Original Article

Relationship of HLA Antigens and Cryoglobulinaemia in Hepatitis C virus Infected Patients

Sabiha Anis[1], Rana Muzaffar[1], Mirza Naqi Zafar[2], Ejaz Ahmed[3], Kiran Fatima[1], Syed Ali Anwar Naqvi[4], Syed Adibul Hasan Rizvi[4]

Department of Microbiology and Immunology[1], Department of Chemical Pathology[2], Department of Nephrology[3], Department of Urology, Sindh Institute of Urology and Transplantation (SIUT)[4], Karachi, Pakistan.

Abstract

Objective: To find the relationship between human leukocyte antigens (HLA) and cryoglobulin positivity in hepatitis C virus (HCV) infected individuals.

Methods: Eligible individuals selected from pre and post renal transplant settings were divided into three groups. Group A (n=301) consisted of normal controls, while group B (n=200) comprised of pathological controls that were HCV antibody (anti-HCV) positive but negative for cryoglobulins. Group C comprised of 56 anti-HCV positive, cryoglobulin positive patients. HLA-A, -B and -DRB1 loci were typed by polymerase chain reaction (PCR) method and relationship between HLA antigens, anti-HCV status and cryoglobulinaemia was analyzed.

Results: HLA-A*02, -B*57 and -DRB1*03 were more frequently found among group C members as compared to groups A and B. Only HLA-B* 57 occurrence reached statistical significance (14.3% versus 6% and 4%, corrected P-value = 0.045 and 0.012 and OR = 2.6 and 4 respectively) No differences in the distribution of HLA antigens were seen among healthy and pathological controls.

Conclusion: The presence of HLA-B*57 confers susceptibility to cryoglobulinaemia in HCV infected patients in our population. HCV positive renal transplant recipients with these alleles should be monitored for cryoglobulin formation (JPMA 57:300;2007).

Introduction

Hepatitis C virus (HCV) is recognized as a major cause of mixed cryoglobulinaemia (MC).[1] MC is characterized by the presence of abnormal proteins called cryoglobulins that reversibly precipitate in serum at temperatures below 37°C.[2] Serologically, cryoglobulins are divided into three types. Type I consists of single monoclonal immunoglobulins, where as types II and III consist of mixed cryoglobulins.[3] In HCV infection, presence of cryoglobulins may result in purpura, arthralgia and weakness, and can lead to glomerulonephritis, carditis, neuropathies and involvement of other organs.[1]

The mechanism of cryoglobulin pathogenesis is not clearly understood. The association of T-cell subsets, somatic mutations in immunoglobulin chains, and gene rearrangements have been investigated.[4-6] Viral genotypes may also contribute to cryoglobulin formation.[7] The presence of certain human leukocyte antigens (HLA) has been shown to be associated with either increased or decreased susceptibility to cryoglobulin formation in HCV infected patients.[8-14]

The association of HCV infection with cryoglobulin formation is of particular interest given the high prevalence of infection in Pakistan. The prevalence is reported to vary between 1-6% in the general population[15], but rates as high as 68% have been reported in renal failure patients.[16] Several of the HLA antigens reported to be associated with cryoglobulin formation, which include -B8[8], -DR3[8,12], -DR6[14], -DR11[9,11] and -DR15[13] are commonly found in Pakistani population.[17,18] As anti-HCV positive individuals frequently undergo renal transplantation, complications due to cryoglobulinaemia may add to challenges associated with accompanying immunosuppression.

The present study investigated the association of specific HLA alleles with cryoglobulin formation in a population of HCV infected patients.

Subjects and Methods

An analytical and case control study was carried out from July 2004 to June 2005. Blood samples were obtained with informed consent. A total of 557 individuals (negative for hepatitis B surface antigen) who visited pre or post renal transplant clinics at the Sindh Institute of Urology and Transplantation (SIUT) were enrolled for HLA testing and distributed into three groups. Group A comprised of 301 healthy kidney donors (normal controls) and group B of 200 anti-HCV positive patients who were negative for cryoglobulins (pathological controls). Group C consisted of 56 patients positive for both anti-HCV and cryoglobulins. In group B 74 patients were on maintenance haemodialysis and 126 were renal transplant recipients. In group C, 20 patients were recruited from maintenance haemodialysis and 36 were renal transplant recipients. Demographic characteristics including age, sex and language were noted for all patients. Native language was used to identify ethnic groups. Anti-HCV was determined by third generation micro particle enzyme immunoassay (MEIA) (Axsym Abbott USA).

