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Characterization of the Genetic Basis of Unresponsiveness to BCG- Vaccinated Sudanese Health Workers in Khartoum State

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Abstract

Tuberculosis (TB) is a chronic disease caused by *M. tuberculosis*. The World Health Organization (WHO) estimated that *Mycobacterium tuberculosis* infects a one third of the world's human population and kills two million people each year. The number of active infection was 14.4million, in Sudan the indicator of 1% corresponds to an approximate incidence of 50cases of pulmonary symptoms smear-positive TB/100,000 population. We attempt to characterize the human leukocyte antigen (HLA) among Healthcare Workers (HCWs) in Khartoum State who have vaccinated with BCG and failed to show any response to the vaccine and Tuberculin Skin Test. One-hundred thirty-seven HCWs were screened by conventional methods (microscopy, TST, HIV test and Chest X-Ray), all subjects were apparently healthy and allocated according to the TST results into three groups (strong TST-positive, moderate TST-positive, and negative groups). Link regards to HLA genotypes distribution, the present study educated that alleles associated with TB-exposed group DRB1*0701, DRB1*1501, DQB1*0501 and DQB1*0301. DRB1*0701 and DQB1*0301 with high frequency among strong TST-positive group, DRB1*0701 and DQB1*0601 alleles were with high frequency among moderate TST-positive group and DRB1*0701, DRB1*1501, DQB1*0601 alleles were with high frequency among TST-negative group. Some of these alleles (DRB1*1501, DQB1*0701, DQB1*0301 were shown exclusively in TB-exposed subjects (100%). It seems that they act as susceptibility genes to TB. Moreover, DRB1*0201 and DQB1*01 and DRB1*0301 alleles were found to be associated with control group.

Subjects of the study group were divided into three sub-groups based on the ethnic group; Nilotic, Kordufan and Darfur, and Nile Nubian groups. DRB1*03, DRB1*13, DQB1*01, DQB1*04, DQB1*02 and DQB1*06 were exclusively found in the Nilotic tribes (100%). While DRB1*07 was exclusively found in Kordufan and Darfur tribes (100%), DRB1*15 allele was found to be higher (53.3%) among Nubian tribes but further investigation using large sample size is recommended to verify these findings.

Keywords: HLA; TB; BCG; Tuberculin Skin Test

Abbreviations: TB: Tuberculosis; WHO: World Health Organization; HLA: Human Leukocyte Antigen; HCWs: Healthcare Workers; ARI: Annual Risk of Infection; TMRI: Tropical Medicine Research Institute; NCR: National Centre for Research; AFB: Acid-Fast Bacilli; SSP: Sequence Specific Primer; SPSS: Statistical Package for Social Science; ZN: Zeil-Nelseen; NHS: Normal Healthy Subject.

Introduction

Tuberculosis is a chronic granulomatous disease affecting man, many other mammals, birds, fishes, amphibians and reptiles. Mycobacterium tuberculosis and the regional variants or sub-types of Mycobacterium africanum and *"M. canettii"* which are primarily pathogenic for man cause mammalian tuberculosis. *M. bovis* and *M. microti* are the causative agents of TB in animals, and it can be transmitted to humans [1,2]. Most *Mycobacterium tuberculosis* infections are caused by inhaling droplets or dust particles containing the bacilli. Tuberculosis usually attacks the lungs but can also affect other organs of the body. The primary site of infection in the lungs is called the *"Ghon focus"*, and is located in either the upper part of the lower lobe, or the lower part of the upper lobe [3]. Primary TB can be divided into progressive-primary and post-primary forms based on the natural history of the disease.

Tuberculosis (TB) remains a major global cause of morbidity, second only to HIV as a single leading infectious cause of death worldwide [4] and estimated to have caused 8.6million new infections and 1.3million deaths in 2012 [5]. The incidence of new cases was estimated to be 9.2million, corresponding to a prevalence rate of 139/100,000 and 12 of the 15countries with the highest estimated TB incidence was in Africa, where the TB incidence rate was 363/100,000 [6]. In health care facilities, certain health care workers (HCWs) may become infected with *Mycobacterium tuberculosis* through repeated exposure to TB patients. Many studies indicate that health care setups are often at higher risk of TB infection [7]. The indicator of the TB issue in Sudan is the average annual risk of infection (ARI). The ARI of 1% corresponds to an approximate incidence of 50cases of pulmonary symptoms smear-positive TB/100,000 of the population, which is the proportion of the population that is likely to be newly infected over one year. The prevalence of TB infection is probably variable in different regions of Sudan [8]. One of the most important groups which are not studied in Sudan is the health care workers group, so this study was designed especially for this group.

