Study the HLA Genotyping in Pulmonary Tuberculosis Patients in Babylon Province-Iraq

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Abstract

This study aimed to investigate the role of Human Leucocyte Antigen (HLA-DRB1) alleles in susceptibility to pulmonary tuberculosis in Babylon/Iraq population. Whole blood samples collected from 50 pulmonary tuberculosis patients who are admitting to consultant clinic for respiratory diseases in Hilla – Babylon province during the period from February 2016 to February 2017. Out of the pTB patients, there were 27 males and 23 females, the patients age range was 12-80 years. Sequence specific oligonucleotide (SSO) was used to genotyping HLA-DRB1. The distribution of HLA-DRB1 genotypes in 50 patients with pulmonary tuberculosis and 50 healthy controls studied. HLA-DRB1*13 was a significantly increased in pulmonary tuberculosis patients 48% as compared with healthy controls 16% while HLA-DRB1*15 was a significantly increased in healthy controls 24% as compared with pulmonary tuberculosis patients 6%. The genotyping of all HLA-DRB1 alleles found more common in females 77% than males 23% from total number of patients. Also, HLA-DRB1*13 was more common in females 75% than males 25% with pulmonary tuberculosis patients while HLA-DRB1*15 was common in females 100% and no appeared in males.

Keywords: Pulmonary Tuberculosis, HLA-DRB1, SSO.

Introduction

Tuberculosis (TB) caused by Mycobacterium tuberculosis (Mtb), remain a leading cause of morbidity and mortality globally [1].

Estimated 9.0 million cases, 5.7 million new cases, and 1.5 million deaths were attributing to TB in 2013 [2]. Iraq is one of the countries with a relatively high rate for TB incidence (64/100,000) and low case finding rate (48%). The WHO assumed that the incidence rate of TB in Iraq is constant. Iraq has a higher incidence of TB than majority of neighboring countries (WHO, 2011).

Several risk factors such as diabetes mellitus, HIV-infected individuals smoking under-nutrition, alcohol, and host immunogenetic factors were associated with susceptibility to TB. Host genetic factors were strongly associated with the development of TB as hardly 5% to 10% of MTB-infected persons develop the disease.

The outcome of TB infection may depend on host genetic factors, as well as environmental and bacterial factors [3]. Numerous studies have associated alleles of the Human Leukocyte Antigen (HLA) class II to TB. HLA class II molecules have crucial role for modulating the adaptive immune response, as well as their association with TB, has been described [4].

HLA class II molecules expressed by antigen-presenting cells and reactive of lymphocytes to class II molecules express CD4 and often helper T cells [5]. Host factors associated with TB pathogenesis complex and a number of genes contribute to initiation and orchestration of the immune response to TB [6].
The human leukocyte antigen (HLA) gene family, i.e., the major histo-compatibility complex (MHC) in humans, plays an important role in immune modulation and is essential in initiating an efficient cell mediated immune response. The HLA system is highly polymorphic due to selective influence by infectious diseases, and HLA polymorphisms may influence antigen presentation specificity by modifying peptide-binding motifs. Increased binding of the pathogen peptide to binding motifs of the MHC leads to enhanced immunogenicity compared to weak MHC-peptide binding [5].

Several studies on the role of HLA II alleles in conferring resistance or susceptibility to TB have been producing conflicting results [7]. There are now numerous studies from various geographical and ethnic settings on the relation between HLA and TB, and several HLA loci and/or alleles have been associated with both susceptibility and resistance to TB. Human leukocyte antigen (HLA) typing, utilizing the sequence-specific oligonucleotide (SSO) and sequence-specific primer (SSP) technologies, has been in routine use in many tissue typing laboratories worldwide for more than 20 years since the development of the polymerase chain reaction [8].

Hybridization of PCR-amplified DNA with sequence specific oligonucleotide (SSO) probes was the first molecular typing method used to identify HLA Class II alleles. The SSO technique has proven to be very robust, reliable and accurate. Good amplification forever gives a clean and clear-cut SSOP hybridization, whereas almost all of problematic typing results regarded to poor amplification. In a quality control exercise, the percentage of correct HLA typing was 99.8% for HLA-DR [9].

Materials

Population of study-included patients with pulmonary tuberculosis (pTB) admitted to consultant clinic for respiratory diseases in Hilla – Babylon province during the period from February 2016 to February 2017. Out of the pTB patients, there were 27 males and 23 females the patient’s age range was 12-80 years. Exclusion Criteria: Any patients with DM, malignancy, allergic and pregnant women excluded from this study. In addition to fifty apparently healthy control groups who had no history of pulmonary Tuberculosis.

