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# HLA antigen profile in pulmonary tuberculosis patients & their spouses

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**HLA-A, -B, -DR and -DQ antigen profile was studied in pulmonary tuberculosis patients (n=209) and their spouses (family contacts; n=50) and healthy volunteers (n=72). An increased frequency of HLA-A-10, B7, B15, DR2 and DQ1 was seen in the pulmonary-TB (PTB) patients when compared to the total control subjects (n=122). However, a significant increase was seen only with HLA-DR2 ($P < 0.001$; $Pc < 0.01$; Relative Risk 2.3) and -DQ1 ($P < 0.005$; $Pc<0.015$; Relative Risk 2.8). Among the spouses and the corresponding patients, a similar increase of HLA-DR2 was seen. A decreased frequency of HLA-A19, B8, B17, B35, DR5 and DR6 were seen in PTB as compared to control groups. The present study suggested that HLA-DR2 and DQ1 genes/gene products may be associated with the susceptibility to tuberculosis either alone or in combination with other HLA or non-HLA genes.**

**Key words** Antigen profile - HLA-A, B, DR & DQ - pulmonary TB patients - spouses (family contacts)

Pulmonary tuberculosis is a granulomatous lung disease caused by *Mycobacterium tuberculosis*. Susceptibility to tuberculosis has been suggested to be multifactorial. Though environmental and socio-economic factors are primarily related, numerous studies have emphasised the importance of host resistance and hereditary susceptibility[1,2].

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 by Selby *et al* [3] showed an increased frequency of HLA-B8 in tuberculosis patients in Canada. A large number of studies have also been carried out in various populations[4-8]. All these studies suggest that different HLA antigens are associated with pulmonary tuberculosis in different populations.

Interestingly, studies carried out in Asian populations revealed an increased frequency of HLA-DR2 in Indian[8-10], Indonesian[11] and Russian[12] pulmonary tuberculosis patients. Further, DR2 subtyping revealed no deviation in the frequency of common DR2 subtypes (1501 and 1502) in pulmonary TB (PTB), suggesting that the whole DR2 molecule or its closely linked gene(s) may be involved in PTB[10]. Earlier HLA studies in pulmonary TB in India were based on general population or hospital contacts as controls, where the gene frequency of HLA-DR2 is high[13].

The present study was carried out to delineate HLA-DR2 association with pulmonary TB using the spouses of the pulmonary TB patients considered to be a resistant population as the control population.

## Material & Methods

*Study subjects :*

(i) Active tuberculosis patients (ATB) – Patients attending the Tuberculosis Research Centre (TRC), Chennai, with respiratory symptoms and radiographic

abnormalities suggestive of pulmonary TB were studied. These patients had sputum positive for *M. tuberculosis* by smear and culture.

(ii) Inactive tuberculosis patients (ITB) – These patients had had active pulmonary TB and had received short course chemotherapy for 6-8 months, 10-15 yr earlier. At the time of blood sample collection all the patients were in the quiescent stage of the disease.

(iii) Controls – The group comprised spouses of the ITB patients living together for 10-15 yr and spouses of ATB patients (living together for 1-2 yr at the time of assessment) and staff members working in TRC for more than 3 yr.

There was no consanguinity between the patients and spouses. The other control subjects were not related to any of the patients. All the family contacts and other control subjects were clinically normal at the time of assessment. All subjects belonged to the same ethnic origin and the patients and controls were randomly selected from the same ethnic group and were mainly Tamil speaking south Indians living in and around Chennai. The study group comprised all communities belonging to the same ethnic group.

Subjects included in this study were 61 active pulmonary tuberculosis patients, 148 inactive tuberculosis patients (cured/quiescent) (total pulmonary TB patients 209) and 122 healthy controls including 50 family contacts (spouses) and clinicians, social workers, health visitors, laboratory volunteers and other staff (n=72) working in TRC for more than 3 yr. Of the 50 family contacts studied, six were spouses of ATB patients and 44 the spouses of ITB patients.

Among the total PTB patients, 164 were males and 45 were females. The mean age (±SE) was 39.8±0.8 yr for males and 35.9±1.4 for females. Among the family contacts 13 were males and 37 were females. The mean age was 35.5±2.2 for males and 35.8±1.3 for females. Among the TRC volunteer control subjects, 40 were males and 32 were females. The mean age was 40.3±1.2 for males and 39.1±1.8 for females.

Twenty millilitres of peripheral blood in heparin (20 units/ml) was drawn from each patient. The mononuclear cells were separated using ficoll-hypaque density gradient centrifugation and washed in TC 199 tissue culture medium and used for HLA typing. The nylon wool non-adherent cells (enriched T cells) were used for HLA-A, -B typing and the adherent population (enriched B cells) were used for HLA-DR and -DQ typing. HLA phenotyping was performed by a two stage microlymphocytotoxicity assay[14]. The antisera for HLA-A, -B, -DR and -DQ antigens were purchased from Biotest, Germany. In addition to the above sera, well established sera of local origin and sera procured from co-operative HLA laboratories in India were also used. At least three sera were included for each specificity studied.

The frequencies of HLA alleles in patients and controls were determined by direct allelic count method. A 2 x 2 contingency table was constructed and Chi-square analysis with Yates correction ($X^2_y$) was performed to find out the significance for HLA antigens. Significant P-values were further corrected (Pc) by multiplying the P-value for the number of antigens studied for each locus. Relative Risk (RR) values were calculated to find out the strength of association of HLA antigens and the occurrence of pulmonary tuberculosis.

## Results & Discussion

Among the active-TB and inactive-TB patients, no difference was observed on the phenotype frequency of various HLA antigens. The data were amalgamated and analysed as one group as pulmonary TB patients.

