Original Article

Analysis of HLA association among North Indian HIV-positive individuals co-infected with *Mycobacterium tuberculosis*

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ABSTRACT

Background: Genetic variation in HLA genes influence the immune response and may thus contribute to differential development of tuberculosis (TB) in HIV-infected individuals. The study was designed to determine whether HLA polymorphisms influence the development of *Mycobacterium tuberculosis* infection in HIV-infected individuals. **Materials and Methods:** Fifty HIV-positive individuals without TB (HIV+TB–), 50 HIV patients co-infected with TB (HIV+TB+) and 50 control subjects (HIV-TB-) were analyzed for HLA Class I and II polymorphisms. **Results:** In HLA Class II, frequency of occurrence of DRB1*13 (OR 3.165, CI 1.176–8.518, *P* value 0.019), DRB5 (OR 2.253, CI 1.011–5.019, *P* value 0.045) and DQB1*06 (OR 2.705, CI 1.197–6.113, *P* value 0.016) were increased in HIV+TB+compared to HIV+TB–. HLA DQB1*02 (OR 0.436, CI 0.185–1.029, *P* value 0.05) on the other hand conferred a protective role. In HLA Class I, frequency of B*15 (OR 2.705, CI 1.040–7.036, *P* value 0.038) was increased, whereas B*51 (OR 0.148, CI 0.031–0.706, *P* value 0.007) was decreased in HIV+TB+group compared to HIV+TB–. These differences however were not significant when compared with healthy controls. **Conclusion:** HLA polymorphisms independently did not account for the susceptibility to either of the disease mostly, although they seem to play a role once the infection(s) has established in a particular individual. Further studies are needed on a larger sample size to confirm these observations.

KEY WORDS: HIV-TB co-infection, HLA association, north India

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INTRODUCTION

India bears the burden of 2.5 million people infected with HIV, 40% of which are co-infected with tuberculosis (TB).[1,2] Rightly termed the "accursed duet,"[3] HIV-TB co-infection has had far reaching impact on the epidemiologic progression and impact on national health. The risk of co-infection with TB is about 20–70 times higher among those infected with HIV according to the WHO estimates and about one in four deaths in HIV-infected individuals are attributable to TB.[4,5] Among the opportunistic infections affecting HIV-infected individuals, TB is the commonest and remains the leading cause of mortality among HIV-infected individuals. In 2012, people living with HIV accounted for 1.1 million (13%) of the estimated 8.7 million people developing TB globally. Of the 2.8 million people with TB tested for HIV in 2012, 20% turned out positive for HIV.[6]

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The association between HIV and tuberculosis is complex and bidirectional. The probability of recently acquired TB infection of progressing to active disease is increased with the co-occurrence of HIV. TB produces a state of continuous ongoing systemic inflammation-induced stimulation of the host immune system resulting in accelerated CD4 lymphocyte turnover augmenting HIV replication. Unlike other opportunistic infections, TB may occur at any level of HIV-associated immunodeficiency, and has been clearly

shown to be associated with enhanced morbidity and mortality.[7-10]

Host genetic factors including genetic polymorphisms within the HLA class I and II loci are known to influence disease progression. Because the HLA system plays an important role in the modulation of the immune response, a possible association between HLA antigens and TB or HIV has been examined in several populations.[11-14] Results however have not yielded any unifying results.

We studied HIV sero-positive individuals with and without co-infection with TB to determine the type of HLA that contributes to the development of TB in a North Indian cohort of HIV-positive individuals.

MATERIALS AND METHODS

Fifty HIV-infected patients without TB or any other infectious diseases (stage II in the Centers for Disease Control and Prevention classification system for AIDS), 50 HIV-infected patients co-infected with TB (AIDS CDC stage IVC), and 50 healthy controls over a period of 2 years were included in this study. The groups were designated HIV+TB−, HIV+TB+, and HIV−TB−, respectively. HIV infection was diagnosed following CDC criteria (NACO Strategy III). In all cases, the diagnosis of TB was confirmed by sputum microscopy, and/or culture of the *M tuberculosis*. Patients with diabetes, silicosis, cirrhosis, or any systemic disease and patients who abused alcohol were excluded. Consent was obtained from all subjects as per the guidelines of research review committee of the Postgraduate Institute of Medical Education and Research, Chandigarh. Fifty control subjects matched in age, gender, and similar ethnic background were recruited from healthy volunteers. Five milliliters of venous blood was obtained by venepuncture for DNA extraction and HLA typing.