Collection of Samples

The human DNA extraction for genetic studies, 2ml of the whole blood samples were transferred to EDTA tube and mixed well for several times. After that, the sample was stored at 4 °C in the refrigerator until the process of human DNA purification and genetic studies carried out.

In this study, the collected samples; both blood from patients and controls were subjected to DNA extraction procedure. It made according to protocols recommended by manufacturer (Gene aid-Korea). DNA abstract was stored at 2-8°C For while using it.

Methods

Extraction of Genomic DNA from Fresh Blood Sample

Fifty blood samples (50) patients utilized for detection of genetic susceptibility and (50) healthy; fresh venous blood samples obtained from them. DNA extraction protocol according to the instructions of Manufacturer Company (Gene aid - Korea).

His to Spot SSO System

Description of Product

The system of HISTO SPOT SSO is an in-vitro diagnostic test for tissue typing of HLA alleles on basis of molecular genetics and provides resolution-typing result from low to medium. It consists of the MR.SPOT processor, the HISTO MATCH interpretation software, the HISTO SPOT typing kit and the HISTO SPOT reagent kit.

The HISTO SPOT typing kits contain all elements required for the PCR reaction and test wells with immobilized sequence-specific oligonucleotide probes for the detection of the PCR products. The HISTO SPOT reagent kits have the reagents for the hybridization and detection and utilized in combination with HISTO SPOT typing kits. The MR.SPOT processor is specifically design to be utilized with the HISTO SPOT kits with the purpose of process between 1 and 96 samples, automating the progression from hybridization, detection through to result
interpretation. The HISTO MATCH software is essential for interpretation of the results.

**Statistical Analysis**

The data were analyzed with a chi-square and t-student tests, and the level of significance was set at p<0.05 [10]. Relative risk (RR) was calculated as follow equation [11].

**Ethical Approval**

Written and verbal consent were obtained from each subjects participated in this study. Moreover, this study was approved by ethical research committee in collage of medicine, Babylon University and Babylon health directorate.

### Results and Discussions

**HLA-DRB1 Genotyping for Pulmonary Tuberculosis Patients**

The HLA-DRB genotyping achieved for 50 PTB patients and 50 healthy controls by using SSO system. The HLA-DRB1 allele frequency for PTB patients and healthy controls shown in table (4-1). Allele frequencies of DRB1*07 and DRB1*13 showed significant (P< 0.05) increase in PTB patients compared with controls. While allele frequencies of DRB1*15 was significant (P< 0.05) increased in controls compared with patients. All other genotypes of HLA-DRB1 (*03,*04,*08,*10 and *11), showed no significant (P> 0.05) for all studied groups.

<table>
<thead>
<tr>
<th>HLA-DRB Allele</th>
<th>PTB patients (n=50) Frequency</th>
<th>Controls (n=50) Frequency</th>
<th>RR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Positive (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*03</td>
<td>18 36</td>
<td>16 32</td>
<td>1.19</td>
<td>0.732</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>21 42</td>
<td>12 24</td>
<td>2.29</td>
<td>0.117</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>15 30</td>
<td>4 8</td>
<td>4.92</td>
<td>0.012*</td>
</tr>
<tr>
<td>DRB1*08</td>
<td>0 0</td>
<td>4 8</td>
<td></td>
<td>0.180</td>
</tr>
<tr>
<td>DRB1*10</td>
<td>0 0</td>
<td>4 8</td>
<td></td>
<td>0.180</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>9 18</td>
<td>8 16</td>
<td>1.15</td>
<td>0.808</td>
</tr>
<tr>
<td>DRB1*13</td>
<td>24 48</td>
<td>8 16</td>
<td>4.84</td>
<td>0.005*</td>
</tr>
<tr>
<td>DRB1*15</td>
<td>3 6</td>
<td>12 24</td>
<td>0.20</td>
<td>0.020*</td>
</tr>
</tbody>
</table>

In this study showed an association between HLA- DRB1*07 and DRB1*13 with susceptibility to PTB in Babylon province. Several HLA-DRB1 alleles were accounted to relate with risk of TB, in spite of some inconsistency between different ethnic groups. The recent meta-analysis that was took in consideration the ethnicity of each case-control study proposes that, in Asian populations, the DRB1*08: 03 and HLA- DRB1*15 alleles present an increased risk of TB, whereas DRB1*03 and DRB1*07: 01 are protective.

