Allelic Distribution of Human Leukocyte Antigen in Patients with Pulmonary Tuberculosis in Baghdad City

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ABSTRACT:

BACKGROUND:

Host genetic factors such as human leukocyte antigens (HLA) and non-HLA genes that are associated with the susceptibility to tuberculosis (TB) will serve as genetic markers to predispose or predetermine the development of the disease.

OBJECTIVE:

The aim of this study is to analyze the association between particular HLA-typing class I and the incidence of pulmonary tuberculosis in Baghdad city.

PATIENTS & METHODS:

Blood samples were collected from one hundred patients; 50 samples from patients with pulmonary tuberculosis referred to the Chest and Respiratory Diseases Institute in Baghdad city and 50 samples from apparently healthy individuals. All samples were submitted to the lymphocytotoxicity test (NIH) and examined in Major Histocompatibility Laboratory in AL- Karama Hospital in Baghdad city.

RESULT:

It was found that HLA (15) expression was significantly higher in recently infected patients with tuberculosis than in the controls ($p<0.01$) and HLA-A (33) was significantly lower in those patients than the controls ($p<0.05$). HLA-A (1) was high significantly lower in historical TB patients than the controls ($p<0.01$). HLA-B (17) was significantly higher in recently infected patients with TB than the controls ($p<0.05$), HLA-B(35) was high significantly lower in historical TB patients than the controls ($P<0.01$). This study concluded that frequencies of HLA-A (15), HLA-B (17), in recently diagnosed pulmonary TB patients were significantly increased compared with those in the control group.

CONCLUSION:

The development of pulmonary tuberculosis infection is partly controlled by genetic factors. Sophisticated techniques such as (PCR) are needed for more assurance to verify this association. Further studies are required to investigate a possible relation between HLA-typing class II and the incidence of pulmonary tuberculosis in Iraq.

KEY WORDS: leukocyte antigens, pulmonary tuberculosis, lymphocytotoxicity.

INTRODUCTION:

Mycobacterium tuberculosis is the causative pathogen for human tuberculosis (TB). Though environmental and socio-economic factors are primarily related, numerous studies have emphasized the importance of host resistance and hereditary susceptibility [1,2,3]. Studies on HLA and susceptibility to tuberculosis have been carried out in various populations as well as families by different groups in different parts of the world. The first report was by Selby et al showed an increased frequency of HLA-B8 in tuberculosis patients in Canada[4]. A large number of studies have also been carried out in various populations [5]. Data from the literature concerning the correlation between tuberculosis and antigens of the HLA system are not consistent and indicate divergences in various ethnic groups [6,7]. Other studies showed an increased frequency of HLA-B5, HLA-B15 and HLA-DR5 in the North American blacks [8, 9], HLA-A2 and HLA-B5 in the Egyptian population [10] and HLA-B27 in the Greek population [11]. A negative association has been reported for HLA-DR6 in American blacks [12].

Several studies of HLA association with pulmonary tuberculosis have been carried out in Chinese [13], Indonesian [14] and Russian patients [15]. A significantly increased frequency of HLA-DR2 was seen in the major studies which have revealed HLA-DR2 association with higher susceptibility to tuberculosis. In a small study of tuberculosis in

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Vietnam, a susceptibility association with the HLA-DQB1 *0503 allele was reported [16]. Another study carried out in Thais revealed the association of HLA-DQB1*0502[17].

Data concerning positive or negative association between HLA alleles and pulmonary tuberculosis from Iraq are scarce. The present study was designed to compare frequencies of respective HLA class I alleles in patients with pulmonary tuberculosis (PTB) and apparently healthy controls in the same ethnic group and to show which HLA type may be involved in the development of pulmonary tuberculosis or association with protection against PTB in a group of Iraqi patients in Baghdad city.