The human immune system is regulated by molecules coded by some genes, among which, are the genes of the human histocompatibility system, which code for human leukocyte antigens (HLA). These genes are located in the short arm of chromosome 6 Furusho, et al. and are divided into three classes, I, II and III [9]. HLA class I is responsible for coding the molecules HLA-A, -B and -C, present in almost all somatic cells Furusho, et al. The expression of particular class I and class II MH Calleles in an individual determines the ability of that individual to respond to particular (mycobacterial) antigens and epitopes. Certain allelic human leukocyte antigen (HLA) variants have been associated with tuberculosis Ravi Kumar M, et al. [10]. HLA polymorphism may explain the vulnerability of certain isolated populations like Amazonian Indians whom have only recently been exposed to tuberculosis [11]. The HLA-DR2 antigen (expressed by the alleles HLA-DRB2*1501 and DRB1*1502) is associated with the development of severe and multi-bacillary forms of tuberculosis, as well as with the high prevalence of drug therapy-resistant [12]. This antigen increases the risk of developing tuberculosis, while the antigen HLA-DR13 protects against the disease [13]. In India, the results are controversial, Brahmajothi found that HLA-A10, -B8 and-DR2 antigens presented with greater frequency in persons with tuberculosis than in healthy controls [14].

This study was designed to investigate the possible genetic factor/factors affecting the outcome of BCG vaccination in HCWs immune responses using HLA-Class II typing.

Materials and Methods

Study Population

Ethical Clearance was obtained from the Committee of Tropical Medicine Research Institute (TMRI), National Centre for Research (NCR) and subjects were included in the study following informed consents. One hundred thirty-seven HCWs (20-70years old) who were vaccinated with BCG at birth, presented to the National Health Laboratory-Fedral Ministery of Health, Ummdawban hospital and Alshaab teaching hospital Khartoum State, Sudan. They were screened for the presence of acid-fast bacilli (AFB), HIV and had Chest X-Ray; TST negative subjects were revaccinated with BCG. They were presented as healthy, tuberculosis-free according to the results of the above tests. X-ray examinations were performed for all subjects. All subjects were examined for HIV using the Trinity Biotech Unit-Gold HIV test.

Sputum specimens from the first productive deep cough of the morning were obtained from TB exposed health care workers in sterile screw-top plastic urine cup containers without being contaminated by saliva or nasal secretions. Direct smears of sputa were stained with ZN method to detect presence of (AFB), Waxy materials in the cell walls of *M. tuberculosis* resist decolourization with acidified alcohols

and strong acids mineral [15]. Moreover Mantoux test (TST) was done for all subjects.

Thirty HCWs, who were found negative to Mantoux test, HIV test, and showed AFB in their sputa samples with normal Chest X-ray results, were revaccinated. BCG vaccine (Live BCG vaccine: 20X20 Doses BCG, Batch, Lot No. 305-2) was given as a single intradermal injection over the deltoid muscle of the arm; it was administered using 1.0mL plastic disposable syringe with a 26-gauge needle with the level facing upwards. The recommended dose was 0.1mL (0.1mg) [16].

Molecular Biology Test

Genomic DNA samples were extracted from all patients using QIAamp Blood Kit. Extracted DNA samples were tested for concentration and purity.

Genotyping of Major Histocompatibility Complex (MHC) Class II Genes DR- DQ Regions

For the determination of the allelic polymorphism by PCR amplification Sequence-Specific Primers (PCR-SSP) was used, the typing method is based on the principle that a completely matched primer will be more efficiently used in the PCR than ones with one or several mismatches, especially in the first critical cycles Bodmer JG, et al. [17]. Thus, the specificity of the typing system is part of the PCR. Assignment of alleles is based on the presence of amplified product, which may be detected by agarose gel electro phctrophoresis [18]. By sequence specific primer (SSP) based methodology [(One Lambda Micro SSP™DNA typing class11 generic kits, Onelambda, Inc. PCR-SSP (Cat # SSP2L, Inc-U.S.A)], class11 typing was assigned, following the manufacture instructions.

