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IL-10-592 A/C Gene Polymorphism and Cytokine Levels are Associated with Susceptibility to Drug Resistance in Tuberculosis

Sitokin Seviyelerinin ve IL-10-592 A/C Polimorfizminin Tüberkülozda İlaç Direnci ile İlişkisi

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Abstract

Introduction: Tuberculosis (TB) and especially resistant forms of it have a substantial economic burden on the community health system for diagnosis and treatment each year. Thus, investigation of this field is a priority for the world health organization (WHO). Cytokines play important roles in the relationship between the immune system and tuberculosis. Genetic variations especially single nucleotide polymorphisms (SNPs) impact cytokine levels and function against TB.

Material and Methods: In this research SNPs in IFN-γ (+874 T/A) and IL-10 (-592 A/C) genes, and the effects of these SNPs on cytokine levels in a total of 87 tuberculosis patients and 100 healthy controls (HCs) were studied. TB patients divided into two groups: 1) 67 drug-sensitive (DS-TB) and 2) 20 drug-resistant (DR-TB) according to drug sensitivity test using polymerase chain reaction (PCR). For the genotyping of two SNPs, the PCR-based method was used and IFN-γ and IL-10 levels were measured by ELISA in pulmonary tuberculosis (PTB) and control group.

Results: In -592A/C SNP, only two genotypes (AA, AC) were observed and both genotypes showed statistically significant differences between DR-TB and HCs (p=0.011). IL-10 serum levels in PTB patients were higher than HCs (p=0.02). The serum levels of IFN-γ were significantly higher in DS-TB patients than that of the other two groups (p<0.001); however, no significant differences were observed for allele and genotype frequencies in IFN-γ +874.

Conclusions: Our results suggest that the SNP at -592 position of IL-10 gene may be associated with the susceptibility to DR-TB. However, further investigation is necessary.

Keywords: Polymorphism, IFN-γ, IL-10, tuberculosis, drug-resistant tuberculosis

Öz

Giriş: Tüberküloz (TB) ve özellikle dirençli tiplerinin tanı ve tedavisi halk sağlığı sistemine önemli bir ekonomik yüke neden olmaktadır. Bu neden ile, bu konuda araştırma yapılması Dünya Sağlık Örgütü (DSÖ) tarafından önceliği olduğu bildirilen bir konudur. Sitokinleri bağışıklık sistemi ve tüberkülozda önemli rol oynamaktadır. Özellikle tek nükleotidde saptanan polimorfizmlerin (TNP) olduğu genetik farklılıklar tüberküloza karşı bağışıklık ve sitokin üretiminde etkilidir.

Gereç ve Yöntemler: Bu çalışmada, 87 hasta ve 100 sağlıklı bireyde IFN-γ (+874 T/A) ve IL-10 (-592 A/C)'un genlerinin TNP'leri ve bu TNP'lerin oluşan sitokinlerin salınımına olan etkileri irdelendi. TB hastaları 2 ayrı gruba ayrıldı: Polimeraz zincir tepkimesi (PZT) ile ilaca hassas (İH) olduğu belirlenen 67 ve ilaca dirençli (İD) olduğu saptanan 20 tüberkülozlu hasta.TNP'ni saptamada PZT temelli bir metod, IFN-γ ve IL-10 düzeyleri için ise, ELISA yöntemi kullanıldı.

Bulgular: Sağlıklı bireyler ve İD TB hastalar arasında -592A/C TNP açısından istatistiksel açıdan anlamlı farklılık saptandı (p=0.011). Akciğer tüberkülozu olan olgulardaki IL-10 düzeyleri sağlıklı bireylerdekine göre yüksek olarak saptandı (p=0.02). İH TB hastalarında serum IFN-γ düzeylerinin diğer gruplardakine göre daha yüksek olduğu saptandı (p<0.001); buna karşın bu hastalarda IFN-γ 874 polimorfizmi açısından allel ya da genotip farklılığı bulunmadı.

Sonuçlar: Çalışmamız, IL-10 geninin 592.pozisyonundaki TNP'nin İD-TB ile ilgili olabileceğini düşündürtmektedir. Ancak, ileri çalışmalara gerek duyulmaktadır.

