ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 09, pp.8331-8334, September, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DISTRIBUTION OF HLA-DQB1 ALLELES IN A SAMPLE OF IRAQI PULMONARY TUBERCULOSIS PATIENTS

[1*]Ali H. Ad'hiah, [2]Khulood K. Hasan, [3]Ahmed Asmar and [4]Hala K. Alsamray

[1]Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq
[2]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[3]Institute of Chest and Respiratory Diseases, Baghdad, Iraq
[4]DNA Research Centre, Al-Nahrin University, Baghdad, Iraq

ARTICLE INFO

Article History:
Received 05th June, 2014
Received in revised form
23rd July, 2014
Accepted 10th August, 2014
Published online 18th September, 2014

Key words:

Pulmonary tuberculosis,
HLA-DQB1 alleles.

ABSTRACT

Studies carried out in various populations have reported an association between some HLA specificities and susceptibility to pulmonary tuberculosis (PTB). In this study, the distribution of class II HLA-DQB1 alleles was determined in 94 PTB patients and 80 apparently healthy subjects from Iraqi population by single specific primer-polymerase chain reaction (SSP-PCR). Out of the five encountered HLA-DQB1 alleles, DQB1*03 showed a significant (P = 0.005) increased percentage frequency in PTB patients compared to controls (71.3 *vs*. 50.0%). The RR of such positive association was 2.48; while the EF was 0.43. The present study suggests that HLA-DQB1*03 allele may be associated with susceptibility to PTB in Iraqi population.

INTRODUCTION

Tuberculosis (TB) remains a major global health problem, causing ill-health among millions of people each year. The latest estimates revealed that there were 8.6 million new *Mycobacterium tuberculosis* infected cases in 2012 and 1.3 million TB deaths (WHO, 2013). In Iraq and based on 2012 estimates, the incidence of TB was 45 per 100,000 populations per year, but as a result of deteriorating socioeconomic conditions during the last decade, the incidence is expected to rise (WHO, 2014). The infection usually takes place in lungs (pulmonary TB, which is the most common), and begins as an alveolar inflammatory reaction that progresses to a typical delayed type granulomatous reaction (Santucci *et al*., 2011). The well-established observation that only 10% of the population infected by *M. tuberculosis* will develop TB has led to an intense search for factors that determine its development in individuals; therefore a genetic component that confers resistance or susceptibility has been suspected (Schurr *et al*., 2011). Genetic studies demonstrated a significant hereditable component in variations observed between individuals in their response to *M. tuberculosis*. Evidence includes twin studies that showed a higher concordance rate in monozygotic twins than in dizygotic twins (Qu *et al*., 2011), and racial differences in susceptibility to infection by the pathogen have also been documented (Nahid *et al*., 2011). Candidate gene approach and association studies have identified various host genetic factors that affect TB susceptibility, especially those genes that control immunological functions (Möller and Hoal, 2010). Major histocompatibility complex (MHC) harbors some of these genes and studies demonstrated their substantial and central role in controlling TB infection (Robinson *et al*., 2011). There are a number of genetic characteristics of HLA genes, but most importantly they are highly polymorphic (Sadki *et al*., 2012). The other characteristic feature of HLA alleles is their association with diseases. These studies have demonstrated that certain HLA alleles showed either positive (increased frequency) or negative (decreased frequency) associations with different infectious diseases (viral, bacterial, fungal and parasitic infections), in addition to autoimmune diseases (Blackwell *et al*., 2009).

Tuberculosis has been a subject of HLA-disease association studies, and alleles encoded by HLA genes have been suggested to play crucial roles in host susceptibility or resistance to TB infection, especially those alleles of HLA-class II genes (Yim and Selvaraj, 2010). HLA-DQB1 alleles play an important role in the modulation of the immune response, and a possible association between DQB1 alleles and PTB has been examined in different ethnic groups, but the results have been inconsistent (Dubaniewicz *et al*., 2003; Lombard *et al*., 2006; Wang *et al*., 2001; Wu *et al*., 2013). In

Corresponding author**: **Ali H. Ad'hiah
Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq.

Iraqi patients, a significant association with DR1, DR8 and DQ3 has recently been described, but at the serological level (Mohammed *et al*., 2014). The specific aim of this study was to investigate the associations of HLA-DQB1 gene polymorphism with PTB in Iraqi population to determine which DQB1 alleles may be involved in susceptibility or resistance to PTB.