DRB1, DQB1 alleles: For the genetic typing of the DR alleles by PCR-SSP™ Kit (Micro SSP HLA DNA Typing Trays, Inc-U.S.A), PCR was set according to manufacturer's instructions. The Micro SSP™ DNA Typing Trays provide sequence-specific oligonucleotide primers for amplification of HLA alleles and the human β- globin gene (internal control) by the PCR. Preoptimized primers are presented in different wells of a 96 well in 0.2ml thin walled tube tray for PCR; each tray included a negative control reaction tube. One µl DNA diluent was added to the negative control reaction tube on the primer set tray and 2µl recombinant Taq polymerase (5Unit/µl) were added to the Micro SSP™ D-mix tube (dNTPs buffer mixture). Nine µl of D-mix were added to the negative control reaction tube, 39µl DNA were added to 360µl D-mix tube vortexed for 5sec and then 10µl of D-mix were added into each tube of the tray. The PCR was then run with the temperature profile as described by the manufactured.

Visualization of the PCR Product: Absence or presence of PCR products was visualized by electrophoresis. After the addition of 3µl loading buffer (3% (v/v) glycerol stained with bromophenol blue), the PCR mixtures were loaded in 2.5% Agarose gel to 100ml of 1xTris Borate EDTA buffer (1xTBE, 0.01MTris HCl and 0.01M sodium bromide (in a 500ml to 500ml of dH_2O , pH adjusted to 7.5) with 0.5µg/ml ethedium bromide (in a 500ml glass bottle) was added to 2.5g electrophoriesis agarose and heated until a homogenous solution was formed, 30ml of the gel were added to the gel box and 10µl of genomic DNA samples (no addition of electrophoresis dye was necessary) loaded into the agarose gel wells. The gel was run at 35mA and 100V for 40 minis. A Digital graphic printer then examined the gel.

Statistical Analysis: Collected data were analysed using the Statistical Package for Social Science (SPSS) (SPSS, Chicago, IL, USA; Version 16). Chi-square test was used to determine the relation between the HLA-D alleles among different types of exposed (positive and negative to tuberculin skin test healthcare workers) and non-exposed subjects. The genotype distribution of the INF-γ gene in TB-exposed HCWs among different work durations in TB wards was analysed using one-way ANOVA. Charts were done using Excel software.

Results

The present study is a controlled study performed among health care workers to investigate the possible genetic factors that could have affected the outcome of BCG vaccination in relation to HLA typing. A total of 157 individuals were included in this study, 137 of them were Healthcare workers that exposed to Tuberculosis. One hundred and seven individuals (78.1%) were positive & (21.9%) 30 individuals were negative. Females among Healthcare workers of Tuberculosis were more than males, with ratio of exposed and non-exposed in females and males of 7:3. The mean age of the exposed healthcare workers group was 33.9 (19-70 Yrs.) whereas of the control group subjects was 31(21- 65 Yrs.). The family history of the exposed Healthcare workers was classified into three categories according to degree of closeness. First, second and third degree relatives. Only 8.1% (n=11) had TB history in their families. Subjects included in the study were from 39 Sudanese tribes according to the stock of Sudanese tribes. About (94.1%) of the subjects were from Nilotic, Kordufan, Darfur and Nubian tribes and the rest were from the other tribes (Busharia, Saadawi, Beja, 5.9%).

Microbiological Results

One-hundred-Thirty-seven (137) sputum specimens from Exposed healthcare workers were investigated and processed for direct microscopically examination using the Zeil-Nelseen (Z.N.) staining procedure. None of the sputum specimens showed the characteristic AFB appearance of serpentine cords on oil immersion fields and they were considered as negative. All subjects were HIV-negative.

Tuberculin skin test was performed on all exposed healthcare workers. 97 out of 137 (70.6%) were positive. All subjects were further classified into three categories according to the diameter circumference, strongly positive, 15-25mm, moderately positive 6-15mm, and negative 0.00-5mm.

BCG Vaccination and Revaccination

Eighty-three exposed healthcare workers out of one hundred and thirty-seven (69.5%) were vaccinated at birth, 9(6.6%) were revaccinated during the study course because they were negative results to tuberculin skin test and were not vaccinated at birth. Forty-five subjects (32.8%) who had not been vaccinated at birth were excluded from the study.