Anahtar Sözcükler: Polimorfizm, IFN-γ, IL-10, tüberküloz, ilaca dirençli tüberküloz

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Introduction

Drug-resistant TB (DR-TB) disease develops by strains of *Mycobacterium tuberculosis* (Mtb) resistant to anti-TB treatment. Based on the last World Health Organization (WHO) report, the number of new cases resistant to rifampin that is the most effective first-line anti-TB medication is 558.000, of which nearly 458.000 had multidrug-resistant TB (MDR-TB).[1] Although the incidence of TB is notably lower in Iran (21 cases per 100.000 populations) in comparison to neighboring countries, immigration from high TB-burden countries (Afghanistan, Pakistan, Armenia, and Azerbaijan), makes TB controlling programs necessary for preventing the spread of drug-resistant forms of the disease.[2] Previous studies have demonstrated the importance of host genetics in Mtb infection.[3] Although genetic features of bacteria has been shown to be important[4], the effect of host genetic differences on the development of resistant forms of TB remains to be clarified.

The immune interactions with Mtb are associated with the secretion of a variety of cytokines.[5] Some cytokines were reported to exert either anti– or pro-inflammatory effects that lead to the immune-related responses and Mtb infection.[5] In a group of Th1 cytokines, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) were described as the most effective anti-Mtb cytokines.[6] They act cooperatively to form granulomas and activate macrophages.[6] Accordingly, both TNF-α and IFN-γ deficiencies are related to poor granuloma formation, multibacillary lesions, and progressive infections.[6,7] In this regard, a single nucleotide polymorphism (SNP) was described by Pravica et al. at +874 position (A/T; rs2430561) of the first intron of the *IFN-γ* gene, which is the site for nuclear factor-kappa B (NF-κB) transcription factor was found to be related to the production of IFN-γ.[8]

On the other hand, macrophages and T cells produce interleukin 10 (IL-10), which serves as an anti-inflammatory factor.[9] IL-10 down-regulates IFN-γ production of T cells, the secretion of TNF-α and nitric oxide, the expression of co-stimulatory and MHC class II molecules on macrophages.[10] Also, IL-10 can inhibit the infected myeloid cell migration to the lymph nodes and Th1 cells migration from lymph nodes to the lungs by inhibiting the cytokines and chemokines required for this process.[11] In TB patients, elevated levels of IL-10 were detected in serum[12], sputum[13], and pulmonary tissue.[13,14] Moreover, increased IL-10 in TB patients led to progression of the disease and longer survival of Mtb in hosts.[15] This finding suggests that Mtb induces IL-10 production to suppress effective immune responses.[15]

There is the SNP at position -592 (A/C; rs1800872) in the regulatory region located at the upstream of the *IL-10* encoding gene.[16] According to the previous study the -1082G/-819C/-592C (GCC), ACC, and ATA haplotypes were associated high, intermediate, and low IL-10 levels, respectively.[16] The present study investigates IL-10 (-592A/C) and IFN-γ (+874T/A) SNPs and the serum levels of IL-10 and IFN-γ in DS-TB and DR-TB patients.

Materials and Method

Patient Selection

Eighty seven patients with PTB from the tuberculosis division at Masih Daneshvari Hospital, Tehran, Iran, diagnosed between January 2017 and December 2018 were analyzed. The PTB patients were bacteriologically selected as smear-positive with clinical and radiological findings confirming PTB. All patients were HIV-negative and none of them had other infectious diseases or immunosuppressive conditions. According to the drug sensitivity test by PCR, the patients were divided into two different groups; 67 patients were susceptible to the treatment and classified as the DS-TB group, and 20 patients who were resistant to one or more drugs were classified as the DR-TB. Pulmonary infiltrations and cavitation were evaluated by chest radiography (Table 1). The healthy controls (HT) were composed of 100 individuals without a history of TB. The study was validated by the Ethics Committee of Iran University of Medical Sciences (IR. IUMS. REC1394.94–01–30–25728) and all subjects filled and signed written informed consent voluntarily.