Cryoglobulins were detected by collecting 10-12 ml of venous blood in a pre-warmed tube and allowing the blood to clot at 37°C. The separated serum was stored at 40C with sodium azide and observed for cryoprecipitation, daily, for a period of seven days. Serum samples showing cryoprecipitation were checked for resolubility by warming at 37°C.[19]

HLA typing for class I antigens was performed using a standard micro lymhocytotoxicity assay (One Lamda Inc., Canago Park, CA, USA). Typing of HLA-DRB1* locus was performed by Polymerase Chain Reaction (PCR) amplification with sequence specific primers (PCR-SSP) using a commercially available kit (CTS-PCR-SSP Tray and kit, Collaborative Transplant Study, Heidelberg Germany). Briefly, genomic DNA was extracted from 3 ml whole blood by using a commercially available kit (Pure gene, Gentra Systems USA). DNA was amplified, electrophoresed on 2% agarose gel, bands visualized and HLA-DRB1* alleles were assigned according to the table provided in the kit.

The qualitative data were expressed as prevalence rates and quantitative data was calculated as mean and standard deviation. The numbers of HLA antigens were counted in each group as 2n. The percentages were calculated per total number of subjects (n). Chi-square test with Yates correction or Fisher exact test was applied to compare the HLA alleles between the groups. Bonferroni and Turkey HSD methods were applied for correction of multiple testing at HLA-A, -B and DR loci. Corrected P-values (Pc) obtained after Turkey HSD correction is shown in the tables. Pc = 0.05 was considered significant. Odds Ratio and Relative Risk were also calculated. Data was analyzed using SPSS statistical software version 13.

The ethical Committee of Sindh Institute of urology and transplantation (SIUT) approved this study.

Results

The mean age for the three groups was 31.34 ± 9.7 years; group A 32.85 ± 9.14 years, group B was 29.4 ± 10.1and group C 30.21 ± 10.26 years. There were 363 males and 194 females in the three groups. The number of

Table 1. Distribution of HLA-A Alleles in Controls and Patients.

HLA-A	Group A HCV -ve, CG -ve n =301 (%)	Group B HCV +ve, CG -ve n = 200 (%)	Group C HCV +ve, CG+ve n = 56 (%)	Pc1	Pc2	Pc3
A1	69 (22.9)	42 (21)	9 (16)	0.865	0.488	0.708
A2	121 (40)	79 (39.5)	30 (53.6)	0.987	0.149	0.142
A3	35 (11.6)	30 (15)	5 (8.9)	0.578	0.807	0.452
A9	87 (28.9)	48 (24)	12 (21.4)	0.443	0.475	0.921
23	3 (0.99)	2 (1)	2 (3.6)			
24	84 (28)	46 (23)	10 (17.9)			
A10	44 (14.6)	38 (19)	11 (19.6)	0.403	0.625	0.993
A11	97 (32.2)	64 (32)	20 (35.7)	0.998	0.866	0.860
A19	105 (34.9)	69 (34.5)	15 (26.8)	0.996	0.469	0.529
29	5 (1.6)	10 (5)	2 (3.6)			
30	14 (4.6)	10 (5)	3 (5.4)			
31	29 (8.7)	19 (9.5)	4 (7.1)			
32	26 (8.6)	11 (5.5)	2 (3.6)			
33	31 (10.3)	18 (9)	4 (7.1)			
74	0	1 (0.5)	0			
A28	44 (14.6)	28 (14)	10 (17.9)	0.98	0.805	0.753
A43	0	2 (1)	0	N.A	N.A	N.A

Abbreviations: HLA-A = human leukocyte antigen A, HCV -ve = HCV antibody negative, CG -ve = cryoglobulin negative, HCV +ve = HCV antibody positive, CG +ve = cryoglobulin positive, Pc1= corrected P value between groups A and B; Pc2 = corrected P value between group A and C; Pc3 = corrected P value between group B and C; NA = not applicable
Note: Allele subtypes not shown due to insignificant numbers and P values.

Table 2. Distribution of HLA-B Alleles in Controls and Patients.