Chest X-Ray

Chest X-Ray was performed on all Healthcare workers (137subjects). The following organs were observed and checked, namely: Heart, the upper lobe of the lung, the lower lobe lung, ribs, intercostal, clavicles, lung and air in trachea. 119 subjects out of the 137(86.9%) were normal and 18(13.1%) subjects were TB positive but they were totally treated.

Molecular Biology Results

HLA alleles

Frequency of DR and DQ alleles: The frequency of the HLA-typing DRB1* alleles among 60 exposed healthcare workers, DRB1*13 allele the highest among the group where DRB1*09, DRB1*14 alleles were the lowest whereas DQB1* alleles among exposed healthcare workers group showed that the allele with higher frequency was DQB1*03 and DQB1*04 allele was the lowest frequent allele.

HLA-DRB1* & DQB1* alleles: Tuberculin skin test results were divided into three categories: a strongly positive, moderately positive and Negative group, among these groups showed the high frequency of HLA DRB1* & HLADQB1* alleles and compare those highest alleles with Control group. The high frequency of the HLA DRB1* alleles among strongly positive group of TST was DRB1*0701 and DRB1*0401 and DRB1*0301, DRB1*1102 were with the lowest frequency, HLA -DQB1*0301 allele frequency was higher than the other alleles among strongly positive tuberculin skin test group.

The comparison between exposed and non-exposed subjects Tuberculosis showed that combination DRB1*0201 & DQB1*0301 alleles were observed with high frequency among control (non-exposed) but not among exposed group, DRB1*1501 & DRB1*0701 were found to be highly frequent in positive group (81.8%), lowest frequency in negative group (18.2%) and was detected in the control group. The frequency of DRB1*0801 was highest among Positive (60%) and control group (30%) but lowest frequency among the negative group (10%). The frequency of DQB1*05 alleles was high in negative group and lowest frequency in group (36.4%), and it was low in the control group (63.6%) (Non - exposed).

Thirty-one (53.2%) of the exposed healthcare workers, had the allele DQB1*02 compared to 40% of control group with a relative risk factor of 1.2, 44.4% of the exposed healthcare workers had DRB1*15 compared to 0% of control group with a relative risk factor 0.0, and 40.7% of the exposed had DRB1*07 compared to 0% of control group with a relative risk of 0.0, while the allele DRB1*03 found to be a protective as only 5.4% of the exposed healthcare workers had the allele but 66.7% of the control group had the allele as shown in (Table 1).

HLA allele	Study Groups		p. value	RR/OR
	Exposed	Control		
DRB1*01	7.60%	33.30%	0.06	1
DRB1*02	2.60%	42.90%	0.009	0.812
DRB1*03	5.40%	66.70%	0.002	0.81
DRB1*04	18.20%	5.00%	0.002	1.31
DRB1*07	40.70%	0%	0.035	0
DRB1*08	18.20%	17.60%	0.104	8.6
DRB1*10	7.60%	17.60%	0.37	1
DRB1*11	14.70%	17.60%	0.52	0.51
DRB1*13	50.00%	40.00%	0.25	0.354
DRB1*14	2.60%	0.00%	0.26	0
DRB1*15	44.40%	0.00%	0.01	0
DQB1*01	33.30%	20.00%	0.01	0.431
DQB1*02	53.80%	40.00%	0.01	1.21
DQB1*03	30.80%	30%	0.5	1.91
DQB1*04	5.40%	5.20%	0.34	0.4
DQB1*05	30.00%	0.00%	0.01	0

Table 1: HLA-DR/DQ alleles (n=16) frequency in exposed healthcare workers of Tuberculosis disease compared to control group, RR=relative risk, OR=odd ratio.

HLA alleles and Work Duration in Chest Wards

The target group was further divided into two sub-groups according to the work duration in the chest wards,medium risk group which includes workers and other medical and nursing staff, physiotherapists, radiographers, paramedical and ambulance staff and students involved in direct patient care, non-clinical staff in regular close contact with patients and community nurses working with at medium risk groups

less than 5 years, and highrisk group which includes all staff working in respiratory system diseases clinics and laboratories and specific tuberculosis treatment areas; staff working in the intensive care units, emergency departments ; all those who regularly work with TB patients for more than 5 years.