Drug Susceptibility Testing

Since PCR–single-strand conformational polymorphism (PCR-SSCP) assessment test was established in 1989, which has been a reliable method for detecting point mutations.[17,18] Mutations in several known genes are responsible for DR-TB. For isoniazid (INH) resistance, different mutations in *katG*, *inhA*, *ahpC*, *kasA* genes, and for rifampin (RIF) resistance, mutations in *rpoB* gene were involved.[19,20] For detecting DR-TB, PCR based

Table 1. Demographic, clinical, and laboratory characteristics of the patient and control groups

Characteristics	DS-TB n=67	DR-TB n=20	Control n=100
Age, median (min-max)	58.5 (20–88)	38.8 (19–74)	46 (23–80)
Gender (Male/Female)	48/19	13/7	64/36
TB exposure, No/Yes %	90/10	70/30	100/0
Smoking, No/Yes %	43/57	55/45	86/14
BK smear, %			–
0–10	13.6%	14.3%	
1+	9.1%	21.4%	
2+	19.7%	21.4%	
3+	57.6%	42.9%	
Pulmonary infiltration, %			– –
>1:3	77%	61%	
≤1:3	23%	39%	
Cavity lesion, %			– –
One side	34%	56%	
Two side	66%	44%	
WBC (10^3/μL), median (IQR)	8.94 (6.74–11.67)	8.18 (6.65–11.05)	5.35 (4.80–8.10)
Absolute neutrophil count (10^3/μL), median (IQR)	6.44 (4.43–8.76)	4.96 (3.48–7.08)	3.45 (3.12–4.15)
Absolute lymphocytes count (10^3/μL), median (IQR)	1.47 (1.15–2.01)	1.89 (1.04–2.67)	1.70 (1.55–1.92)
ADA (U/L), median (IQR)	44 (38–52)	35 (30–41)	10 (6–13)
CRP (mg/L), median (IQR)	64 (47–72)	37 (12.5–58)	2 (0.8–3)
ESR (mm/h), median (IQR)	88 (58–108)	49.5 (21–68)	6 (4–8)

ADA, adenosine deaminase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; TB, tuberculosis; WBC, white blood cell.

drug susceptibility testing (DST) was performed for Mtb culture-positive cases. In the first step, genomic DNA of Mtb isolates was extracted and then different primers were used as follows: The primers were used to detect rifampin resistance TB: rpoB516:5'-CAG CTG AGC CAA TTC ATG GA-3', RIRm: 5'-TTG ACC CGC GCG TAC AC-3', rpoB526:5'-CTG TCG GGG TTG ACC CA-3', rpoB531:5'-CAC AAG CGC CGA CTG TC-3'. Primers were used to detect isoniazid resistance TB: katG5R: 5'-ATA CGA CCT CGA TGC CGC-3', katGOF: 5'-GCA GAT GGG GCT GAT CTA CG-3', inhAP-15:5'-GCG CGG TCA GTT CCA CA-3', inhAPF2:5'-CAC CCC GAC AAC CTA TCG-3'. Finally, PCR was optimized for detecting clinical isolates and the PCR products were denatured and run on the polyacrylamide gel.

Genotyping of IL-10 and IFN-γ Polymorphisms

Five mL of EDTA-containing whole blood samples were taken and by the salting-out method, genomic DNA was extracted. Genotyping of IL-10 SNP at -592A/C (rs1800872) positions and IFN-γ+874A/T (rs2430561) was carried out on DNA samples by PCR-sequence specific primer (PCR-SSP) method. In brief, two tube reactions were prepared for each SNP, one for the normal allele and another for the variant allele. To check the success of PCR amplification, human growth hormone (hGH) primers were used as the internal control. The PCR mixtures consisted of Taq DNA Polymerase 2x (Ampliqon, Denmark) having 1.5 mM $MgCl_2$ in final concentration, 100 ng/μL DNA sample, different amounts of each specific primer, and hGH primers. Forward and reverse primers are shown in Table 2. The PCR reactions for IFN-γ+874 SNP included; initial denaturation at 95°C for 1 min, 10 cycles (95°C for 15 sec, 62°C for 50 sec, and 72°C for 40 sec), followed by 20 cycles (95°C for 20 sec, 56°C for 50 sec, and then 72°C for 20 sec) with a final extension for 5 min. Furthermore, IL-10–592 SNP genotyping was investigated under the condition of initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 67°C for 25 sec, and extension at 72°C for 30 sec. The genotype of each SNP was determined by the length of the amplicon after gel electrophoresis on 2% agarose gel which stained with 0.01 μg/mL safe DNA gel stain (Figure 1). The genotypes were selected for sequencing to confirm the SNPs. The PCR product for sequencing was prepared in a 50 μL reaction mix. In order to confirm the validity of