DNA was extracted using blood genomic DNA isolation kits from Axygen Biosciences, Union City, CA, USA. The quality of DNA was judged by spectrophotometry. DNA-based HLA typing was done using low-resolution sequence-specific primer (SSP) method for class I (HLA-A, -B, -C) and class II (HLA-DR and -DQ) using kits from Inno-Train Diagnostik GmbH, Cronberg, Taunus, Germany, according to manufacturer's protocol.

Statistical methods

Data were entered in Microsoft Excel and checked for completeness. Analysis was done using SPSS version 17. Descriptive analysis was done. For comparing the proportions within groups, Chi-square and Fischer's exact tests was used. For statistical significance, $P < 0.05$ was considered.

RESULTS

In the HIV+TB+group, sitewise distribution of TB were as follows: Pulmonary 31 (including 1 miliary), gastrointestinal 3, lymph nodal 10, disseminated 4, and one each of bone and liver. HLA class I and class II alleles distribution among HIV+TB+con-infected individuals compared with HIV+TB− and HIV−TB− healthy control subjects are represented in Tables 1–4. The findings revealed that HIV+TB+group had statistically higher frequencies of DRB1*13 (34% vs 14%), DRB5 (58% vs 38%), DQB1*06 (56% vs 32%), and B*15 (34% vs16%) compared with only HIV+TB− group [Table 1]. Patients of HIV+TB+group had higher odds of expressing DRB1*13 [OR 3.16, 95% CI; 1.17–8.5], DRB5 [OR 2.25, 95% CI; 1.01–5.02], DQB1*06 [OR 2.70, 95% CI; 1.19–6.11], and B*15 [OR 3.16, 95% CI; 1.17–8.5] compared with only HIV+TB− group [Table 1].

Among the HIV+TB− and HIV− TB− control groups, frequencies of expression of HLA classes differed significantly [Table 2]. Frequencies of expression of A*02 (24% vs 6.7%), B*13 (22% vs 0%), B*51 (22% vs 0%), CW*02 (12% vs 0%), and CW*04 (20% vs 0%) were more in HIV+TB− group, whereas those of DQB1*06 (32% vs 54%) and CW*06 (2% vs 16.7%) were more in the control group [Table 2].

Across the three groups, that is, HIV+TB+, HIV+TB−, and HIV−TB− controls, frequencies of DQB1*04 (94% vs 100% vs 100%) and DQB1*06 (44% vs 68% vs 46%) were statistically different (0.047 and 0.029, respectively) [Table 3]. Compared with control group, A*02 (28% vs 6.7%; OR 5.4, 95% CI; 1.14–25.9) and B*13 (16% vs 0%; OR 1.7, 95% CI; 1.4–2.1) were more expressed in HIV+TB+group and this difference was statistically significant [Table 4].

DISCUSSION

Host genetic factors are known to influence the immunopathogenesis of both TB and HIV. Research has indicated certain HLA types in susceptibility to infection and progress of TB in HIV. Parallels however cannot be drawn between susceptibility to HIV and TB. Exposure to *Mycobacteria* is environmental and much more widespread, whereas exposure to HIV is of chance. Whereas presence or overexpression of a certain HLA allele can be attributable to both susceptibility and disease progression of TB in HIV, lack of a certain HLA allele in HIV infection cannot be attributed to protection from the disease, unless correlated with rate of disease progression.

In the present study, DQB1*06 was over-represented in the HIV+TB+group compared with HIV+TB− group ($P = 0.016$) indicating that DQB1*06 rendered HIV+individuals to develop TB. DQB1*06 was however also found in equal frequencies in the healthy controls and this observation though could be interpreted as a reduced frequency in HIV+TB− group, cannot be taken as having a protective role against HIV infection. Similar trends were observed with DRB1*13, DRB5,

Table 1: Distribution of HLA classes across study participants in HIV+TB+and HIV+TB− groups

