Toll-Like Receptor-2 Gene *[TLR2]* Polymorphism and Susceptibility to *Mycobacteria Tuberculosis* Infections in Sudanese Patients

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ABSTRACT--- Tuberculosis [TB] is a major public health threat and a leading cause of death worldwide. Innate immunity plays an important role in the host defense against *M. tuberculosis* through recognition Toll-Like Receptors [TLRs]. Polymorphisms in *TLR2 [Arg753Gln]* gene have been reported to be associated with increased susceptibility to TB. This study aimed to determine possible roles for *TLR2 [Arg753Gln]* gene polymorphism in susceptibility to *M. tuberculosis* infections in a cohort of Sudanese individuals. Following informed consent, 141 individuals with smear positive tuberculosis and latent TB infections [mean age 19±38.3 years; male: female ratio 2:1] and 197 apparently healthy individuals [mean age 30±14; male female ration of 1:5] were enrolled. DNA was extracted from EDTA-blood using the guanidine chloride method. PCR was carried out using *TLR2 gene* specific primers, Forward: [5'CATTCCCCAGCGCTTCTGCAAGCTCC-3'] and Reverse: [5'GGAACCTAGGACTTTATCGCAGCTC-3')]. Genotyping was done by RFLP using *MSP1*restriction enzyme. The *TLR2 Arg753Gln* mutant allele was not detected in any of the study groups. *TLR2 Arg753Gln* gene polymorphisms plays little or no role in host susceptibility to *Mycobacterium tuberculosis* infections in Sudanese population.

Keywords--- *TLR2 Arg753Gln* gene polymorphisms, *Mycobacterial* infections.

1. INTRODUCTION

Tuberculosis [TB] is a common and often deadly contagious disease caused by various strains of *Mycobacteria* usually *Mycobacterium tuberculosis* in humans. *Mycobacterium tuberculosis* is an obligatory aerobic intracellular pathogen which has a predilection for the lung tissue rich in oxygen supply. Approximately 1.4 million people died of TB in the year 2010, of which 1.1 million deaths were among HIV-negative people. In the same year, there were an estimated 12.0 million prevalent cases of TB and 8.8 million incident cases. In Sudan, according to WHO statistics in 2010 that were taken before Sudan partition, mortality from TB [excluding HIV] was 8.3 thousand with a rate of 19 per 100 000 population, prevalence [including HIV] was 82 thousand with a rate of 188 per 100 000 population and incidence [including HIV] of 52 thousands with a rate of 119 per 100 000 population [1, 2]. The exact reasons as to why only some of the individuals exposed to *M. tuberculosis* develop uncontrolled disease and others eradicate or limit the disease remains unknown. The evidence suggests that genetic factors may be important determinants of increased susceptibility to progressive disease development [3, 4]. Toll-like receptors [TLRs] comprise a family of mammalian cell-surface proteins that stimulate pro-inflammatory cytokine gene transcription in response to various microbial ligands. TLRs mediate cellular responses to microorganism, but are not required for phagocytosis. Members of the mammalian TLR family have been implicated in the activation of macrophages by a variety of chemically diverse bacterial products [5, 6]. *In vitro* studies have shown that TLR2 activation directly leads to intracellular killing of *M. tuberculosis* by alveolar macrophages [7]. A novel polymorphism in the *TLR2* gene [Arginine to Glutamine substitution at residue 753 [Arg753Gln] leads to a decreased response of macrophages to bacterial peptides, resulting in an attenuated immune response in the host [8]. The present study aimed to investigate the role of *TLR2* gene [Arg753Gln] polymorphism in host susceptibility to mycobacterial infections in Sudanese population.

2. MATERIALS AND METHODS

The study population were recruited from the TB Lymphadenitis Clinic at the Institute of Endemic Diseases, University of Khartoum; Abu Anja Hospital, Omdurman; Khartoum North Teaching Hospital, Khartoum North and Alshab Teaching Hospital, Khartoum. Following informed consent, 141 individuals with smear-positive pulmonary tuberculosis and latent TB and 197 Manteux un-reactive apparently healthy individuals were enrolled. Demographic data was collected in a specially designed form. Four ml blood in EDTA tubes were collected. DNA was extracted using the guanidine chloride method. The samples with DNA concentration above 50 Nanogram and purity above 1.7 were used. PCR was carried out using primers specific for *TLR2 gene* [TLR2 primers; forward [5'CATTCCCCAGCGCTTCTGCAAGCTCC-3'] and reverse [5'GGAACCTAGGACTTTATCGCAGCTC-3'] primers [Inqaba Biotic, South Africa]. RFLP-PCR technique was carried for genotyping using *MSP1* enzyme. Digested products were characterized using 15% polyacrylamide gel electrophoresis. The molecular work was conducted at the laboratories of the Institute of Endemic Diseases, University of Khartoum, Sudan.