MATERIALS AND METHODS

Study population

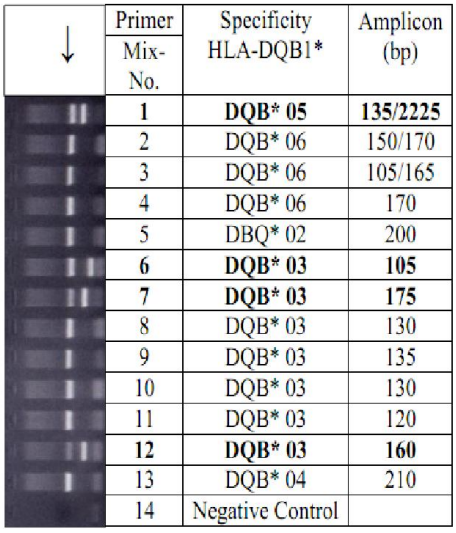
Ninety four Iraqi Arabs patients with PTB were enrolled in the study. They were referred to the Institute of Chest and Respiratory Diseases in Baghdad for diagnosis and treatment during the period May-October 2012. Patients included were clinically and radiologically diagnosed for PTB and confirmed by conventional sputum smear and culture for *M. tuberculosis*. Among the patients, 70 were males (mean age ± S.E. = 43.5 ± 1.7 years) and 24 were females (mean age ± S.E. = 36.6 ± 2.6 years). A control sample of 80 clinically healthy individuals with no signs, symptoms or history of previous mycobacterial infection was also included. They were blood donors and matched patients for gender (60 males and 20 females) and ethnicity (Iraqi Arabs). The male age mean was 40.2 ± 2.8 years, while for females, it was 38.3 ± 3.5 years. An informed consent was obtained from each participant.

DNA extraction

Three milliliters of venous blood were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant and kept frozen until use. Genomic DNA was extracted from frozen whole blood using Blood gDNA Miniprep kit (Promega, USA). Extracted DNA was quantified by spectrophotometery, checked for purity and stored at -20ºC until further analyses.

HLA-DQB1 polymorphism typing

HLA-DQB1 polymorphism was determined by polymerase chain reaction with sequence specific primers (SSP-PCR) using Heidelberg University HLA-DQB1 genotyping (Low Resolution) kit (Heidelberg-Germany). Each kit contained 14 PCR Primer Mixes (13 allele-specific mixes and 1 negative control mix) that were able define five alleles of HLA-DQB1 gene (HLA-DQB1*02, HLA-DQB1*03, HLA-DQB1*04, HLA-DQB1*05 and HLA-DQB1*06) (Figure 1). Amplification was carried out using a PCR 9600 thermal cycler (applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 94ºC for 2 minutes; denaturation at 94ºC for 15 seconds; annealing+extension at 65ºC for 1 minute (10 cycles); denaturation at 94ºC for 15 seconds; annealing at 61ºC for 50 seconds; extension at 72ºC for 30 seconds (20 cycles). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. Each of the primer mixes contained an internal positive control, which was a primer that amplified a part of the C-reactive protein (CRP) gene that produces a 440-bp amplicon.



↓	Primer Mix-No.	Specificity HLA-DQB1*	Amplicon (bp)
	1	**DQB* 05**	**135/2225**
	2	DQB* 06	150/170
	3	DQB* 06	105/165
	4	DQB* 06	170
	5	DBQ* 02	200
	6	**DQB* 03**	**105**
	7	**DQB* 03**	**175**
	8	DQB* 03	130
	9	DQB* 03	135
	10	DQB* 03	130
	11	DQB* 03	120
	12	**DQB* 03**	**160**
	13	DQB* 04	210
	14	Negative Control	

Figure 1. Amplification patterns of HLA-DQB1 alleles detected by the HLA-DQB1 Heidelberg Low Resolution kit after 2% agarose gel electrophoresis for a pulmonary tuberculosis patient. The arrow refers to the interior positive control band, which was a fragment of the human C-reactive protein gene (440 bp), while bands below it refer to the PCR amplified products. The HLA-DQB1 genotype is interpreted as DQB1*03, 05 in this patient.

Statistical analysis

HLA-DQB1 alleles were presented as percentage frequencies, and significant differences between their distributions in PTB patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between an allele with the disease. The RR value can range from less than one (negative association) to more than one (positive association). If the association was positive, the EF was calculated, while if it was negative, the PF was given (Ad'hiah, 1990). These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package (including the programs and their manuals) is available free online at http://www.brixtonhealth.com.