When including other populations besides Asian, namely European and North and South American, this same meta-analysis was found consistent results with increased risk of TB for individuals with DRB1*08: 03 and HLA-DRB1*15 alleles. On the other hand, in this non-grouping evaluation, beside DRB1*03 and exclusion of DRB1*07: 01, other alleles were emerged as conferring the protection against TB, namely DRB1*11, DRB1*11: 03 and DRB1*12:02.

The association with HLA-DRB1*07 was reported in Iranian patients with pulmonary tuberculosis where HLA-DRB1*07 allele apparently was conferred susceptibility to TB [5]. Studying of [12] was reported an association between HLA-DRB1*13 with susceptibility to PTB in Iraq population. In South Africans was observed a significant
interaction between HLA-DRB1*1302 allele and susceptibility to TB [13]. By contrast, In Polish population HLA-DRB1*13 alleles apparently were conferred resistance to TB [5].

In this study found that HLA-DRB1*15 associated with protection against pTB in Babylon province. This result matched with that recorded by [12] who mentioned that this allele associated with protection against pTB in Iraqi population. By contrast [14] was found association of HLA-DRB1*15 with pTB in north Chinese. The other HLA allele was reported to be related with susceptibility to pTB, HLA-DRB1*4 were associated with pTB in population of Amazon Brazilian [15] and Syrian population [16].

HLA-DRB1 Alleles Distribution according to Sex for Patients Groups

Table 2: HLA-DRB1 alleles distribution according to sex for patients groups

<table>
<thead>
<tr>
<th>HLA-DRB1 Alleles</th>
<th>Sex</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Total of all alleles</td>
<td>69</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>77%</td>
<td>23%</td>
</tr>
</tbody>
</table>

*= Significant (P< 0.05)

Table 3: Comparison between sex and HLA-DRB1*07 allele for patients groups

<table>
<thead>
<tr>
<th>HLA-DRB1*7</th>
<th>Patients Groups(n=50)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>80%</td>
<td>20%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

*= Significant (P< 0.05)

Table 4: Comparison between sex and HLA-DRB1*13 allele for patients groups

<table>
<thead>
<tr>
<th>HLA-DRB1*13</th>
<th>Patients Groups(n=50)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>75%</td>
<td>25%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

*= Significant (P< 0.05)

Table 5: Comparison between sex and HLA-DRB1*15 allele for patients groups

<table>
<thead>
<tr>
<th>HLA-DRB1*15</th>
<th>Patients Groups(n=50)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

*= No Significant (P > 0.05 )

HLA genotyping was studied for other diseases, the narcolepsy was a higher risk in females carrying DRB1*1501 than in males in Mexican patients [17]. As well as [18] was found that the association of HLA-DRB1*0301 with breast cancer in women. Liu et al [19] was found that HLA-DRB1*07, HLA-DQB1*02 and HLA-DQB1*03 were the differentially expressed gene in HPV16 infected women with advanced cervical cancer.

While [20] was found a significant association between the HLA-B7-HLA-DRB1*1501-HLA-DQB1*0602 haplotype and increased risk of HPV type 16 and 18 infection in women. The study of [21] was found that HLA-B*57 frequency in cohort of HIV was significantly higher among females without tuberculosis (21.6%, 19/88) than males (1.7%, 1/59) thus was suggests that HIV positive women with HLA-B*57 have less incidence of TB as compared to the males.
Studying of [22] on Graves' disease in Iraq was appeared that the Genotyping HLA-DRB1*03 allele was more common in females (n=25) (41.7%) than males (n=8) (24.2%) from a total number (n=33) of patients and control groups.

The World Health Organization reported that nearly twice as many men as women have been diagnosed with TB globally. The possible impact of sexual hormones and the differences between men and women in immunological reactions have also proposed as factors causing men to be more susceptible to Mycobacterium tuberculosis infection [23]. TB prevalence in males versus females varies widely among countries, and in some as prevalence is higher among women. In Pakistan, the majority of TB patients across all age groups are females, and in Peru, a higher prevalence of TB found in women of reproductive age compared to males in the same age range. These countries are an exception, however. In Africa and Eastern Europe, males seem to represent the majority of adult patients with TB [24].

Conclusions
- The HLA-DRB1*07 and DRB1*13 alleles are most common in pTB.
- The HLA-DRB1*15 alleles are most common in apparently healthy controls.

References