Table 2. Oligonucleotide primers for amplification of IL-10 and IFN-γ SNPs by PCR-SSP

Gene name (nucleotide change)	Primer 5′-3′	Ta (°C)	Amplicon (bp)
IL-10–592 (A/C)	Forward (A): CTG TGA CCC CGC CTG TA Forward (C): CTG TGA CCC CGC CTG TC Common reverse: CAA GCC CCT GAT GTG TAG A	67	600
IFN-γ+874 (A/T)	Forward (A): TTC TTA CAA CAC AAA ATC AAA TCA Forward (T): TTC TTA CAA CAC AAA ATC AAA TCT Common reverse: TCA ACA AAG CTG ATA CTC CA	56/62*	262
Internal control (hGH)	Forward: GCC TTC CCA ACC ATT CCC TTA Reverse: TCA CGG ATT TCT GTT GTG TTT C	62	429

* two-step
IL-10, interleukin-10; IFN-γ, interferon-gamma; hGH, human growth hormone.

primers functions (the SNP products), we used confirmed sequences as controls for these SNPs.

Cytokine Assay

IL-10 and IFN-γ serum levels were measured by commercial assay kits (BD Opt EIA for human, USA) according to the instructions of the manufacturer. In brief, 100 µL diluted capture antibody (Ab) added to each well and incubated at 4°C overnight. Each well was aspirated and washed three times with phosphate-buffered saline (PBS). Then we blocked wells using 200 µL assay diluent (PBS with 10% fetal bovine serum) at 25°C for 1 h, after repeating the wash step, 100 µL either standard or sample was added to each well and incubated for 2 h at 25°C; subsequently, the wash was repeated five consecutive times. At this step, 100 µL working detector (detection Ab + Streptavidin-HRP) was added to each well and the plates were incubated at 25°C for 1 h and each well was washed seven times. Then 100 µL substrate solution was added to each well and incubated at 25°C for 30 min (protected from light). We added 50 µL stop solution to each well and read the plate at 450 nm against 630 nm (reference filter) by microplate ELISA reader (Biohit, Finland). Cytokine concentrations were determined based on standard curves and expressed in pg/mL.

Statistical Analysis

To compare demographic, clinical, and other variables between DS-TB and DR-TB patients as well as HCs, binary logistic regression (odds ratio), t-test, and