PATIENTS AND METHODS:

Fifty blood samples were collected from patients with PTB in the microbiology laboratories of Chest and Respiratory Diseases Institute in Baghdad during the period from (1st October 2007 to 1st January 2008).These samples were examined at the major histocompodibility laboratory in Al-Karama hospital. The selection of patients was achieved according to clinical and radiological criteria with diagnosis of pulmonary tuberculosis. Numbers of patients group was (50) cases (43 males and 7 females); their ages ranged from (17-55) years with mean age of 33 years. All suspected cases were present with clinical features suggested of pulmonary tuberculosis that's including chronic cough, haemoptysis, chest pain, low grade fever, loss of weight, fatigue, night sweats and all patients were able to produce sputum. Number of control group was (50) apparently healthy individuals (26 males and 24 females); their ages ranged from (17-65) years with mean age of 31 years. These healthy controls had no history or clinical evidence of PTB and any other chronic disease. Five milliliters (ml) of venous blood were collected from each patient as well as controls and immediately transferred in plastic tube containing EDTA then transmitted to the laboratory for examination.

Lymphocytotoxicity test (NIH)

Anti-HLA-ABC sera were used for tissue typing of HLA class I antigens according to the method of (Terasaki and McClelland, 1964) modified by (Dick and Bender, 1984)[18].

The listings of available Anti-HLA sera and test results were available on request according to the diagnostic kit (BAG-Biologische Analysensystem Gmbh Amtsgerichtsstrabe 1-5 D-35423 lich post fach 1152. D-35419 lich, Germany). Test procedure including isolation of lymphocytes from heparinized blood and NIH technique was done according to manufacturer instructions.

Statistical analysis

Descriptive statistics was used for creating statistical tables (observed frequencies, percentages) and creating contingency tables. Inferential statistics was used in order to accept or reject the statistical hypotheses they include: Binomial test for testing the difference between two ratios related to binary nominal responding with pointed their *P*-values, and Chi-square test for testing independency between the two categories factors in the contingency table with pointed their *P*-values. All the statistical analysis was done by using SPSS computer program version 10 and Excel application.

RESULTS:

It was shown that the number of pulmonary TB infections in the males was more than in females. In recently diagnosed infection of PTB there were 34 (68%) males, 6 (12%) females, whereas in historical infection there were 9 (18%) males, 1 (2%) females. The results revealed that age groups (30 – 39) years and (20 – 29) years were more affected in recent and historical infections, respectively. Patients with age below (20 years) showed decreased percentage of infection in recently diagnosed PTB 3 (6%), and 1 (2%) in historical PTB infection.

The results presented in table (1) show a comparison between HLA-A antigen in recently diagnosed PTB and apparently healthy controls. Patients with recently PTB having HLA-A (15) were 9 (18%), whereas no positive results were recorded in controls.

Statistical analysis revealed a high significant difference in HLA–A (15) between recently diagnosed PTB patients and controls ($p<0.01$) that means a positive correlation. On the other hand, apparently healthy controls having HLA-A(33) were more than PTB patients. Significant difference in HLA-A(33) was shown between PTB patients and control group ($p<0.05$) that means a negative correlation .

Table 1: Phenotype frequency of HLA-A antigen in patients with recently diagnosed PTB compared with apparently healthy controls.

Type of HLA-A antigen	Recent PTB		Controls		P-value	Significance
	Positive No.	%	Positive No.	%		
HLA – A (15)	9	18	—	—	0.002	HS(p<0.01)
HLA – A (9)	17	34	13	26	0.112	NS(p>0.05)
HLA – A (10)	1	2	4	8	0.156	NS(p>0.05)
HLA – A (1)	15	30	21	42	0.095	NS(p>0.05)
HLA – A (11)	10	20	2	4	0.016	S(p<0.05)
HLA – A (30)	4	8	4	8	0.273	NS(p>0.05)
HLA – A (2)	9	18	16	32	0.061	NS(p>0.05)
HLA – A (3)	10	20	6	12	0.122	NS(p>0.05)
HLA – A (33)	1	2	8	16	0.018	S(p<0.05)

Table 2 shows a comparison between HLA-B in recently diagnosed PTB and controls. Patients with recently PTB having HLA-B (17) were more than controls. There was a significant difference in HLA–B (17) between recently TB patients and controls ($p<0.05$) that means a positive correlation. Patients with recently PTB having HLA-B (35) were less than controls. Significant difference in HLA-B(35) was shown between patients and control group ($p<0.05$) that means a negative correlation .