An increased frequency of HLA-B7, -B15, -DR2 and -DQ1 was seen in pulmonary tuberculosis patients than the total control subjects (Table). The frequencies of B7 and B15 were only trends and not significant when corrected for the number of antigens studied for that locus (HLA-B7 : PTB vs total controls: $P < 0.05$; $P_C > 0.5$). A similar trend was also observed in spouses (family contacts). A significant increase in the antigen frequency of HLA-DR2 and -DQ1 was seen in pulmonary tuberculosis patients than control subjects (total control vs PTB:HLA-DR2 : $P < 0.001$; $Pc < 0.01$; HLA-DQ1: $P < 0.005$; $Pc < 0.015$). An increase in the frequency of HLA-DR.2 was observed in the PTB patients as compared to their spouses ($P < 0.05$), while the frequencies of HLA-DR5 and -DR6 were decreased

**Table.** Percentage phenotype frequency of deviated HLA-A, -B, -DR and - DQ antigens of control (total) subjects, PTB patients (total), spouses (family contacts) and their corresponding PTB patients

| HLA | % PF | | | |
|---|---|---|---|---|
| | Control n=122 | PTB patients n=209 | Spouses** n=48 | PTB patients** n=48 |
| A10 | 7.4 | 13.9 | 8.3 | 14.6 |
| A19 | 32.0 | 23.0 | 27.1 | 18.8 |
| B7 | 17.2 | 27.8 | 12.5 | 23.0 |
| B8 | 7.4 | 3.8 | 10.4 | 2.1 |
| B15 | 9.8 | 16.3 | 8.3 | 14.6 |
| B17 | 29.5 | 18.7 | 22.9 | 12.5 |
| B35 | 28.7 | 19.1 | 25.0 | 20.1 |
| DR2 | 29.5 | 48.8 | 25.0 | 47.9 |
| DR5 | 19.7 | 16.3 | 22.9 | 8.3 |
| DR6 | 19.7 | 17.7 | 29.2 | 12.5 |
| DQ1 | 68.0 | 85.6 | 70.8 | 83.3 |

I. Total controls vs total PTB patients
   i. HLA-B7 : $P < 0.05$; $Pc > 0.5$;
   ii. HLA-B17 : $P < 0.025$; $Pc > 0.30$; B35 : $P < 0.05$; $Pc > 0.5$;
   iii. HLA-DR2 : $P < 0.001$; $Pc < 0.01$; DR 2 RR value 2.3;
   iv. HLA-DQ1 : $P < 0.005$; $Pc < 0.015$ RR value 2.8.

II. Spouses vs corresponding PTB patients
   i. HLA-B8 : $P < 0.05$;
   ii. HLA-DR2 : $P < 0.05$; RR value : 2.7;
   iii. HLA-DR5 : $P < 0.025$;
   iv. HLA-DR6 : $P < 0.025$

** HLA not done for two patients and the corresponding spouses were not included (total number of spouses studied n = 50)

in the patients as compared to the spouses (DR5 : $P < 0.025$; DR6 : $P < 0.025$). This trend of decreased frequency of DR5 and DR6 was not seen in the PTB patients as a total group when compared to controls as a total group.

Previous investigations of HLA and disease association in pulmonary tuberculosis patients showed a varied picture in different populations, mainly because of the geographical distribution of the antigens and the ethnic background of the populations[3-7]. However, studies carried out in Asian region especially India[8-10]; Indonesia[11] and Russia[12] showed a definite association of HLA-DR2 and -DQ1 with pulmonary tuberculosis. It is well established that DR2 is in linkage disequilibrium with DQ1.

The association of HLA-DR2 with the severity of pulmonary tuberculosis in Indian population has been emphasized. In south Indian patients with PTB, DR2 has been shown to be more strongly associated with far-advanced, smear positive cases than with cases of minimal and moderate radiographic lung lesion[8]. It has been shown that DR2 is associated with patients with chronic fibrocavitory disease who failed to react to PPD in skin test[12]. Further, high frequency of DR2 among north Indian PTB patients was seen in the drug-failure group who received multiple drugs compared with the frequency of healthy controls and drug responders[10]. Though the severity of the disease is associated with HLA-DR2, our earlier study on bacteriological relapse in pulmonary tuberculosis patients treated with short chemotherapy did not reveal any deviation in the DR2 frequency from that of quiescent patients[15].

It has been shown that PTB patients bearing DR2 antigen had higher levels of antibodies to *M. tuberculosis* than other patients[12]. Further, there is evidence for an immune spectrum in tuberculosis wherein the cell-mediated and humoral immunity are mutually exclusive at opposing ends of the spectrum[16]. Our recent study reveals that active tuberculosis patients with DR2 showed a trend towards a higher antibody response to *M. tuberculosis* antigen, suggesting that the higher antibody titre may suppress the CMI response in DR2 positive patients (unpublished). Further, our earlier study revealed a decreased level of plasma lysozyme (lysosomal enzyme) in the DR2 positive active PTB patients as compared to DR2 negative patients[17]. The data suggest that susceptibility to pulmonary tuberculosis is not only influenced by HLA-DR2 genes/gene products alone but also by other non-HLA gene factors.

Recently, the association of multi candidate genes has been suggested for various infectious diseases[18]. A study carried out in north Indian PTB patients revealed that transporter associated with antigen-processing gene TAP2 is associated with PTB along with HLA-DR2[19]. Recently, our studies on non-HLA gene polymorphism in pulmonary tuberculosis

revealed that functional mutant homozygotes of mannose binding protein genes are associated with pulmonary tuberculosis independent of HLA-DR2 (unpublished data). This suggests that HLA-DR2 genes/gene products in combination with other MHC or non-MHC genes, play a major role in susceptibility to pulmonary tuberculosis.

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