HLA-DRB1*15 (n=9) (found just in medium risk group, ≤ 5 years), DRB1*01, DQB1*01 (n=6), DQB1*02 and DRB1*04 alleles found to be with high frequency in the group who worked for less than5 years and not in the groups worked for more than 5 years. HLA-DRB1*07, DQB1*05 was only found in the medium risk group while DRB1*08 was only found in high risk group and not the other group. DRB1*11 allele was found only in the control group and with high frequency (p-value=0.01). DQB1*03 allele was found in the three groups but with high frequency in the medium risk group (p-value =0.05).

HLA alleles and Different Tribes of Exposed Healthcare Workers

The study group was categorized into three groups based on the types of the tribes: Nilotic, Nubian, and Kordufan & Darfur. It was found that DRB1*01, DRB1*13, DRB1*14, DQB1*01, DQB1*04, DQB1*02, DRB1*07 and DQB1*06 were exclusively found in the Nilotic tribes (100%). while DRB1*11 alleles was exclusively found among Kordufan and Darfur tribes (100%). DRB1*15 allele was higher (53.3%) among Nubian tribes and lower among the Nilotic tribes (46.7%), DRB1*03 allele was higher among Nilotic tribes (93.8%) and less frequent among other tribes (6.2%) (p-value=0.01) (Table 2).

DRB1* Alleles	Study Group's Tribes		
	Nilotic (North - central)	Kordofan&Darfur	Nile Nubian
101	100%	-	-
303	93.80%	6.20%	-
401	100%	-	-
701	-	100%	-
801	100%	-	-
1101	-	100%	-
1305	100%	-	-
1402	100%	-	-
1501	46.70%	-	53.30%
DQB1*	-	-	-
1	100%	-	-
2	100%	-	-
3	46.40%	25%	28.60%
4	100%	-	-
5	53.80%	7.70%	38.50%
6	100%	-	-

Table 2: The distribution of HLA DRB1* & DQB1 alleles among tribes of exposed healthcare workers.

HLA alleles and Genders Among Tuberculosis Exposed Health cares:

The high frequency HLA-DR alleles among males were DRB1*0801, DRB1*0701 was exclusively found among males (100%) while DRB1*1301 was found to be higher among males (89.9%) than among females (10.1%). DRB1*0103, DRB1*1402were exclusively found (100%) among females (p=0.03). HLA-DQB1*01 allele was only found (100%) among females while DQB1*0601 was higher (70%) in females than in males (30%). All other alleles DQB1*04, DQB1*03, DQB1*05 and DQB1*02 were estimated to be equally distributed, 50% each, among males and females (p=0.01).

HLA alleles (Risk & protective)

a) Of the positive TST individuals seven (34.80%) had

DRB1*070, compared to only (12.5%) Negative cases, with a relative risk of 6.1 (p=0.5).

b) Fourteen (25.6%) of the TB exposed healthcare workers TST individuals had DRB1*0701, while this allele was completely absent among the control group, with a relative risk of 0 (p=0.04).

c) Six (26.1%) of the positive Tuberculin skin test individuals had the DRB1*1501 compared to only 18.8 % among the Negative individuals, with relative risk of 2.34 (p=0.5).

d) 30.8% of the TB-exposed healthcare workers Tuberculin skin test individuals had DRB1*1501, while this was exclusively absent in the control group, with relative risk of 2.34 (p=0.01).

e) 65.2% of the positive Tuberculin skin test positive individuals had DQB1*0201, compared to only 37.5 % of the negative individuals, with a relative risk of 2.8 (p=0.03).

f) 53.8% of the TB exposed Tuberculin skin test individuals had DQB1*0201 compared to 40% in the control group, with a relative risk of 1.2 (p=0.01).

Distribution of HLA-DR Alleles Among INF-γ Genotypes (+874AA, TT & AT) among TB-Exposed Healthcare Workers

We tried to draw an association between the phenotype frequencies of HLA DR & DQ alleles and INF-γ gene polymorphism at position +874 A/T, AA, TT in TB-exposed healthcare workers. There was a high frequency of DRB1*0701 allele, DRB1*0202 allele and DRB1*1501 allele.