3. RESULTS AND DISCUSSION

The mean age of the patients with smear positive TB was 19±38.3 [median=23] years while that of the apparently healthy group was 30±14 (median=33.5) years. The male: female ratio in the healthy group was 1:5 while that of the patients group was 2:1. The mean of the TST indurations of the healthy group was 0.9±2 mm while that of the latent tuberculosis infection individuals was 13.5±3.5 mm (Median=13 mm). The *TLR2* Arg753Gln mutant allele was not detected in any of the study groups (Fig.1) [*M. tuberculosis* infected and apparently healthy groups]. As far as we are aware, *TLR2*gene (Arg753Gln) polymorphism has not been investigated in Sudan or other Arab countries. This allele has been shown to be associated with susceptibility to TB in Turkish population [9]. This allele has been found not to be associated with susceptibility to TB in Chinese and Korean and India populations [10, 11, 12]. Most probably that the genes associated with susceptibility to TB are outside this area of the genome as was shown by Velez and colleagues in 2010 [insertion (I)/deletion (D) polymorphism at (-196 to -174). [13]. Recently, Zhang and colleagues analyzed a number of studies looking at TLR2 polymorphism reached the same conclusion and reported that the TLR2 polymorphism that increases susceptibility to TB [G2258A is associated with increased TB risk, especially in Asians and Europeans] is outside the area we studies [Arg753Gln]. They also showed that polymorphism in TLR1 G1805T is associated with increased TB in Africans and American Hispanics [14].

In conclusion, *TLR2* Arg753Gln gene polymorphism plays little or no role in host susceptibility to *M. tuberculosis* infections in this cohort of Sudanese population. Other sites in *TLR 2* gene and other *TLR* genes needs to be studied in Sudanese population to find the TLRs polymorphism that are responsible for increased susceptibility.

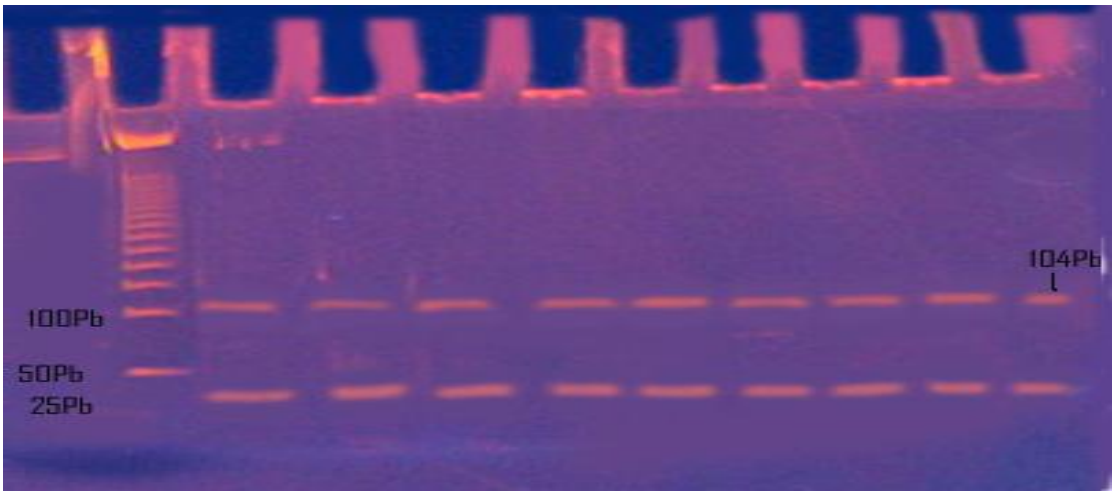

Fig. 1 PCR-RFLP for *TLR2 gene* digested with *MSP1*restriction enzyme, only wild type of the gene was detected.

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