RESULTS

Out of the five encountered HLA-DQB1 alleles, DQB1*03 showed a significant (P = 0.005) increased percentage frequency in PTB patients compared to controls (71.3 *vs*. 50.0%). The RR of such positive association was 2.48; while EF was 0.43 (Table 1).

DISCUSSION

The presented results are in favor of that HLA-DQB1 is a susceptibility locus for PTB in the sample of investigated Iraqi patients, and DQB1*03 allele may confer the individual an immunogenetic predisposition to the development of disease,

Table 1. Observed numbers and percentage frequencies of HLA-DQB1 alleles in pulmonary tuberculosis patients and controls

HLA Alleles	Patients (No. = 94)		Controls (No. = 80)		RR	EF or PF	P	95% C.I.
	No.	%	No.	%				
DQB1*02	48	51.1	48	60.0	0.70	0.18	N.S.	0.38 - 1.27
DQB1*03	67	71.3	40	50.0	2.48	0.43	0.005	1.33 - 4.62
DQB1*04	0	0.0	4	5.0	-	-	-	-
DQB1*05	23	24.5	20	25.0	0.97	0.07	N.S.	0.49 - 1.93
DQB1*06	45	47.9	32	40.0	1.38	0.13	N.S.	0.76 - 2.51

RR: Relative Risk; EF: Etiological Fraction; PF: Preventive Fraction; P: Two-sided Fisher's Exact Probability; N.S.: Not Significant; C.I.: Confidence Interval.

especially if we consider RR value of 2.48 and EF value of 0.43. Such results confirm what is known that HLA alleles are one of the important components of susceptibility and resistance to many infectious diseases including TB. In the case of PTB disease, case-control association studies have found significant associations between HLA polymorphisms and the disease etiopathogenesis, and the major susceptibility locus is suggested to be within HLA class II region compared to those located in HLA class I section (Kettaneh *et al*., 2006; Yim and Selvaraj, 2010). Indeed, both negative and positive association are observed between TB and HLA class II polymorphisms; suggesting a strong influence of class II alleles in the modulation of immune response to *M. tuberculosis* infection through cell-mediated immunity. With respect to HLA-DQB1 gene, there have been many reports of associations between the frequency of DQB1 alleles and the susceptibility to PTB, but the results have been inconsistent and this has been related to the impact of ethnicity (Magira *et al*., 2012). In the present study, the suggested susceptibility allele was DQB1*03, and similar finding has been reported by Mohammed *et al*. (2014), but their determination was based on a microlymphocytotoxicity test using a panel HLA anti-sera, while the present study adopted a molecular approach. Reviewing the literature for similar studies in other world populations revealed further associations.

A significant association was found between the occurrence of DQB1*0501, DQB1*0502 and DQB1*06 alleles and TB in subjects from North India (Rani *et al*., 1998). In South India the results of studies on the association of DQB1 alleles with the development of clinical TB have been inconsistent. Ravikumar *et al*. (1999) found a higher frequency of DQB1*0601 in those with TB while Sanjeevi *et al*. (1992) found no correlation between TB and DQB1 alleles. In Mexican TB patients, a significant positive association with DQB1*0501 was reported by Teran-Escandon *et al*. (1999). In a Thai population, DQB1*0502 was found more frequently in subjects with TB (Vebaesya *et al*., 2002), and in Cambodia, Poland and Brazil, an association was found between TB and DQB1*05 but not with DQB1*06 (Goldfeld *et al*., 1998; Dubaniewicz and Moszkowska, 2003; Figueiredo *et al*., 2008). A negative association between the presence of *M. tuberculosis* and DQB1*03 in Thai subjects and DQB1*04 in Mexicans was also noted (Teran-Escandon *et al*., 1999; Vebaesya *et al*., 2002). The DQB1*02 allele was associated with susceptibility to TB in Chinese patients (Wu *et al*., 2013). DQB1*06 allele was associated with the progression of TB in Korean patients (Kim *et al*., 2005). In a Portuguese population, there has been no association between DQB1 alleles and TB (Durate *et al*., 2011). However, the finding in the present study of a high frequency of DQB1*03 in patients with PTB is in agreement with a previous study carried out in South Africa (Lombard *et al*., 2006). They found that DQB1*03 allele was significantly higher in cases of TB than controls. In contrast, DQB1*03 allele was associated with a protection against TB in a Thai population (Vebaesya *et al.,* 2002).