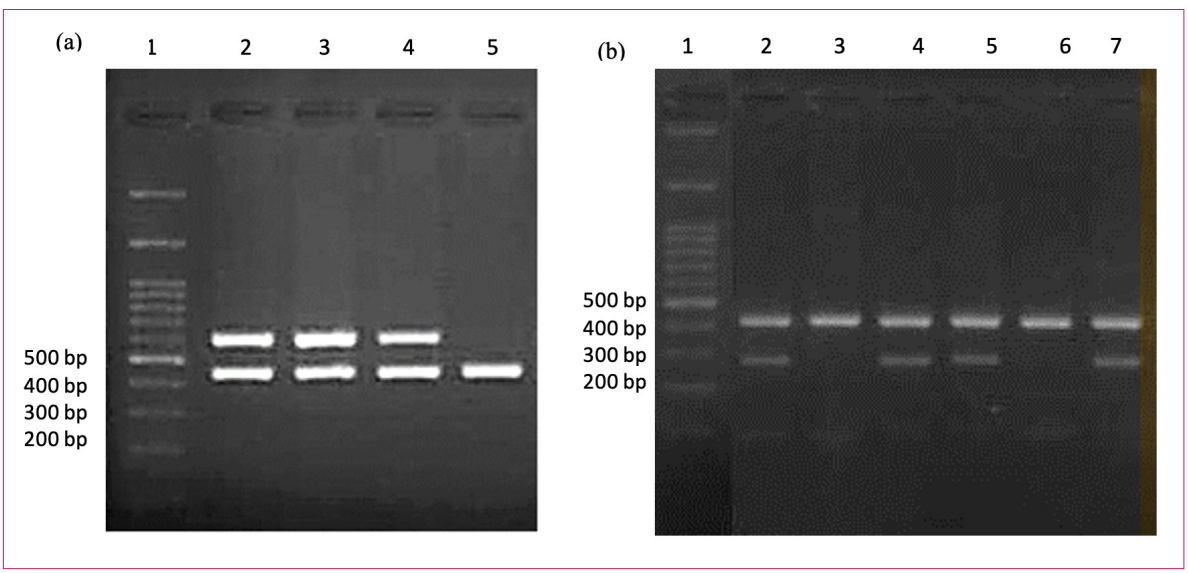

Figure 1. IL-10–592 (A/C) and IFN-γ+874 (A/T) gene polymorphisms. IL-10–592 (A/C), lane 1–100 bp ladder; lane 2 and 3: AC genotype; lane 4 and 5: AA genotype (a). IFN-γ+874 (A/T), lane 1–100 bp ladder; lane 2 and 3: TT genotype; lane 4 and 5: AT genotype and lane 6 and 7 AA genotype (b).

ANOVA were used. By direct counting, the genotype and allele frequencies observed in the control and patient groups were calculated. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed by SPSS version 20 and the graphs were presented using GraphPad Prism software version 6.01.

Results

The clinical and demographic characteristics of the PTB patient and control groups are shown in Table 1. Although 100% of the DR-TB patients indicated a history of TB, the DS-TB group showed only 19.4% with a history of TB (80.6% new cases). The size of the infiltration and incidence of cavitation between the two patient groups did not differ significantly. Also, PTB patients had a significantly higher median in different laboratory parameters (WBC, Neutrophils, ADA, CRP, and ESR) than the HC group.

IL-10 Gene Polymorphism and its Influence on IL-10 Levels

Among the three possible genotypes (AA, AC and, CC), only two genotypes (AA, AC) were observed in both PTB and HCs and the AC genotype was repeated more than others. However, the comparison of the data between three groups showed differences between the DR-TB patients and two other groups, but these dissimilarities were only statistically significant between the HCs vs. DR-TB in both AA and AC genotypes (p=0.011) (Table 3). Consequently, the AC genotype had a protective role against TB (OR=0.2). In addition, the AA genotype was related to a 4-fold increased risk of developing DR-TB in this study (OR=4.4) (Table 3). Moreover, PTB patients showed higher IL-10 serum levels than the HCs (p=0.02) (Figure 2a). There is no difference in serum levels of IL-10 between AA and AC genotypes among the groups studied (Figure 2b–d.). Although, regardless of genotype, the DR-TB patients showed a significant increase in serum levels of IL-10 compared with the DS-TB and HCs (Figure 2a). The Figure 2e shows the comparison of serum IL-10 levels according the genotypes among studied groups.

IFN-γ Gene Polymorphism and its Influence on IFN-γ Levels

Allele frequencies of *IFN-γ* gene polymorphism (IFN-γ+874) did not vary significantly among the HC, DS-TB, and DR-TB groups (Table 3). The frequency of +874 A allele was higher compared with T allele in both PTB and control groups (Table 3). This difference is more obvious in DR-TB patients (Table 3). On the other hand, the AT genotype was significantly overrepresented among the HC, DS-TB, and DR-TB groups. However, no significant differences were found in the frequency of

Table 3. Distribution of IFN-γ +874 (A/T) and IL-10–592 (C/A) genotypes in the study population

