HLA Genotyping in Type-I Autoimmune Hepatitis in Western India

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Abstract

Objectives: To analyze association of different HLA genotypes for predisposition to type-I autoimmune hepatitis in Western India.

Methods: This study was undertaken on patients of type-I autoimmune hepatitis (defined by international criteria by IAHG, 1999). HLA genotyping for class I and II was done in 20 patients of autoimmune hepatitis and 100 healthy controls. Statistics were done using Halden's modification of Woolf's formula.

Result: Significant association of autoimmune hepatitis was found amongst class I antigens - HLA B27 [20 vs. 0 %] & HLA cw4 [40 vs. 15 %] and amongst class II antigens - DRB1*01XX [25 vs. 2 %], DRB1*14XX [30 vs. 12 %], DRB1*15XX [40 vs. 25%] and DRB1*07XX [20 vs. 9 %] at DRB1 locus. Stronger association was found with HLA B27, cw4 & HLA DRB1 *01XX.

Conclusion: Our data indicate that predisposition to autoimmune hepatitis is different in Indian patients and not associated with HLA DRB1*03XX or *04XX, as seen in Western world.

INTRODUCTION

Autoimmune hepatitis (AIH) is an unresolving inflammation of liver of unknown cause. It reflects complex interaction between triggering factors, autoantigen, genetic predisposition and immunoregulatory networks. A genetic predisposition is an absolute prerequisite. Only predisposition is inherited, not the disease itself, which must be triggered by an antigen.1,2

Postulated antigen after entering hepatocyte by endocytosis, fuses with HLA class II molecule and is broken down to peptide by proteolysis. HLA class II - peptide complex is transported to the plasma membrane, expressed in a groove and presented to CD4-T lymphocyte, which initiates inflammatory cascade. Interaction between HLA molecules, antigenic peptide presented in its groove and T cell receptors is crucial. Therefore, HLA alleles determine ability of each class II molecule of MHC to bind and present antigen to CD4 lymphocytes, consequently, they directly influence the immune response and in turn, clinical manifestation and behavior of AIH.2,3 HLA- DRB genes are more strongly associated with the disease than DQA and DQB.1

In India, more and more cases of autoimmune hepatitis are being recognized, prevalence of which is around 5% in chronic liver disease.4 To best of our knowledge, there are no studies from India linking HLA genotype and predisposition to AIH.

We planned this study to analyze HLA genotype predisposition of AIH type- I in western India, as there is wide ethnic variability in genetic predisposition to AIH, throughout the world.

MATERIAL AND METHODS

This prospective study was carried out from January 2002 to July 2002 at Bombay Hospital and Medical Research Center, Mumbai. A total of 20 consecutive patients of autoimmune hepatitis defined by the international scoring system, IAHG, 1999, were included in the study group.5 Various parameters like gender, AP/AST ratio, IgG levels, autoimmune markers, viral markers, drug history, alcohol consumption, HLA typing, presence of coexisting immune disease, histology and treatment response are considered in this scoring system. In the second group - control group comprised of a total of 100 hepatitis-free healthy persons, undergoing HLA genotyping study as a donor in the renal transplantation program at our center. In both the groups, HLA class I and class II genotype analysis were carried out by the following technique. HLA A, B and C allele were identified by using 72 well histo tray ABC (Biotest, Germany) by standard NIH complement dependant microlymphocytotoxicity assay from lymphocytes isolated from heparinised blood. Theogenic
DNA was extracted using commercially procured DNA extraction kit (Qiagen kit). HLA-DRB1 low resolution typing was followed using the commercial DRB1 PCR kit protocol (Dynal, Oslo). Further high resolution typing for samples of HLA DRB1*01XX positive patients was carried out using the high resolution typing kit (Genovision, USA). Genotype-disease association was analyzed by using Halden’s modification of the Woolf’s formula. P value of < 0.001 suggested strong association between HLA genotype and the disease, < 0.05 suggested presence of association and > 0.05 suggested no association.