DRB1*0701 allele was found to be with high frequency among subjects with INF-γ TT, AA, AT genotype, while DRB1*1501and DQB1*0401 alleles were found to be with high frequency among subjects with INF-γ AT genotype, DQB1*0101 was highest frequency (100%) among control group as a protective allele (Table 3). All exposed healthcare workers with tuberculin skin test result positive & negative had DRB1*0701, but it was completely absent among the control group, with a relative risk of 0 (p=0.04), whereas, 53.8% (n=21) of the positive tuberculin skin test individuals had DQB1*0201 , compared to 40% in the control group , with a relative risk of 1.2 (p=0.01).

DRB1* (n=9)	Exposed and non-exposed Groups (Alleles)				
	TT alleles	AA alleles	AT alleles	Control	Total (N)
DRB1*01	-	-	45.50%	54.50%	11
DRB1*02	20%	-	-	80%	7
DRB1*03	7.10%	7.10%	28.50%	57.10%	14
DRB1*04	16.70%	-	66.60%	16.70%	6
DRB1*07	10%	60%	30%	-	10
DRB1*08	20%	20%	40%	20%	10
DRB1*10	14.20%	-	42.90%	42.90%	7
DRB1*11	14.30%	-	57.10%	28.60%	7
DRB1*13	-	-	46.70%	53.30%	15
DRB1*15	14.30%	-	85.70%	-	14
DQB1*(n=6)	-	-	-	-	-
DQB1*01	-	-	-	100%	16
DQB1*02	5.60%	-	50%	44.40%	18
DQB1*03	21.70%	-	52.10%	26.10%	23
DQB1*04	-	-	75%	25%	4
DQB1*05	7.70%	38.50%	53.80%	-	13
DQB1*06	21.70%	13%	65.20%	-	23

Table 3: Genotyping frequencies of HLA-DR and DQ in TB-exposed healthcare workers and their controls group that had INF-γ genotype AA, TT,AT alleles and Total frequency of DRB1*& DQB1* alleles.

Discussion

This study investigated the association between MHC-ClassII DR and DQ regions and vaccinated TB exposed HCWs. Newport (2004) showed that TB is more consistent for MHC-ClassII than for ClassI genes and reported that DRB1 alleles were often associated with increased risk of TB and never with protection. While DQB1 alleles may be linked to TB-susceptibility or resistance [13]. In another study, it was reported that, the presence of DRB1 shared alleles between primary cases and their contacts was a risk factor for tuberculosis. In this study, DRB1*13 allele was found to be the highest frequent allele among the TB exposed HCWs. This allele protect against the TB disease [19]. In the present study, DRB1*0701, DQB1*0301were found to be the highest frequent alleles among strong TST-positive group. No association between DQB1*0301 allele and the presence of *M. tuberculosis* was noted among Thai subjects [13]. In this study DRB1*0701 and DQB1*0601 were found to be highest frequent alleles among moderate TST positive group, HLA-DRB1*0701 and DQB1*0601 alleles were reported to be associated with higher susceptibility to development of pulmonary tuberculosis [19] and in another Ravi Kumar study (1999) it was found that there is an association between DQB1*0601, DQB1*05 and pulmonary tuberculosis. This study also showed that DRB1*1501 (with relative risk (RR=2.34), DRB1*0701 and DQB1*0601 were found to be the highest frequent alleles among TST negative group, this finding is very consistent with Ravi Kumar (1999) who observed association of the DRB1*1501 and DQB1*0601 with TB susceptibility and in another study which was carried out in Indian patients revealed that DRB1*15 and DQB1*06 were positively associated with susceptibility to pulmonary tuberculosis [20]. In the present study some of these alleles (DRB1*1501, DRB1*0701 and DQB1*0601 with high relative risk (RR=6.1) were shown exclusively in TB-exposed HCWs, it seems that they act as susceptible genes to TB. Moreover, DRB1*02 highest frequency (80%) among control and DQB1*01 (exclusively in control) which might be protective genes IFN-γ is one of the most important cytokines involved in macrophage activation, stimulating antitumor and antimicrobial activities, as well as it stimulates the synthesis and expression of MHC-II. There is an association between the genetic background of the human host and the susceptibility or resistance to *M. tuberculosis* infection Stead WW, et al. [21]. A previous study in South Africa shows that both host and pathogen genetics are important in the development of TB Salie M, et al. [22]. Low synthesis of INF-γ has been associated with active tuberculosis [23]. The present study investigated the genetic variation of the intronic region of the INF-γ gene (+874 T/A) and its relationship with expression of MHC-II among TB exposed HCWs and non-exposed individuals. This study showed that DRB1*07 was significantly the highest frequent HLA allele among TB- exposed HCWs with INF- γ (A/A) genotype. A significant association between the presence of the +874 A allele and active TB was observed, reaffirming earlier notion to the association of the +874 A/A genotype with the susceptibility to the bacterium [23], an association between AA genotype in +874T/A region with increased TB in Spanish and South African populations was reported Leandro ACCS, et al. [24]. A high frequency of DRB1*0202 was reported among TB-exposed HCWs who had INF-γ+874T/T genotype, this is in a total agreement with Petrovsky & Harrison (1997) who showed that (employing a similar whole-blood culture system) many HLA alleles (HLA DR1,-2,and-6) associated with high IFN-γ production while few others (DR3,-4,-5,and-7) associated with low IFN-γ production in healthy individuals (Petrovsky and Harrison, 1997). A high frequency of HLA-DRB1*1501, DQB1*03 were observed among TB exposed HCWs with INF-γ (A/T) genotype (with a relative risk 1.2) in this study. HLA-DRB1*1501 predisposes to sputum positive pulmonary tuberculosis [10]. Sri Ram study (2001) revealed that DRB1*1501 may be associated either alone or with other DR2 alleles, with the susceptibility to pulmonary tuberculosis. None of the DR2 alleles influenced the humoral and cellular response to *M. tuberculosis* culture filtrate antigens Sriram U, et al. [25]. In another study a significantly increased IFN-γ response was observed in HLA-DRB1*03-positive normal healthy subjects (NHS) (p=0.03) and decreased IFN-γ response in HLA-DRB1*1501-positive PTB patients (p=0.04) than in respective allele-negative individuals [26].

