positive HCV.

Negative HCV Group n=55			Positive HCV Group n=55			
	n	%	n.	%	P.Value	Sig
HLA-A						
1	10	18.2	10	18.2	1	NS
2	15	27.3	25	45.5	0.39	NS
3	10	18.2	5	9.1	0.54	NS
9	15	27.3	20	36.4	0.65	NS
11	0	0	5	9.1	0.32	NS
19	35	63.6	35	63.6	1	NS
28	15	27.3	0	0	0.1	NS
HLA-B						
5	25	45.5	10	18.2	0.18	NS
7	0	0	5	9.1	0.32	NS
12	0	18.2	15	27.3	0.62	NS
13	0	0.0	10	18.2	0.15	NS
14	5	9.1	0	0	0.32	NS
15	0	0	15	27.3	0.08	NS
17	10	18.2	10	18.2	1	NS
18	10	18.2	0	0	0.15	NS
21	5	9.1	25	45.5	0.07	NS
22	0	0	5	9.1	0.32	NS
27	5	9.1	0	0	0.32	NS
35	15	27.3	10	18.2	0.62	NS
40	0	0	5	9.1	0.32	NS
70	10	18.2	0	0	0.15	NS

All comparisons are made between the patients and controls.
NS – Non Significant

chronic hepatitis in 1). The overall graft survival after 36 months was 82% and 88% respectively for HCV positive and negative subjects. Patient survival after 36 months was respectively 90% and 91% for HCV positive and negative subjects.

Ten HCV positive patients died (Pulmonary embolism in 3, hepatic failure in 2, sepsis and CVA in 2 each Kaposi's sarcoma in 1). Nine control subjects died (sepsis in 3, CVA, pulmonary embolism and DIC in 2 each).

Discussion

Genes located within the MHC play a major role in influencing the immune response against infectious agents. Optimal interactions between T cell receptor, MHC class I or II molecules and antigenic viral peptides are required for an adequate immune response. Thus, the expression of particular HLA specificities might lead to a defective antigen presentation, allowing the HCV infection [21].

Table 2. HLA class II frequencies of serologically typed Egyptian population with negative HCV (control group) and positive HCV.

Negative HCV Group n=55			Positive HCV Group n=55			
	n	%	n.	%	P.Value	Sig
HLA-DR						
1	5	9.1	10	18.2	0.54	NS
2	5	9.1	20	36.4	0.14	NS
310	18.2		10	18.2	1	NS
4	15	27.3	20	36.4	0.65	NS
5	10	18.2	10	18.2	1	NS
6	30	54.5	10	18.2	0.00	NS
7	10	18.2	15	27.3	0.62	NS
8	15	27.3	0	0	0.07	NS
9	0	0	5	9.1	0.32	NS
80	0	0.0	5	9.1	0.32	NS
HLA-DQ						
1	40	72.7	30	54.5	0.39	NS
2	30	54.5	25	45.5	0.68	NS
3	30	54.5	20	36.4	0.4	NS
4	0	0	10	18.2	0.15	NS

All comparisons are made between the patients and controls.
NS = Non Significant

The role of HLA system in predisposing to HCV infection is still unclear. Some studies did not reveal any significant association between HLA phenotypes and susceptibility to HCV infection [16-19] and this agrees with our data presented in this study for the Egyptian population. The absence of a relationship between HLA typing and susceptibility to HCV infection, means that the Egyptian population, from the view of HLA phenotype, are all equally susceptible to HCV infection.

In contrast, other studies have revealed association between some HLA phenotypes and HCV infection [20,21] and our study revealed that in the Saudi population, the frequency of HLA-A19 was higher in HCV positive patients compared to con-

Table 3. HLA class I frequencies of serologically typed Saudi population with negative HCV (control group) and positive HCV