RESULTS

In autoimmune hepatitis group, twenty patients were included with mean age of 44 ± 2.6 years (range of 18 -61 years) and male to female ratio of 1:3. All the patients were definite AIH with pretreatment more than 15 score. Clinically, seven patients (35%) presented as chronic hepatitis, 11 (55%) as cirrhosis and two (10%) as acute hepatitis. Out of 20 patients, 16 (80%) were ANA positive (≥ 1:80) and 13 (65%) ASMA positive. None was anti-LKM positive, one patient (5%) was AMA positive (this patient was primary biliary cirrhosis overlap with AIH) and one patient (5%) was anti-HCV positive, but HCV RNA negative by PCR. All were negative for HBsAg. On liver histology, inflammation in 18 (90%), fibrosis in 16 (80%), liver cell rosetting in 16 (80%) and biliary changes in one (5%) (PBC overlap with AIH patient). Amongst extra-hepatic manifestation, which were seen in 55% patients, rheumatoid arthritis in three (15%), diabetes in three (15%), hypothyroidism in two (10%), vitiligo in two (10%), hemolytic anemia in two (10%), psoriasis in one (5%) and erythema nodosum in one (5%). Only one patient (5%) showed PBC overlap with AIH. In the control group, 100 healthy persons with age of 41 ± 2.8 yrs (17-65 yrs) and male to female ratio 2:1 were included.

On comparing HLA genotyping between AIH and control group, amongst HLA class I molecule- A locus, A1 (20 vs. 5 %), A2 (30 vs. 12 %), A11 (40 vs. 21 %), A19 (30 vs. 14 %) and A28 (20 vs. 5 %) showed association with AIH (p<0.05); whereas A3 (10 vs. 9 %), A9 (20 vs. 16 %), A10 (10 vs. 10 %), A21 (0 vs. 1 %) and A29 (0 vs. 1 %) showed no association (p>0.05).

Amongst class I- B locus, B27 (20 vs. 0 %) showed a strong association with the disease (p<0.001); B7 (25 vs. 12 %), B12 (20 vs. 7 %), B17 (15 vs. 4 %), B35 (30 vs. 12 %), B37 (10 vs. 2 %) and B40 (20 vs. 9 %) showed association with AIH (p<0.05); B8 (5 vs. 3 %), B13 (5 vs. 4 %), B14 (0 vs. 1 %), B15 (0 vs. 4 %), B16 (0 vs. 2 %), B18 (0 vs. 3 %), B21 (0 vs. 4 %), B22 (5 vs. 5 %), B41 (0 vs. 1 %) and B71 (5 vs. 0 %) showed no association with AIH (p > 0.05); whereas B5 (0 vs. 21 %) showed negative association with AIH.

Amongst class I- C locus, cw4 (40 vs. 15 %) showed strong association with AIH (p<0.001); cw1 (10 vs. 2 %), cw3 (20 vs. 8 %) and cw6 (20 vs. 8 %) showed association with the disease (p<0.05); whereas cw2 (0 vs. 2 %), cw5 (5 vs. 0 %), cw7 (30 vs. 33 %) and cw8 (0 vs. 2 %) did not show any association with AIH (p>0.05).

Amongst HLA class II molecules, HLA DRB1*01XX (25 vs. 2 %) had strong association (P < 0.001) with AIH. Further, high resolution typing for HLA B1*01XX revealed presence of HLA B1*0101 in all the five patients. Where as, HLA DRB1*07XX (20 vs. 9 %), DRB1*14XX (30 vs. 12 %) and DRB1*15XX (40 vs. 25 %) showed association with AIH (P < 0.05), but there was no association (p > 0.05) with HLA DRB1*03XX (20 vs.12 %), DRB1*04XX (5 vs. 3 %), DRB1*09XX (0 vs. 2 %), DRB1*10XX (10 vs. 8 %), DRB1*11XX (5 vs. 4 %), DRB1*12XX (5 vs. 2 %), DRB1*13XX (20 vs. 15 %) and DRB1*16XX (0 vs. 4 %).

Amongst patients with extra-hepatic manifestation, frequency of HLA A11, A19, B22, B27, DRB1*14XX and DRB1*03XX alleles was more but was not statistically significant.

DISCUSSION

In our study, HLA B27, HLA cw4 and HLA DRB1*01XX were strongly associated with autoimmune hepatitis type I. HLA DRB1*07XX, DRB1*14XX and DRB1*15XX also were associated with autoimmune hepatitis in our population.