The discrepancies in the results between studies may be caused by different methods and/or the high degree of polymorphism of the DQB1 allele in different ethnic groups, in which significant geographical variations have been observed. Hypotheses have been proposed to explain this geographic variation. It seems likely that evolutionary selection pressures have given rise to frequent polymorphisms in the genes involved in resisting infectious pathogens and so contributed to marked differences in allele frequency at the same loci. When geographic variation in pathogen polymorphism is superimposed on host genetic heterogeneity, considerable variation may occur in allelic associations and ethnic populations showed different frequencies of HLA alleles, including Iraqis (Ad'hiah, 2009). Gene-environment interactions are likely to introduce another layer of complexity. The genes involved in the defense against infectious pathogens evolve more rapidly than others and excessive polymorphism in the human genome may result from selection pressures exerted by infectious diseases. The causative organism, *M. tuberculosis,* also has genetic variation, and therefore all these polymorphic forms might have evolved over time due to host–microbial interaction, and accordingly may show different associations in different populations (Yim and Selvaraj, 2010).

REFERENCES

Ad'hiah, A. H. 1990. *Immunogenetic Studies in Selected Human Diseases*. University of Newcastle upon Tyne. England.

Ad'hiah, A. H. 2009. Distribution of HLA polymorphism in a sample of Iraqi Arabs in comparison with three Arab Gulf populations. *Iraqi Journal of Science*, 50: 120-125.

Blackwell, J. M., Jamieson ,S. E. and Burgner, D. 2009. HLA and infectious diseases. *Clin Microbiol Rev.*, 22:370-385.

Duarte, R., Carvalho, C., Pereira, C., Bettencourt, A., Carvalho, A., Villar, M., Domingos, A., Barros, H., Marques, J., Pinho Costa, P., Mendonça, D. and Martins, B. 2011. HLA class II alleles as markers of tuberculosis susceptibility and resistance. *Rev Port Pneumol*., 17:15-19.

Dubaniewicz A., Moszowska G., Szczerkowska, Z. and Hoppe, A. 2003. Analysis of DQB1 allele frequencies in pulmonary tuberculosis: preliminary report. *Thorax,* 58: 890-891.

Figueiredo, J. D., Rodrigues, M. D., Deghaide, N. H., and Donadi, E. A. 2008. HLA Profile in Patients with AIDS

and Tuberculosis. *The Brazilian Journal of Infectious Diseases*, 12:278-280.

Goldfeld, A. E., Delgado, J.C., Thim, S., Bozon, M.V., Uglialoro, A.M., Turbay, D., Cohen, C. and Yunis, E.J. 1998. Association of an HLA-DQ allele with clinical tuberculosis. *JAMA*, 279: 226–228.

Kettaneh, A., Seng, L., Tiev, K.P., Tolédano, C., Fabre, B. and Cabane, J. 2006. Human leukocyte antigens and susceptibility to tuberculosis: a meta-analysis of case-control studies. *Int. J. Tuberc. Lung. Dis*., 10: 717- 725

Kim, H.S., Parkin, M.H., Songh, E.Y., Park, H., Kwon, S.Y., Han, S.K. and Shim, Y.S. 2005. Association of HLA-DR an HLA-DQ genes with susceptibility to pulmonary tuberculosis in Koreans: preliminary evidence of associations with drug resistance, disease severity and disease recurrence. *Hum. Immunol.,* 66:1074-1081.

Lombard, Z., Dalton, D.L., Venter, P.A., Williams, R.C. and Bornman, L. 2006. Association of HLA-DR,-DQ, and vitamin D receptor alleles and haplotypes with tuberculosis in the Venda of South Africa. *Hum. Immunol.*, 67: 643–654.

Magira, E. E., Papasteriades, C., Kanterakis, S., Toubis, M., Roussos, C. and Monos, D.S. 2012. HLA-A and HLA-DRB1 amino acid polymorphisms are associated with susceptibility and protection to pulmonary tuberculosis in a Greek population. *Hum Immunol*., 73:641-646.

Mohammed, N.A., Qassem, H and Hassen, F. 2014. Association between HLA-class I and HLA-class II alleles and *Mycobacterium tuberculosis* infection in Iraqi patients from Baghdad city. *Iran J. Med. Sci*., 39: 191-195.