	Negative HCV Group = 122		Positive HCV Group n=146			
	n	%	n.	%	P.Value	Sig
HLA-A						
1	32	26.2	28	19.2	0.33	NS
2	34	27.9	64	43.8	0.06	NS
3	20	16.4	14	9.6	0.24	NS
9	32	26.2	34	23.3	0.7	NS
10	18	14.8	12	8.2	0.23	NS
11	6	4.9	4	2.7	0.5	NS
19	38	31.1	78	53.4	0.01	S
28	38	31.1	30	20.5	0.16	NS
HLA-B						
5	44	36.1	64	43.8	0.37	NS
7	12	9.8	8	5.5	0.35	NS
8	20	16.4	8	5.5	0.04	S
12	18	14.8	12	8.2	0.23	NS
13	4	3.3	12	8.2	0.24	NS
14	4	3.3	2	1.4	0.46	NS
15	6	4.9	10	6.8	0.64	NS
16	6	4.9	6	4.1	0.82	NS
17	18	14.8	16	11.0	0.51	NS
18	6	4.9	10	6.8	0.64	NS
21	20	16.4	36	24.7	0.24	NS
22	2	1.6	6	4.1	0.5	NS
27	2	1.6	4	2.7	0.67	NS
35	22	18.0	22	15.1	0.65	NS
37	4	3.3	4	2.7	0.84	NS
40	12	9.8	6	4.1	0.2	NS
41	6	4.9	6	4.1	0.82	NS
42	0	0	0	0	–	–
47	0	0	2	1.4	0.36	NS
48	2	1.6	2	1.4	0.9	NS
53	10	8.2	10	6.8	0.76	NS
70	10	8.2	14	9.6	0.78	NS

All comparisons are made between the patients and controls. NS = Non Significant S=Significant

trol groups (53.4% versus 31.1%) and significant decreased frequencies of HLA-B8, HLA-DR1 and HLA-DR3 were also observed. These results indicate that both HLA-class I which primes CD8 + cytotoxic lymphocytes, and HLA-class II which primes CD4+ helper lymphocytes, are involved in the susceptibility to HCV infection. The HLA molecules bind oligopeptides (8-20 mers) to activate or anergize functional T lymphocytes such as CD8+ CTL and CD4+ helper T cells (both Th1 and Th2). CTL kill virally infected target cells. Th1 CD4+ cells inititate cellular immune responses,

Table 4. HLA class II frequencies of serologically typed Saudi Population With negative HCV (control group) and positive HCV.

	Negative HCV Group = 122		Positive HCV Group n=146			
	n	%	n.	%	P.Value	Sig
HLA-DR						
1	22	18.0	10	6.8	0.048	S
2	40	32.8	68	46.6	0.11	NS
3	44	36.1	26	17.8	0.02	S
4	44	36.1	56	38.4	0.78	NS
5	18	14.8	26	17.8	0.64	NS
6	26	21.3	38	26.0	0.5 3	NS
7	24	19.7	22	15.1	0.48	NS
8	6	4.9	6	4.1	0.82	NS
9	0	0	0	0	–	–
10	4	3.3	14	9.6	0.15	NS
HLA-DQ						
1	86	70.5	104	71.2	0.93	NS
2	56	45.9	50	34.2	0.17	NS
3	52	42.6	78	53.4	0.22	NS
4	8	6.6	2	1.4	0.12	NS

All comparisons are made between the patients and controls. NS= Non Significant S= Significant

whereas Th2 help antibody production. By activating or anergizing these functionally distinct T cell subsets, HLA molecules associated with HCV may control the susceptibility or resistance to HCV infection [21]. Consequently the expression of particular HLA alleles could be associated with susceptibility (HLA-A19) or resistance (HLA-B8; HLA-DR1 and HLA-DR3) to HCV infection.

Our study suggests that there are variations in susceptibility to HCV infection between the Egyptian and Saudi populations; while the Egyptian population showed no relationship between HLA phenotypes and HCV infection, the Saudi population showed significant increased frequency of HLA-A19 and significant decreased frequencies of HLA-B8, HLA-DR1 and HLA-DR3. Further studies are needed on larger numbers of individuals to support the association of the HLA system and HCV infection. HCV positive patients, in our study, had good outcome following trans-

plantation. This is similar to a few other reports [28,29, 32]. However, there are other reports which mention lower graft and patient survival in HCV positive patients after renal transplantation [24,32,33]. The reported enhanced mortality was caused predominantly by sepsis and these studies had longer follow-up period.

Although our study indicates good outcome at short-term more studies with longer follow-up period are required to determine the outcome of HCV-positive patients after renal transplantations.

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