Table 2: Phenotype frequency of HLA-B antigen in patients with recently diagnosed PTB compared with apparently healthy controls.

Type of HLA-B antigen	Recent PTB		Controls		p-value	Significance
	Positive No.	%	Positive No.	%		
HLA – B (35)	7	14	15	30	0.047	S(p<0.05)
HLA – B (B6)	4	8	9	18	0.087	NS(p>0.05)
HLA – B (17)	7	14	1	2	0.031	S(p<0.05)
HLA – B (55)	3	6	—	—	0.0125	NS(p>0.05)
HLA – B (37)	4	8	—	—	0.063	NS(p>0.05)
HLA – B (16)	1	2	6	12	0.055	NS(p>0.05)
HLA – B (8)	7	14	8	16	0.196	NS(p>0.05)
HLA – B (13)	3	6	6	12	0.164	NS(p>0.05)
HLA – B (44)	1	2	—	—	0.5000	NS(p>0.05)
HLA – B (41)	1	2	4	8	0.156	NS(p>0.05)

Table 3 shows a comparison between HLA-A in historically diagnosed PTB and controls. Patients with historically diagnosed PTB having HLA-A (1) were less than controls. Highly significant difference in HLA-A(1) was shown between patients and control group ($p<0.01$) that means a negative correlation . The same result was observed with HLA-A (9).

Table 3: Phenotype frequency of HLA-A antigen in patients with historically diagnosed PTB compared with apparently healthy controls.

Type of HLA-A antigen	Historically diagnosed PTB		Controls		P-value	Significance
	Positive No.	%	Positive No.	%		
HLA – A (11)	1	2	2	4	0.3750	NS(p>0.05)
HLA – A (30)	2	4	4	8	0.2344	NS(p>0.05)
HLA – A (1)	1	2	21	42	0.0000	HS(p<0.01)
HLA – A (32)	1	2	1	2	0.5000	NS(p>0.05)
HLA – A (2)	4	8	16	32	0.0046	S(p<0.05)
HLA – A (24)	1	2	1	2	0.5000	NS(p>0.05)
HLA – A (3)	2	4	6	12	0.1094	NS(p>0.05)
HLA – A (10)	2	4	4	8	0.2344	NS(p>0.05)
HLA – A (9)	1	2	13	26	0.0009	HS(p<0.01)
HLA – A (34)	1	2	1	2	0.5000	NS(p>0.05)

Table 4 shows a comparison between HLA-B in historical PTB and controls. Patients with historically diagnosed PTB having HLA-B (35) were less than controls. Highly significant difference in HLA-B(35) was shown between patients and control group (p<0.01) that means a negative correlation .

Table 4: Phenotype frequency of HLA-B antigen in patients with historically diagnosed PTB compared with apparently healthy controls.