HLA-A	Group A HCV -ve, CG -ve n =301 (%)	Group B HCV +ve, CG -ve n = 200 (%)	Group C HCV +ve, CG+ve n = 56 (%)	Pc1	Pc2	Pc3
B5	112 (37.2)	58 (29)	23 (41)	0.141	0.842	0.214
51	80 (26.5)	41 (20.5)	17 (30.4)			
52	32 (10.6)	17 (8.5)	6 (10.7)			
B7	21 (7)	11 (5.5)	3 (5.4)	0.784	0.891	0.999
B8	57 (18.9)	47 (23.5)	13 (23.2)	0.438	0.751	0.999
B12	32 (10.6)	17 (8.5)	7 (12.5)	0.718	0.905	0.654
B13	14 (4.6)	8 (4)	3 (5.35)	0.937	0.97	0.902
B14	0	1 (0.5)	0	N.A	N.A	N.A
B15	29 (9.6)	15 (7.5)	9 (16)	0.704	0.288	0.13
B16	14 (4.7)	11 (5.5)	3 (5.4)	0.905	0.973	0.999
B18	26 (8.6)	17 (8.5)	5 (8.9)	1.000	1.000	0.995
B21	18 (6)	16 (8)	4 (7.1)	0.469	0.762	0.677
B22	6 (2)	8 (4)	1 (1.8)	0.267	1.000	0.360
B27	14 (4.6)	9 (4.5)	3 (5.35)	1.000	0.737	0.964
B17	55 (18.3)	33 (16.5)	12 (21.4)	0.869	0.839	0.673
57 (17) 1	18 (6)	8 (4)	8 (14.3)	0.634	0.045*	0.012*#
58 (17) 2	36 (12)	19 (9)	2 (3.6)	0.52	0.134	0.457
B35	82 (27.2)	56 (28)	11 (19.6)	0.981	0.467	0.426
*B7	20 (6.6)	13 (6.5)	0	0.998	0.13	0.163
B40	77 (25.6)	60 (30)	14 (25)	0.522	0.996	0.738
B41	2 (0.7)	4 (2)	0	N.A	N.A	N.A
B42	1 (0.33)	0	0	N.A	N.A	N.A
B47	4 (1.3)	3 (1.5)	0	N.A	N.A	N.A
B48	1 (0.33)	0	0	N.A	N.A	N.A
B53	9 (3)	9 (4.5)	1 (1.8)	0.634	0.892	0.585
B70	6 (2)	4 (2)	0	N.A	N.A	N.A
B73	1 (0.33)	0	0	N.A	N.A	N.A
B81	1 (0.33)	0	0	N.A	N.A	N.A

Abbreviations: HLA-B = human leukocyte antigen B, HCV -ve = HCV antibody negative, CG -ve = cryoglobulin negative, HCV +ve = HCV antibody positive, CG +ve = cryoglobulin positive, Pc1 = corrected P value between groups A and B, Pc2 = corrected P value between group A and C, Pc3 = corrected P value between group B and C, 1, 2 = Subtypes of HLA-B allele B17, * = significant P-value = 0.05, = P-value before correction = 0.028, # = P-value before correction = 0.005, NA = not applicable
Note: All allele subtypes not shown due to insignificant numbers and P values.

Table3. Distribution of HLA-DR Alleles in Controls and Patients.

DRB1*	Group A HCV -ve, CG -ve n =301 (%)	Group B HCV +ve, CG -ve n = 200 (%)	Group C HCV +ve, CG +ve n = 56 (%)	Pc1	Pc2	Pc3
*01	34 (11.3)	20 (10)	4 (7.14)	0.888	0.620	0.811
*04	27 (9)	21 (10.5)	5 (9)	0.836	1.000	0.933
DR6	97 (32.2)	78 (39)	21 (37.5)	0.267	0.729	0.977
*13 1	48 (15.9)	33 (16.5)	13 (23.2)	0.986	0.378	0.463
*14 2	42 (14)	30 (15)	6 (10.7)	0.942	0.798	0.694
*07	61 (20.3)	43 (21.5)	5 (9)	0.938	0.122	0.091#
*08	5 (1.7)	5 (2.5)	1 (1.8)	0.787		0.939
*09	4 (1.3)	3 (1.5)	3 (5.4)	0.989	0.998	0.133
*1001	39 (13)	20 (10)	5 (9)	0.457	0.093	0.992
*11	76 (25.2)	46 (23)	17 (30.4)	0.837	0.658	0.500
*12	9 (3)	5 (2.5)	0	0.937	0.697	0.542
*15	107 (35.9)	73 (36.5)	19 (33.9)	0.974	0.39	0.933
*16	23 (7.6)	8 (4)	2 (3.6)	0.209	0.971	0.992
*17	119 (39.5)	64 (32)	24 (42.9)	0.202	0.463	0.298
*18	1 (0.33)	14 (7)	6 (10.7)	1.000	0.884	1.000
					1.000	