Gene polymorphism			DS-TB (%)	DR-TB (%) *p-value*	HCs (%) OR (95% CI)	DS-TB vs. HCs		DR-TB vs. HCs		DS-TB vs. DR-TB	
						p-value	OR (95% CI)	*p-value*	OR (95% CI)	*p-value*	OR (95% CI)
IL-10–592	Allele	A	73 (54.5)	25 (62.5)	107 (53.5)	0.86	1 (0.6–1.5)	0.29	0.7 (0.3–1.4)	0.36	0.7 (0.3–1.5)
		C	61 (45.5)	15 (37.5)	93 (46.5)						
	Genotype	AA	6 (9)	5 (25)	7 (7)	0.64	1.3 (0.4–4.1)	**0.011**	4.4 (1.2–16)	**0.06**	3.4 (0.9–12.6)
		AC	61 (91)	15 (75)	93 (93)	0.64	0.76 (0.2–2.4)	**0.011**	0.2 (0.1–0.8)	**0.06**	0.3 (0.1–1.1)
		CC	0 (0)	0 (0)	0 (0)	-	-	-	-	-	-
IFN-γ+874	Allele	A	76 (56.7)	26 (65)	110 (55)	0.75	0.9 (0.6–1.4)	0.24	0.6 (0.3–1.3)	0.35	0.7 (0.3–1.5)
		T	58 (43.3)	14 (35)	90 (45)						
	Genotype	AA	19 (28.4)	8 (40)	32 (32)	0.61	0.8 (0.4–1.6)	0.48	1.4 (0.5–3.8)	0.32	1.7 (0.6–4.8)
		AT	38 (56.7)	10 (50)	46 (46)	0.17	1.5 (0.8–2.9)	0.74	1.2 (0.4–3.1)	0.6	0.8 (0.3–2.1)
		TT	10 (14.9)	2 (10)	22 (22)	0.25	0.6 (0.3–1.4)	0.22	0.4 (0.1–1.8)	0.6	0.6 (0.1–3.2)

DS: Drug sensitive, DR: Drug resistant, TB: Tuberculosis, HC: Healthy control, Bold font shows statistically significant results