Type of HLA-B antigen	Historically diagnosed PTB		Controls		P-value	Significance
	Positive No.	%	Positive No.	%		
HLA-B (12)	1	2	—	—	0.5000	NS(p>0.05)
HLA-B (51)	2	4	2	4	0.3750	NS(p>0.05)
HLA-B (13)	1	2	4	8	0.0439	NS(p>0.05)
HLA-B (B4)	2	4	4	8	0.2344	NS(p>0.05)
HLA-B (44)	1	2	—	—	0.5000	NS(p>0.05)
HLA-B (17)	1	2	1	2	0.5000	NS(p>0.05)
HLA-B (35)	2	4	15	30	0.0010	HS(p<0.01)
HLA-B (5)	2	4	1	2	0.3750	NS(p>0.05)
HLA-B (7)	1	2	2	4	0.375	NS(p>0.05)
HLA-B (49)	1	2	8	16	0.018	S(p<0.05)

DISCUSSION:

One of the first reports of an association between the HLA-class I antigens and pulmonary tuberculosis was that by Selby et al [4]. The association of tuberculosis with HLA class II antigens may be more relevant than that with HLA-A, B, C antigens because cell-mediated immunity is known to be involved in the pathogenesis of tuberculosis [5].

This study demonstrated that frequencies of HLA-A(15) expression was high significantly higher in recently patients with tuberculosis than
in the tested group of healthy controls (p<0.01) , HLA-A(33) antigen was significantly lower in recently diagnosed patients with tuberculosis than the controls (p<0.05). The results obtained suggest that the presence of HLA-A(15) can extend the risk of developing tuberculosis, whereas HLA-A (33) antigen occurrence was significantly more rare in pulmonary tuberculosis than in healthy individuals. It was observed that HLA-A(1) was significantly lower in historical PTB patients than the controls (p<0.01). HLA-B(17) was significantly higher in recently diagnosed PTB patients than controls (p<0.05). HLA-B(35) was high significantly lower in historical PTB patients than the controls. The results obtained suggest that the presence of HLA-A(15) and HLA-B(17)

antigens can extend the risk of developing tuberculosis, while HLA-A(33) in recently PTB patients and HLA-A(1), HLA-B(35) in historical PTB patients were significantly more rare than in healthy individuals.

It was shown from another study conducted on (44) Iranian sputum smear positive pulmonary TB patients that the frequencies of HLA-B17 and DR14 antigens were higher in TB patients than controls, and the frequencies of HLA-A26 and –B27 were higher in healthy controls than patients [19].

Another study conducted in Italy demonstrated that there was no significant association between HLA alleles and tuberculosis in the population of recently diagnosed TB patients. On the contrary, among the historical TB patients there was a strong association with an increased frequency of the HLA-DR allele alone and/or in the presence of the HLA-B14 allele, as well as with decreased frequency of the HLA-A2+, HLA-B14, HLA- DR allele association [20].

These differences were mainly due to the ethnic variation in HLA phenotypes as well as the specificity and sensitivity of the applied methods[21]. The analysis of the association between particular class II HLA antigens and relative susceptibility or resistance to tuberculosis has been reported rarely and, further determination more precise modern molecular techniques, based particularly on the method of the polymerase chain reaction PCR, should be used [5].

Our study concluded that the patients with recently diagnosed pulmonary tuberculosis, as compared with control group, HLA-A15 antigen was shown to be significantly more frequent. It can be assumed that the presence of this antigen may be connected with a greater risk of pulmonary tuberculosis. Furthermore, in the group of patients, as compared with the control group, the occurrence of HLA-B35 (in recently diagnosed TB), HLA-A1, HLA-A9, and HLA-

B35 (in historical TB) was significantly decreased and the lower risk can be connected to

its relation with the genes of insusceptibility to tuberculosis.

On the best of our knowledge, sophisticated techniques such as polymerase chain reaction (PCR) have not yet been applied in Baghdad for the study of this correlation. Consequently, this technique needs to be performed for more assurance to verify association of HLA phenotypes with PTB. Further studies are advised to be conducted on haplotypes of class-II antigens, rather than phenotypes of class I antigens in our country.

CONCLUSION:

The development of pulmonary tuberculosis infection is partly controlled by genetic factors. Sophisticated techniques such as (PCR) are needed for more assurance to verify this association. Further studies are required to investigate a possible relation between HLA-typing class II and the incidence of pulmonary tuberculosis in Iraq.

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