Abbreviations: HLA-DR = human leukocyte antigen DR, HCV -ve = HCV antibody negative, CG -ve = cryoglobulin negative, HCV +ve = HCV antibody positive, CG +ve = cryoglobulin positive, Pc1 = corrected P value between groups A and B, Pc2 = corrected P value between group A and C, Pc3 = corrected P value between group B and C, 1, 2 = Subtypes of HLA-DR allele 6, = P value before correction = 0.045 (RR = 2.270; 95%confidence interval = 0.955 - 5.396), # = P value before correction = 0.033 (RR = 2.408; 95%confidence interval = 1.001 - 5.790)

males in groups A, B and C were 174, 151 and 38 and females 127, 49 and 18 respectively. No statistically significant differences in age, sex or linguistic background were found among the three groups.

The distribution of different HLA alleles identified is given in Tables 1-3. The most frequent HLA-A alleles among the three groups were -A*02 (41.3%), -A19 (*29, *30, *31, *32, *33) (34%) and -A*11 (32.5%) (Table 1). At the HLA-B locus, the most recurrent alleles were -B5 (*51, *52) (34.6%), -B*40 (27.1%) and -B*35 (26.7%) (Table 2), while -DRB1*03 (40.9%), -DR2 (DRB1*15 and *16) (41.7%), (35.7%) and DRB1*11 were the most frequently identified alleles at the DR locus (Table 3). HLA-B* 57 frequency was significantly higher in group C compared to other groups (14.3% versus 6% and 4%, Pc = 0.045 and 0.012 by Turkey HSD correction, 0.05 and 0.013 by Bonferroni correction, OR = 2.6 [95%confidence interval: 1.1-6.4] and 4.0 [95%confidence interval: 1.4-11.2] respectively) (Table2).

Only 20 (35.7%) patients in group C had symptoms related to cryoglobulinaemic vasculitis. The most frequent alleles were HLA-DRB1*03 identified in 11 (55%), -A*02 and A19 (*29, *30, *31, *32 and *33) in seven [35%], and B*08 and -B17 (*57 and *58) in 5 (25%) patients.

Discussion

MC is the most frequent extra hepatic manifestation of HCV infection.[1] The exact mechanism leading to cryoglobulin formation is still not clear but there are several possible explanations. It has been proposed that the interaction between E2 viral envelope protein of HCV and CD81 receptors on B cells and hepatocytes can lead to cryoglobulin formation. The monoclonal component of cryoglobulin has been shown to be a product of VH1-69 gene of B-cells and it has been hypothesized that restricted and excessive use of this gene may lead to lymphoma formation.[5] It has also been suggested that bcl-2 gene rearrangement (t14: 18 translocation) in B-lymphocytes may play an important role in cryoglobulin formation. This translocation can occur due to an intense or prolong antigenic stimulation of these cells.[6] Similarly, deficiency of CD4+CD25+ regulatory T cells has been demonstrated in MC pathogenesis of.[4] But these observations do not explain why only a subset of HCV infected patients develops MC, and are also insufficient in deciphering the phenomenon of induction of cryoglobulin formation. Several studies have shown an association of a specific HCV genotype with MC formation but a consensus has not been reached.[7,13,14] Furthermore, cryoglobulin formation may relate to the quasispecies nature of the virus1 rather than a specific genotype.

HLA system encoded by the MHC is the most polymorphic genetic system known. Presentation of viral peptides by HLA class I and class II causes activation of CD8+ and CD4+ T lymphocytes respectively, and results in the activation of both cytotoxic T lymphocytes and antibody production. Response to viral antigens is partly determined by the

genetic makeup of an individual.[10,12]

Variable and contradictory reports are found in the literature on the association of HLA haplotypes with MC. Several antigens including HLA-A33, -B8, -B65, -DR2, -DR3, -DR6 and -DR11 have been shown to be associated with MC.[9-15] Some of these antigens (HLA-B8, -DR3, -DR6 and -DR11) are linked to increased susceptibility for cryoglobulin formation, while others may have a restrictive role. We were unable to find any association of the above mentioned alleles with cryoglobulin formation in HCV infected patients. In the present study, HLA-B*57 was found more frequently in the patient population investigated, as compared to both normal and pathological controls (Pc = 0.045 and 0.012 respectively). Another important finding was the lower frequency of HLA-DRB1*07 in the patient-group (group C) than in the control groups (groups A and B) studied (9% vs. 20.3% and 21.5%; RR= 2.27 and 2.4 respectively), indicating a protective role of -DRB1*07 against cryoglobulin formation as previously suggested.[9] The frequencies of HLA alleles determined in the normal controls were comparable to previously published rates of occurrence in Pakistani population.[17,18] However, HLA-B*57 identified in 14.3% of the patient group is not a commonly occurring HLA allele in Pakistani populations. This is in contrast to an Italian study, where HLA-B17 (serotype of HLA-B*57) was a frequently occurring antigen in normal controls, but was conspicuously absent in patients with HCV associated MC.[8]