Möller, M. and Hoal, E. G. 2010. Current findings, challenges and novel approaches in human genetic susceptibility to tuberculosis. *Tuberculosis (Edinb),* 90: 71-83.

Nahid, P., Horne, D. J., Jarlsberg, L. G., Reiner, A. P., Osmond, D., Hopewell, P. C. and Bibbins-Domingo, K. 2011. Racial differences in tuberculosis infection in United States communities: the coronary artery risk development in young adults study. *Clin Infect Dis.*, 53:291-294.

Qu, H.Q., Fisher-Hoch, S.P. and McCormick, J.B. (2011).Knowledge gaining by human genetic studies on tuberculosis susceptibility. *J Hum Genet*., 56:177-182.

Rani, R., Mukherjee, R. and Stastny, P. 1998. Diversity of HLA-DR2 in North Indians:the changed scenario after the discoveryDRB1*1506. *Tissue Antigens*, 52:147–152.

Ravikumar, M., Dheenadhayalan, V., Rajaram, K., Lakshmi, S.S., Kumaran, P.P., Paramasivan, C.N., Balakrishnan, K., Pitchappan, R.M. 1999. Associated of HLADRB1, DQB1 and DPB1 alleles with pulmonary tuberculosis in south India. *Tuber. Lung Dis*, 79: 309–317.

Robinson, J., Mistry k., McWilliam, H., Lopez, R., Parham, P. and Marsh S.G. 2011. The IMGT/HLA database. *Nucleic Acids Research*, 39: D1171-1176.

Sadki, K., Bakri, Y., Tijane, M. and Amzazi, S. 2012. MHC Polymorphism and Tuberculosis Disease, Understanding Tuberculosis - Analyzing the Origin of Mycobacterium Tuberculosis Pathogenicity, Dr.Pere-Joan Cardona (Ed.), ISBN: 978-953-307-942-4, InTech, Available from: http://www.intechopen.com/books.

Sanjeevi, C. B., Narayanan, P.R., Prabakar, R., Charles, N., Thomas, B.E., Balasubramaniam, R. and Olerup, O. 1992. No association or linkage with HLA-DR2 or DQ genes in South Indians with pulmonary tuberculosis. *Tubercle*, 73: 280–284.

Santucci, N., D'Attilio, L., Kovalevski, L., Bozza, V., Besedovsky, H., del Rey, A., Bay, M.L. and Bottasso, O. 2011. A multifaceted analysis of immune-endocrine-metabolic alterations in patients with pulmonary tuberculosis. *PLoS ONE*, 6: e26363.

Schurr, E. 2011. The contribution of host genetics to tuberculosis pathogenesis. *Kekkaku*, 86:17-28.

Teran-Escandon, D., Teran-Ortiz, L., Camarena-Olvera, A., González-Avila, G., Vaca-Marín, M.A., Granados, J. and Selman, M. 1999. Human leukocyte antigen-associated susceptibility to pulmonary tuberculosis: molecular analysis of class II alleles by DNA amplification and oligonucleotide hybridization in Mexican patients. *Chest*., 115: 428–433.

Vebaesya, S., Chierakul, N., Luangtrakool, K., Srinak, D. and Stephens, H.A. 2002. Associations of HLA class II alleles with pulmonary tuberculosis in Thais. *Eur. J. Immunogenet* , 29: 431–434.

Wang, J., Song, C. and Wang, S. 2001. Association of HLA-DRB1 genes with pulmonary tuberculosis. *Zhonghua Jie He He Hu Xi Za Zhi,* 24: 302-305.

WHO 2013. Global tuberculosis report 2013. http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf.

WHO 2014. World Health Organization (WHO) estimates of tuberculosis incidence by rate, 2012 (sorted by rate). http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317140584841.

Wu, F., Zhang, W., Zhang, L., Wu, J., Li, C., Meng, X., Wang, X., He, P. and Zhang, J. 2013. NRAMP1, VDR, HLA-DRB1, and HLA-DQB1 gene polymorphisms in susceptibility to tuberculosis among the Chinese Kazakh population: a case-control study. *Biomed Res Int.*, 2013:484535.

Yim, J. J. and Selvaraj, P. 2010. Genetic susceptibility in tuberculosis. *Respirology,* 15: 241–256.

****