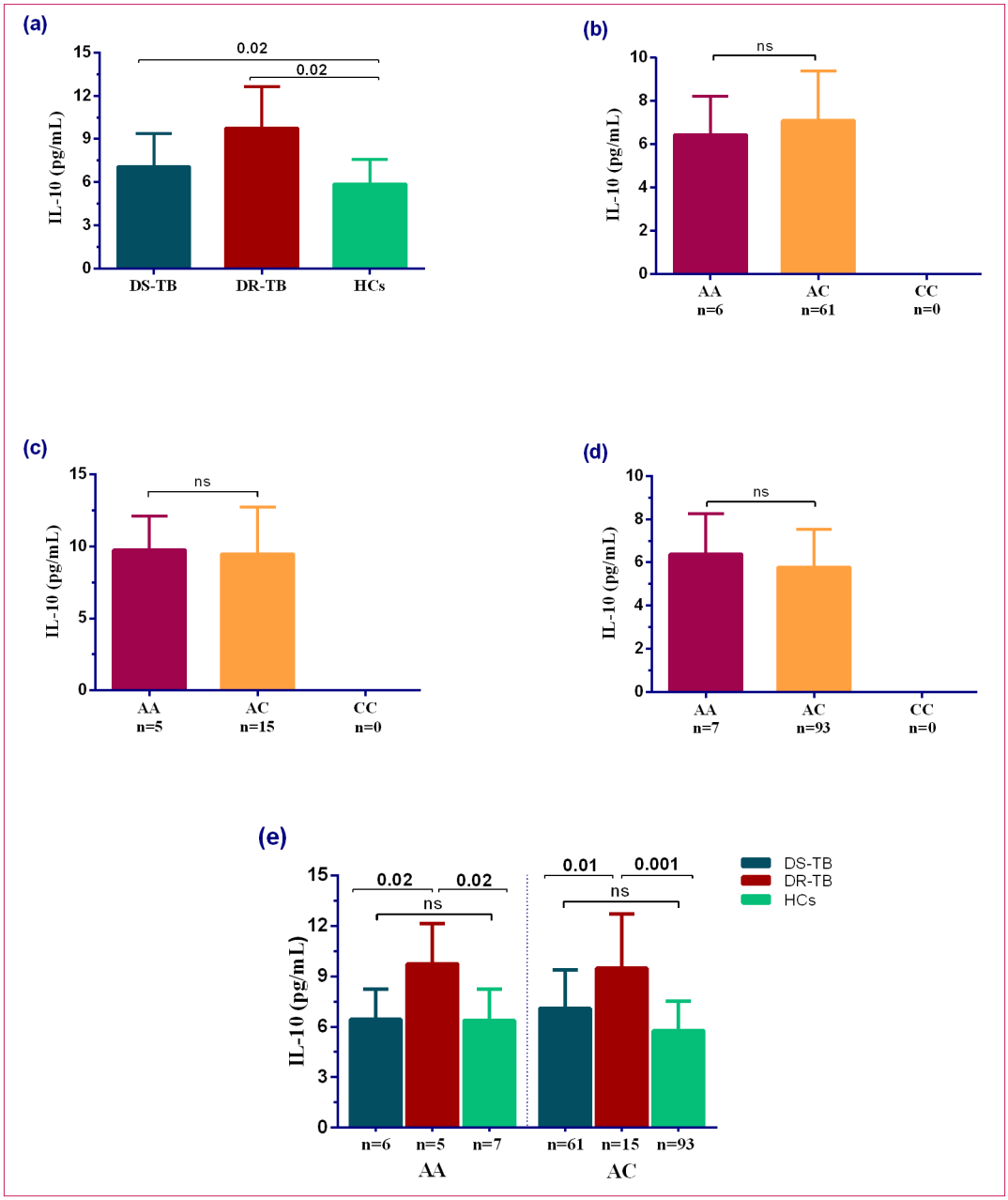

Figure 2. Serum concentrations of IL-10 among three groups (a), in each group [DS (drug-sensitive) (b), DR (Drug-resistant) (c), and HCs (Healthy controls) (d)], and between different groups (e), according to the genotypes. Significant data show in the figure, ns: non-significant

Table 4. Relationship of IFN-γ and IL10 SNPs with serum cytokine levels

Cytokine Polymorphism	Genotype	DS-TB	DR-TB	HCs	Genotypes Comparison of serum Cytokine level	DS-TB	DR-TB	HCs
		Serum cytokine level				*p*-value		
IL10–592	AA	6.4 (5.6–8.2)	9.8 (8.6–12.1)	6.4 (5.9–8.2)	AA vs. AC	ns	ns	ns
	AC	7.1 (5.7–9.4)	9.5 (8.9–12.7)	5.8 (5.3–7.5)	AA vs. CC	ns	ns	ns
	CC	-	-	-	AC vs. CC	ns	ns	ns
IFN-γ +874	AA	8.7 (8.3–16.3)	2.3 (1.8–3.1)	5.8 (1.5–6.8)	AA vs. AT	ns	ns	ns
	TT	17.5 (12.2–32.9)	7.4 (1–13.8)	6.3 (2.3–7.2)	AA vs. TT	**0.02**	ns	ns
	AT	13 (7.3–23.3)	1.9 (1.8–7.9)	6.2 (1.3–8.3)	AT vs. TT	ns	ns	ns

DS: Drug sensitive, DR: Drug resistant, TB: Tuberculosis, HC: Healthy control, ns: non-significant results, Bold font shows statistically significant results