The indigenous population of the Sudan is an Arab-Negriod admixture comprising semi-nomadic ancient tribes attracted to the River Nile Valley where this admixture occurred [27]. This study showed that the distribution of HLA alleles was associated with TB susceptible alleles among TB-exposed HCWs. The study group was categorized into three sub-groups based on the types of the tribes Nilotic, Nubian, and Kordufan and Darfur groups. In another study it was observed that a high negative Mantoux reactivity makes vaccine efficacy questionable; but it could probably be explained by ethnic difference, highlighting the need for more research to investigate the efficacy of BCG vaccination in multi-ethnic countries like Sudan. Interestingly, DRB1*13, DQB1*01, DQB1*02 and DQB1*06 were exclusively found in Nilotic tribes, while DRB1*07 allele was exclusively found among Kordufan and Darfuri tribes. DRB1*15 allele was higher (53.3%) among Nubian tribes than Nilotic tribes (46.7%), DRB1*03 allele was highest among Nilotic tribes (93.8%), some studies suggested that the HLA-DQB1*03:03 allele may be associated with resistance to TB Wamala D, et al. [28]. In most studies the HLA-DQB1*03:03 allele is of relatively low frequency [10,29,30], or completely absent Lombard Z, et al. [31], Escandon DT, et al. [32].

The high skin non-reactivity may indicate a need for re-vaccination as practiced elsewhere. The current BCG vaccine schedule in Sudan has adequate coverage, but may need to be modified to include revaccination, and the probable explanation for high negative Mantoux reactivity may be due to differences in ethnicity [33,34]. TB infection was reported to be distributed among the majority of the tribes with varying rates, where the tribes of Dainka (12.9%), Gaalin (12.2%), and Nuba (10.9%) were found to have significantly higher incidence rates than the other tribes El Eragi, et al. The possibility of genetic predisposition of members of these tribes should be considered, since many reports worldwide stated that genetic factors were involved in the process of TB infection [35-40].

Conclusion

HLA-typing revealed that DRB1*02 allele was shown exclusively in the control group which might work as a protective gene, on the other hand DRB1*0701, DRB1*1501 and DQB1*0501 alleles were high among TB-exposed HCWs while DRB1*14 allele was found exclusively in TST-negative group which might be considered as susceptible genes. There is an association between TB-susceptible genes and Sudanese different ethnic groups but further studies with bigger sample size are highly recommended.

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