RESEARCH ARTICLE

The Role of IL-6 Gene Polymorphism in Multidrug-Resistant Tuberculosis Patients in Iraq

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ABSTRACT

Background: Tuberculosis (TB) remains to be a leading cause of morbidity and mortality in developing countries, and the incidence of the disease is increasing in developed. One-third of the world's population is infected by *Mycobacterium tuberculosis* (Mtb). Still, only about 5% of infected individuals develop the disease within the first year of infection, and another 5% develop the disease later in life.

Aim of the study: Is to investigate the relationship between the levels of Interleukin 6 (IL-6) and detection of multidrug-resistant tuberculosis (MDR-TB) in Al-Diwaniyah population.

Patients and methods: The current study included 120 patients with tuberculosis who were classified into two groups. The first group included 60 TB patients who were sensitive to anti TB drugs and 60 patients with multi-drug resistance (MDR) based on gene Xpert. The study also included 60 healthy individuals serving as a control group. Demographic characteristics of study and control groups were retrieved. IL-6 (-572G/C) genotype polymorphism was carried out using polymerase chain reaction (PCR) and according to the instruction of the providing company. Serum level of IL-6 was also measured by ELISA according to the instruction of the providing company.

Results: It has been observed that genotype CC was more frequent in sensitive TB patients than both MDR TB patients and control subjects, 73.3 % versus 60 % and 61.7 %, respectively. Serum IL-6 level was significantly higher in MDR TB patients than in control group ($p = 0.007$), and there was no significant difference in its level between sensitive and MDR TB patients ($p = 0.284$).

Conclusion: IL-6 gene polymorphism is not associated with multidrug resistance in TB patients; however, Serum IL-6 level was significantly higher in MDR TB patients than in the control group.

Keywords: IL-6 gene polymorphism, Mycobacterium tuberculosis, Tuberculosis.

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INTRODUCTION

Tuberculosis (TB) remains to be a leading cause of morbidity and mortality in developing countries, and the incidence of the disease is increasing in developed countries.[1] One-third of the world's population is infected by *Mycobacterium tuberculosis* (Mtb), but only about 5% of infected individuals develop the disease within the first year of infection, and another 5% develop the disease later in life. Candidate gene and association studies have identified various host genetic factors that play significant roles in susceptibility to TB.[2] Identifying the host genes responsible for susceptibility and resistance to TB may lead towards a better understanding of the pathogenesis of TB and the development of prophylactic or treatment strategies.[3]

The emergence of antimicrobial resistance against Mycobacterium tuberculosis, the leading cause of mortality due to a single microbial pathogen worldwide, represents a growing threat to public health and economic growth. The global burden of MDR-TB has recently increased by an annual rate of more than 20%. According to the World Health Organization (WHO), approximately only half of all patients treated for MDR-TB achieved a successful outcome.[4]. For many years, patients with resistant TB have received standardized treatment regimens, thereby accelerating the development of MDR-TB through drug-specific resistance amplification. Comprehensive drug susceptibility testing (phenotypic and/or genotypic) is necessary to inform physicians about the best drugs to treat individual patients with tailor-made treatment

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regimens. Phenotypic drug resistance can now often, but with variable sensitivity, be predicted by molecular drug susceptibility testing based on whole genome sequencing, which in the future could become an affordable method for the guidance of treatment decisions, especially in high-burden/resource-limited settings.[5] More recently, MDR-TB treatment outcomes have dramatically improved with the use of bedaquiline-based regimens. Ongoing clinical trials with novel and repurposed drugs will potentially further improve cure-rates and may substantially decrease the duration of MDR-TB treatment necessary to achieve a relapse-free cure.[4]

Most studies have not differentiated TB disease according to its natural history. Therefore, it is difficult to evaluate the differences in their results. In the present study, groups were stratified into sensitive TB infection and multidrug resistant TB disease in addition to a control group. The influence cytokine gene polymorphisms were investigated in association with the susceptibility of individuals to TB disease and also to their association with multidrug resistance. Therefore the current study was aiming at investigating the relationship between the levels of some cytokines and detection of MDR-TB in Al-Diwaniyah population.