These alleles are different from what Western literature has noted. In Western Europe and North American Caucasian population, most common haplotype is A1-B8-DRB3-DQ2. HLA DRB1*0301 is associated with presentation at young age, high frequency of treatment failure, higher response after treatment withdrawal and higher need for liver transplant, whereas DRB1*0401 is associated with presentation at older age, higher frequency of concurrent autoimmune disease and better response to steroid treatment. But, studies from other parts of the world differ in HLA genotyping for AIH type I. In Mestizo Mexicans DRB1*0404 was found, in Argentinean adults DRB1*0405, in Argentinean children DRB1*1301, in Brazil DRB1*03XX and DRB1*1301 and in Japan DRB1*0405 and HLA B54. This ethnic variability may be explained by ‘shared motif hypothesis’ - different HLA DR3 and HLA DR4 alleles encode the same or similar amino acid sequence within critical site that is antigen binding groove of class II MHC molecule, so they each can affect susceptibility to AIH. There is strong association of a single amino acid residue, lysine, at position of HLA DRβ6 polypeptide. Lysine DRB71 is encoded by DRB1*0301 and DRB1*0401 as well as other DRB alleles. Susceptibility alleles in other ethnic group (Japanese, Mexican and Argentinean adults) encode similar motif with expect of arginine for lysine at DRβ71, as arginine is a polar residue that is strongly similar to lysine, so is unlikely to affect immunogenesis. But, studies from Asia - Japan and Argentina have challenged this hypothesis. Presence of HLA DRB1*1301 in Argentinean children and Brazilian patients encode different amino acid sequence. In Japan, different basic amino acids at position 13 of HLA DRB polypeptide are responsible for the different HLA DR4 genotype being associated with late onset of AIH type I.

Not all the patients with AIH have these alleles and the
existence of other nonspecific promoters of autoimmunity is likely. There promoters may provide a permissive environment for the emergence of disease specific triggers and they may reside within or outside of MHC.8 ‘Autoimmune promoter hypothesis’ suggests autoimmune promoters such as polymorphism of tumor necrosis factor - α gene (TNF A * 2) and CTLA - 4 gene (CTLA - 4) may synergise with each other or the principal class II HLA risk factors to increase susceptibility to the disease.9 North American patients have a significant overrepresentation of a polymorphism for the gene encoding cytotoxic T lymphocyte antigen - 4 (CTLA - 4). This polymorphism involving a guanine (G) for adenine (A) exchange at position 49 in exon - 1 was associated with higher AST, higher antibodies and more common occurrence of HLA DRB1 * 0301. Since CTLA-4 is a T cell surface molecule that competes with CD28 molecule for ligands B7 -1 and B7 - 2 on antigen presenting cells and modulates CD4 T helper cell activation. G alleles may result in product, which is less effective in preventing immuncytocyte activation and may promote autoimmune process.10 The other autoimmune promoter is polymorphism of tumor necrosis factor gene (TNF -A), which involves guanine to adenine substitution at position - 308 (TNF α 2). This variant results in high inducible and constitutive TNF α levels, which in turn enhance cellular cytotoxicity. TNF α 2 polymorphism is in strong linkage dysequillibrium amongst HLA A1- B8- DR3 haplotype and there is interdependent association between TNF α 2 and DRB1 * 0301.11 TNF α 2 polymorphism is also associated with less frequent remission, more common treatment failure and more frequent cirrhosis.12

Other HLA loci are also studied. HLA - C gene - CW * 0701 was found to be associated with AIH type 1. HLA - C antigen enhance target recognition by NK cells and that may be pertinent in pathogenesis of AIH, unfortunately linkage dysequilibrium amongst CW * 0701, B8 and DRB1 * 0301 may account for its increased frequency in type 1 AIH.13 Other HLA gene studied is HLA class III - complement polymorphism. C4A-Q0 alleles are associated with low complement level, since C4 is involved in immune complex clearance, low level may indicate involvement of viral agent in pathogenesis of the disease.12 Also, studies on Argentinean children, where hepatitis A is endemic, suggest protracted, but not acute, infection with hepatitis A virus is strongly associated with DRB1 * 1301, which results in enhanced exposure to hepatic self antigen and induces the disease.14

So, discrepancies from different ethnic group may suggest different diseases, diverse selection factors or distinctive environmental or etiological agents in the various ethnic groups and different geographic region.7

In western series from North America and Western Europe, HLA DRB1*03XX and DRB1*04XX are considered as a criteria for diagnosis of AIH3 as well as a predictor for prognosis and for response to therapy.15 This may not be applicable all over the world, as HLA genotype is host-specific and not disease-specific. This fact is well supported by our study and many more above mentioned studies. In our study, despite small sample size and genetically heterogeneous Indian population, HLA DRB1 * 03XX and DRB1 * 04XX alleles are distinctively uncommon in western Indian AIH patients. Because of small sample size, correlation of HLA prevalence with various aspects of the disease was not possible in our study and further larger studies are warranted to address this issue and to reconfirm findings of our study.

Acknowledgements

We acknowledge support of staff of Gastroenterology and Tissue Typing Departments, Bombay Hospital, Mumbai. We also thank all the patients for consenting and participating in this research. This research work was supported by research grant from the medical research fund, Bombay hospital, Mumbai.

References