The variation in HLA associations between the studies could be attributed to several factors. Differences in patient populations may be one contributing factor. In the Lenzi et al study[8], patient selection was based on the presence of MC with one or more symptoms of cryoglobulinaemic vasculitis, whereas in the present study patients were selected on the basis of presence of cryoglobulins, with or without symptoms. The inclusion of patients without cryoglobulinaemic symptoms may dilute strong HLA linkage with disease but is unlikely to explain the major differences seen in HLA association with MC. This is clearly shown by the fact that in a Chinese population comprising of symptomatic as well as asymptomatic patients, HLA-DR3 was found significantly higher in HCV infected patients with MC.[12]

Another important contributing factor is the presence of wide geographical and ethnic variation in the distribution of HLA alleles. This may reflect an adaptive immune response directed towards different pathogens[17], or can be due to the preferential use of alleles in viral eradication.[20] While Tillman and colleagues[21] reported the association of HLA-DRB1*11 with reduced risk of end stage liver disease due to viral clearance, others[9,11] observed a significantly higher association of -DRB1*11 with MC in chronic HCV infection. Similarly, association of HLA-B*57 with HCV clearance was reported in both Caucasian and African-American populations.[22] In the present study, this allele was found to be associated with an increased risk of cryoglobulin formation emphasizing the need to study more than one ethnic group to establish these associations. It may well be the case that our patient population comprising of renal failure patients on replacement therapy, despite the presence of HLA-B*57 falls just short of complete viral clearance resulting in cryoglobulin formation.

The important ethnic differences in the distribution of HLA antigens among different populations are further emphasized by a recent report from China1[2] where frequency of HLA -DR 3 was significantly higher in HCV positive patients with MC (36.5%), compared to 11% of the healthy controls.[12] We did not find any association of HLA-DRB1*03 with cryoglobulin formation despite the fact that HLA-DRB1*03 prevalence in our healthy controls and in a larger Pakistani population is 39% and 40.6% respectively.[18]

Another factor that may influence the relationship between HLA and MC is the nature of associated diseases present. Congia et al[10] found a protective role of -DRB1*16 against HCV associated MC in a group of thallasemic Italian patients, while Amoroso et al[11] found a very different relationship between HLA and HCV associated MC in patients with or without chronic liver disease. Although the frequency of -DRB1*11 was significantly higher in HCV infected patients with MC, it was decreased in those with severe liver disease. In the present study all patients (groups B and C) had end stage renal disease (ESRD) and were on renal replacement therapy. Patients with ESRD, who are immunosuppressed due to nonspecific effects of dialysis and drugs, may have a more complex interaction with HCV antigens, which may not be entirely dependent on genetic makeup of the patient.

In conclusion our results support the association of HLA alleles with cryoglobulin formation in HCV infected patients. However, this association should be interpreted with caution, and in consideration of HLA distribution in the healthy population of the geographical area. Presence of other diseases that influence the immune system is likely to influence the presentation of viral peptides resulting in immune complex mediated disorders such as MC. Further studies are required in these patients to clarify the role of HLA antigens once

they are infected with the HCV.

Acknowledgement

We are grateful to Jaffar Baquar for his help with the statistical analysis.