PATIENTS AND METHODS

This study included 120 patients with tuberculosis who were classified into two groups. The first group included 60 TB patients who were sensitive to anti TB drugs and 60 patients with MDR based on gene Xpert. The study also included 60 apparently healthy individuals serving as a control group.

Demographic characteristics of study and control groups were retrieved. IL-4 (-589C/T) genotype polymorphism was carried out using PCR and according to the instruction of the providing company. The serum level of IL-4 was also measured by ELISA according to the instruction of the providing company.

The study was approved by the institutional ethical approval committee, and verbal consent was obtained from all participants. Data were analyzed using statistical package for the social sciences (SPSS) version 23. Chi-square test was used to study association among categorical variables, whereas independent samples t-test was used to the evaluated difference in mean serum interleukin level. The level of significance was considered at $p \leq 0.05$.

RESULTS

The distribution of patients with TB and control subjects, according to IL-6 (-572G/C) genotypes and alleles and alleles is shown in Table 1. It has been observed that genotype CC was more frequent in sensitive TB patients than both MDR TB patients and control subjects, 73.3% versus 60 and 61.7%, respectively. However, in terms of allelic variation, there was no significant difference in the distribution of patients and control subjects ($p > 0.05$).

Serum Levels of Interleukins in Control And Patients Groups

Serum IL-6 levels were 364.04, 405.46, and 301.07 in sensitive TB patients, MDR TB patients and control subjects, respectively, table 2. There was no significant difference in its level between sensitive TB patients and control subjects ($p = 0.104$). The level was significantly higher in MDR TB patients than in the control group ($p = 0.007$); there was no

Table 1: Distribution of patients and control groups according to IL-6 (-572G/C) genotypes and alleles

IL-6 (-572G/C)	*Sensitive n = 60*		*MD TB patients n = 60*		*Control n = 60*			
Genotype	*n*	*%*	*n*	*%*	*n*	*%*	*P*	
CC	44	73.3	36	60.0	37	61.7	Sensitive vs control	0.007
CG	8	13.3	16	26.7	21	35.0	mdTB vs control	0.117
GG	8	13.3	8	13.3	2	3.3	Sensitive vs mdTB	0.177
Allele	*Sensitive n = 120*		*MD TB patients n = 120*		*Control n = 120*			
C	96	80.0	88	73.3	95	79.2		
G	24	20.0	32	26.7	25	20.8		
Comparison	*P*	*OR*	*95 % CI*					
Sensitive vs Control	0.873	0.95	0.51	1.78				
mdTB vs Control	0.288	1.38	0.76	2.51				
Sensitive vs mdTB	0.222	0.69	0.38	1.26				

Table 2: Serum levels of interleukin 6 (IL-6) in control and patients groups

	Sensitive n = 60		*MD TB n = 60*		*Control n = 60*		*p value*		
Interleukin	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Se vs. C*	*MD vs. C*	*S vs. MD*
IL-6	364.04	220.63	405.46	230.23	301.07	179.28	0.104 NS	0.007 HS	0.284 NS

n: number of cases; SD: standard deviation; Se: sensitive; MD: multi-drug resistance; C: control; S: significant at $P \leq 0.05$; NS: not significant at $P \leq 0.05$; HS: highly significant at $P \leq 0.01$