HLA allele	Number of subjects (%)		Odds ratio (95% CI)	*P*
	HIV+TB +	HIV+TB−		
DRB1*13	17 (34)	7 (14)	3.16 (1.17-8.5)	0.034
DRB 5	29 (58)	19 (38)	2.25 (1.01-5.02)	0.045
DQB1*06	28 (56)	16 (32)	2.70 (1.19-6.11)	0.016
DQB1*02	12 (24)	21 (42)	0.436 (0.18-1.02)	0.056
B*15	17 (34)	8 (16)	2.7 (1.04-7.03)	0.038
B*51	2 (4)	11 (22)	0.14 (0.03-0.70)	0.007

HIV + TB: HIV + tuberculosis, CI: Confidence interval, HLA: Human leucocyte antigen

Table 2: Distribution of HLA classes across study participants of HIV+TB− and HIV−TB− healthy controls

HLA allele	Number of subjects (%)		Odds ratio (95% CI)	*P*
	HIV+TB−	HIV−TB−		
DQB1*06	16 (32)	27 (54)	0.40 (0.17-0.90)	0.026
A*02	12 (24)	2 (6.7)	4.4 (0.91-21.3)	0.048
B*13	11 (22)	0 (0)	-	0.006
B*51	11 (22)	0 (0)	-	0.006
CW*02	6 (12)	0 (0)	-	0.049
CW*04	10 (20)	0 (0)	-	0.009
CW*06	1 (2)	5 (16.7)	0.10 (0.01-0.92)	0.016

HIV + TB: HIV + tuberculosis, CI: Confidence interval, HLA: Human leucocyte antigen

Table 3: Distribution of HLA classes across study participants of HIV+TB+and HIV+TB− and HIV−TB− healthy controls

HLA allele	Number of subjects (%)			*P*
	HIV+TB+	HIV+TB−	HIV−TB−	
DQB1*04	47 (94)	50 (100)	50 (100)	0.047
DQB1*06	22 (44)	34 (68)	23 (46)	0.029

HIV + TB: HIV + tuberculosis, HLA: Human leucocyte antigen

Table 4: Distribution of HLA classes across study participants of HIV+TB+and control groups

HLA class	Number of subjects (%)		Odds ratio (95% CI)	*P*
	HIV+TB+	Control		
A*02	14 (28)	2 (6.7)	5.4 (1.14-25.9)	0.021
B*13	8 (16)	0 (0)	-	0.021

HIV + TB: HIV + tuberculosis, CI: Confidence interval, HLA: Human leucocyte antigen

DQB1*02, and B15. Because the differences between individual groups lost its statistical significance when compared with healthy controls, it can hence be inferred that the polymorphisms independently did not account for susceptibility to either of the disease though they probably play a role in disease progression once the infection (s) has established in a particular individual.

Previous studies have shown that HLA-DQB1*06:01:01:01 is associated with susceptibility to pulmonary TB as well as development of pulmonary TB in HIV-positive individuals[12,13] and an earlier association with susceptibility to pulmonary TB[15] corroborated the findings in South Indian cohort. DQB1*06:01:01:01 however has been shown to have a protective role against HIV disease progression in Europians.[16] HLA-DRB1*13 has been previously associated with susceptibility to HIV-1 infection in Argentinian population.[17]

HLA-A2 and -B13 were over-represented in both the HIV+TB− and HIV+TB+groups compared with healthy controls. On the other hand, HLA B*51 was over-represented in the HIV+TB− group compared with both HIV+TB+and healthy controls, probably signifying a protective role of HLA B*51 against development of TB in HIV-positive individuals. In a study from North India, HLA-A2 was significantly increased in pulmonary TB (PTB) as compared with controls, and this increase was more pronounced in the sputum-negative patients suggesting its possible role in the mediation of CD8+suppressor T-cell activity against *M. tuberculosis* resulting in limited disease in these patients and hence suggesting a protective role. In a study from Mexico, DQA1*0101, DQB1*0501, and DRB1*0501 were significantly increased in non-immunosuppressed PTB, whereas DRB1*1101 allele was found in HIV-positive subjects. AIDS patients with PTB however did not show any of these relationships, suggesting that the severe acquired immunosuppressive state predisposes individuals to the development of TB despite the lack of genetic susceptibility.[18]

Based on these observations, it can be concluded that various HLA alleles may show either susceptibility or resistance to either of the disease independently but once an individual is exposed to both the pathogens, synergistic role of these alleles in disease progression is likely. Further studies using high-resolution typing and studying actual rate of disease progression instead of studying mere presence or absence of the disease would throw more light on the role of HLA molecules in HIV-TB co-infection.

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