References

1. Ferri C, Sebastiani M, Giuggioli D, Cazzato M, Longombardo G, Antonelli A, et al. Mixed cryoglobulinemia: demographic, clinical and serologic features and survival in 231 patients. Semin Arthritis Rheum 2004; 33: 355-74.
2. Meltzer M, Franklin EC, Elias K, McChuskey RT, Cooper N. Cryoglobulinaemia a clinical and laboratory study. ii.Cryoglubulins with rheumatoid factory activity. Am J Med 1966; 40: 837-56.
3. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of Cryoglobulins. Am J Med 1974; 57: 775-88.
4. Boyer O, Saadoun D, Abriol J, Dodille M, Piette JC, Cacoub P, et al. CD4+CD25+ regulatory T-cell deficiency in patients with hepatitis C - mixed cryoglobulinemia vasculitis. Blood 2004; 103: 3428-30.
5. Chan CH, Hadlock KG, Foung SK, LevyS. V(H)1-69 gene is preferentially used by hepatitis C virus-associated B cell lymphomas and by normal B cells responding to the E2 viral antigen. Blood 2001; 97: 1023-26.
6. Zignego AL, Ferri C, Giannelli F, Giannini C, Caini P, MontiM, et al. Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B cell lymphomas. Ann intern med 2002; 137: 571-80.
7. Antonescu C, Mayerat C, Mantegani A, Frei PC, Spetini F, Tissor JD. Hepatitis C virus infection: serum rheumatoid factor activity and HCV genotype correlate with Cryoglobulin clonality. Blood 1998; 92: 3486-8
8. Lenzi M, Frisoni M, Mantovani V, Ricci P, Muratori L, Francesconi R, et al. Haplotype HLA-B8-DR3 confers susceptibility to hepatitis C virus-related mixed cryoglobulinaemia. Blood 1998; 91: 2062-6.
9. Cacoub P, Renou C, Kerr G, Hue S, Rosenthal E, Cohen P, et al. Influence of HLA-DR phenotype on the risk of hepatitis C virus-associated mixed cryoglobulinaemia. Arthritis Rheum 2001; 44: 2118-2124.
10. Congia M, Clemente MG, Dessi C, Cucca F, Mazzoleni AP, Frau F, et al. HLA class II genes in chronic hepatitis C virus-infection and associated immunological disorders. Hepatology 1996; 24: 1338-41.
11. Amoroso A, Berrino M, Canale L, Cornaglia M, Guarrera S, Mazzola G, et al. Are HLA class II and immunoglobulin constant region genes involved in the pathogenesis of mixed cryoglobulinaemia type II after hepatitis C virus infection? J Hepatol 1998; 29: 36-44.
12. Hwang SJ, CHU CW, Huang DF, Lan KH, Chang FY, Lee SD. Genetic predispositions for the presence of cryoglobulinemia and serum autoantibodies in Chinese patients with chronic hepatitis C. Tissue Antigens 2002; 59: 31-37.
13. Perrone A, Deramo MT, Spaccavento F, Santarcangelo P, Favoino B, Antonaci S. Hepatitis C virus (HCV) genotypes, human leucocyte antigen expression and monoclonal gammopathy prevalence during chronic HCV infection. Cytobios 2001; 106: 125-34.
14. Sebastiani GD, Bellisai F, Caudai C, Rottoli P, Valensin PE, Pippi L, et al. Assiciation of extrahepatic manifestations with HLA class II alleles and with virus genotype in HCV infected patients. J Biol Regul Homeost Agents 2005; 19: 17-22.
15. Hamid S, Umar M, Alam A, Siddiqui A, Qureshi H, Butt J. PSG consensus statement on management of hepatitis C virus infection-2003. J Pak Med Assoc 2004; 54: 146-50.
16. Gul A, Iqbal F. Prevalence of hepatitis C in patients on maintenance hemodialysis. JCPSP 2003; 13: 15-18.
17. Mohyuddin A, Ayub Q, Khaliq S, Mansoor A, Mazhar K, Rehman S, et al. HLA polymorphism in six ethnic groups from Pakistan. Tissue antigens 2002; 59: 492-501.
18. Zafar N, Khan S, Qadir A, Raza Y, Naqvi A, Rizvi A. HLA frequencies in Pakistani population groups. J Pak Med Assoc 1996; 46: 12-13.
19. Musset L, Diemert MC, Taibi F, Thi Huong Du L, Cacoub P, Legar JM, et al. Characterization of cryoglobulins by immunoblotting. Clin Chem 1992; 38: 798-802.
20. Yee LJ. Host genetic determinants in hepatitis C virus infection. Genes Immun 2004; 5: 237-45.
21. Tillmann HL, Chen DF, Trautwein C, Kliem V, Grundey A, Berning-Haag A, et al: Low frequency of HLA-DRB1*11 in hepatitis C virus induced end stage liver disease. Gut 2001; 48: 714-8.
22. Thio CL, Gao X, Goedert JJ, Vlahov D, Nelson NE, Hilgartner MW, et al. HLA-Cw *04 and hepatitis C virus Persistence. J Virol 2002; 76: 4792-7.