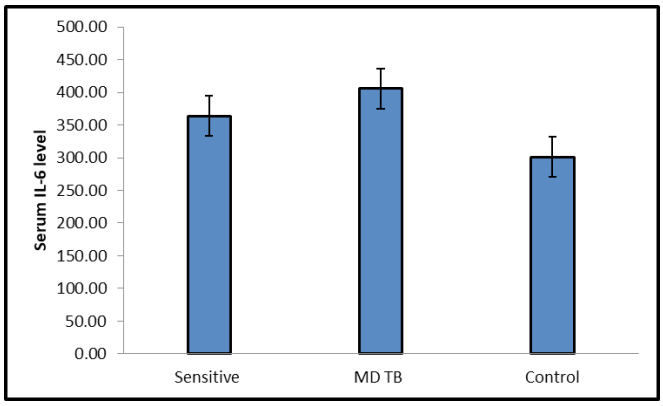

Figure 1: Serum levels of interleukin 6 (IL-6) in control and patients groups

significant difference in its level between sensitive and MDR TB patients (p = 0.284), Table 2 and Figure 1.

Discussion

In the current study, it has been observed that genotype CC was more frequent in sensitive TB patients than both MDR TB patients and control subjects, 73.3 % versus 60 and 61.7%, respectively. However, in terms of allelic variation, there was no significant difference in the distribution of patients and control subjects (p > 0.05). When the results of the current study are taken into consideration, it has been observed that the homozygous GG genotype of IL-6 (-572G/C) was more frequent in a control group than both TB groups, 3.3% versus 13.3 and 13.3%, respectively; and that significant variation was obtained when sensitive TB group contrasted against a control group (p = 0.007); however, no significant level was obtained when contrasting MDR TB group against control group (p = 0.117). Therefore, GG genotype may be associated with less risk of pulmonary TB in comparison with GC and CC genotypes. In accordance with these findings, Wang[6] in their meta-analysis have found that genotype GG is associated with significantly more susceptibility to pulmonary TB than both GC and CC genotypes. In agreement with the finding of the current study, it has been shown than genotype CC is associated with less risk of pulmonary tuberculosis.[7] Similar results were also described in a Chinese study.[8]

It has been reported that IL-6 played a critical role in protection against murine *M. tuberculosis* infection, while it has also been suggested to downregulate macrophage microbicidal activity and promote the growth of *Mycobacterium avium* in vitro. Additionally, environmental factors may interact with gene polymorphism and influence tuberculosis susceptibility. How to adjust the environmental effects on tuberculosis risk is still being investigated. More studies should be performed to investigate the mechanism of how *IL-6* polymorphism regulates IL-6 production and plays a role in tuberculosis susceptibility.[6]

The current study has not supported a role for IL-6 gene polymorphism in patient susceptibility to MDR pulmonary tuberculosis, since contrasting both genetic allelic variation in MDR TB group against both control and drug-sensitive TB patients has failed to show statistical significance. In accordance with these findings, Milano found no significant association between susceptibility to MDR TB and genotype or allelic polymorphism associating IL-6 (-572G/C) gene locus.[9]

In the present study, there was no significant difference in serum IL-6 level between sensitive TB patients and control subjects (p = 0.104); the level was significantly higher in MDR TB patients than in control group ($p = 0.007$) and there was no significant difference in its level between sensitive and MDR TB patients (p = 0.284). These findings are also in agreement with Verbon who stated that serum IL-6 levels are elevated in patients with active TB and those who are already receiving anti-TB treatment.[10] Our results are also in agreement with Joshi, who found that serum IL-6 was elevated in TB patients in comparison with control subjects.[11]

Similar results were obtained in serum from pulmonary tuberculosis patients compared to healthy controls.[12-13] Another study also reported increased IL-6 in subjects with active TB disease compared to those with latent tuberculosis infection.[14] An Asian study showed high IL-6 concentrations in patients with pulmonary cavities than in patients without cavities signifying the disease severity.[15] Another Chinese study also reported increased IL-6 concentration in active TB compared with Latent TB infections, which were family contacts.[16] The increased IL-6 concentration in patients and household contacts when compared with controls may be due to the release of IL-6 into circulation during early stages of infection-causing systemic symptoms and hence the levels may also vary depending upon the clinical status of the patients or contacts. As stated, earlier production of IL-6 is to a large extent under the control of TNF-α and IL-1, and thus its concentration in blood may reflect local production of these cytokines in the lungs.

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