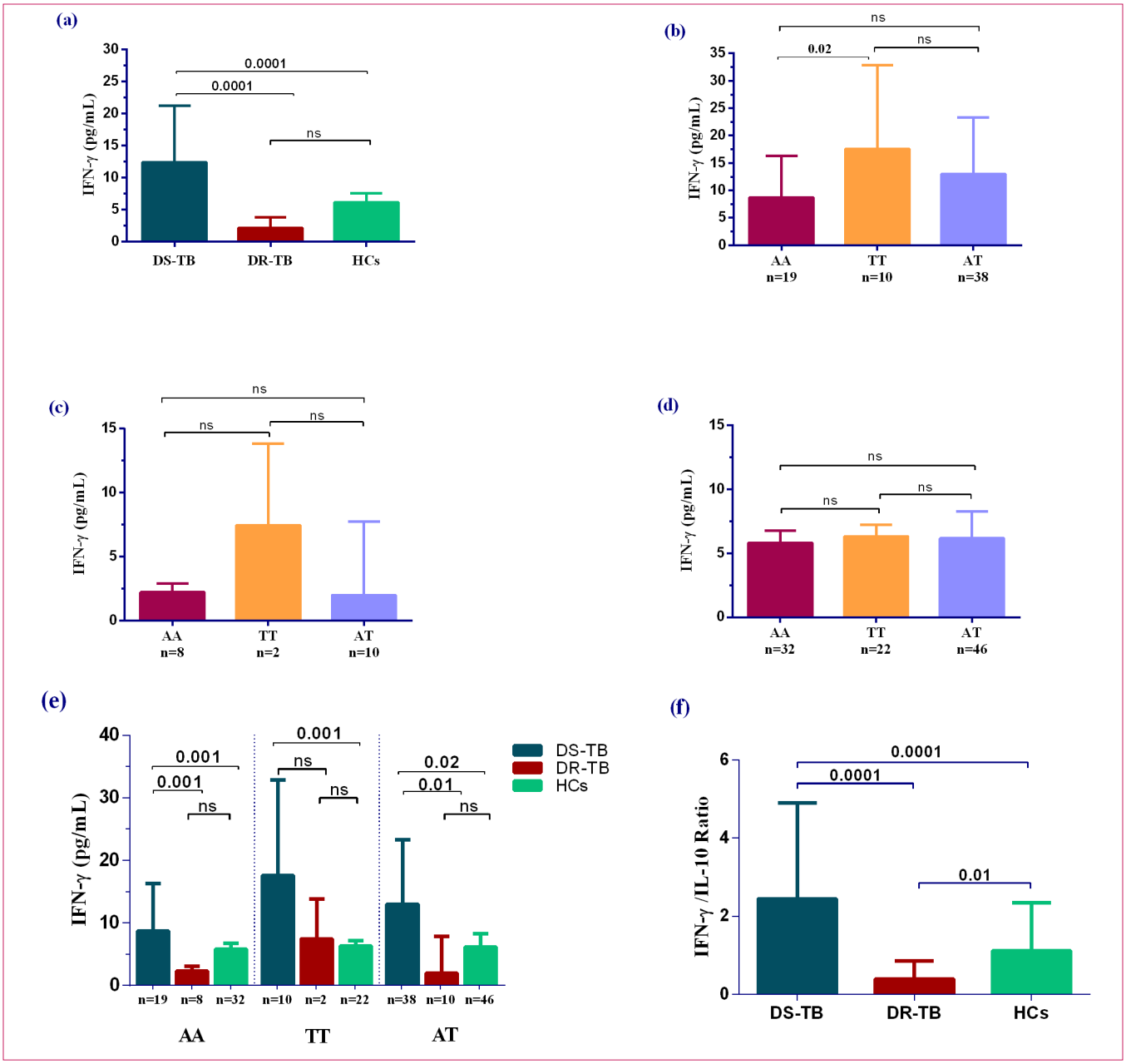

Figure 3. Serum concentrations of IFN-γ among three groups (a), in each group [DS (drug-sensitive) (b), DR (Drug-resistant) (c), HCs (Healthy controls) (d)], and between different groups (e), according to the genotypes. IFN-γ/IL-10 cytokine ratio in serum of the DS-TB, DR-TB, and HCs (f). Significant data show in the figure, ns: non-significant

IFN-γ (+874T/A) genotype among studied groups (Table 3).

The IFN-γ serum levels were higher in DS-TB patients compared with two other groups (p<0.0001) (Figure 3a). At rs2430561 a trend was seen with TT genotype regarding high serum IFN-γ level followed by AT and AA genotypes.[21] Interestingly, our data showed almost the same results in both DS-TB and HC groups, which TT and AA genotypes were the highest and the lowest serum IFN-γ producers, respectively (Figure 3b, 3d, and 3e). However, TT genotype was the highest IFN-γ producers and AT genotype showed the lowest IFN-γ level for the DR-TB patients (Figure 3c and 3e). Genotype comparison in the PTB patients having only TT genotypes showed an increase in IFN-γ levels (p=0.02). Regardless of genotypes, the DS-TB showed higher IFN-γ levels than the DS-TB and HC groups (Table 4 and Figure 3a). The ratio of IFN-γ/IL-10 in serum was significantly higher in the DS-TB group compared with two other groups (p<0.0001),

while the DR-TB group had the lowest ratio among the three groups (Figure 3f).

Discussion

Cytokines, as key regulators of the immune system, have important roles in a variety of immune responses to different pathogens.[22] Mtb is not an exception and any alterations in cytokine genes may lead to inadequate immune responses.[22] DR-TB is now a serious problem in the control and management of Mtb infection, especially in developing countries.[23] Current study showed the association of *IFN-γ* and *IL-10* gene polymorphisms concerning their levels in two different (DS and DR-TB) groups compared to HCs.

IL-10, as an important anti-inflammatory cytokine, has the ability to downregulate Th1 responses.[24] Controversial results were reported in association with *IL-10* gene promoter SNPs and TB.[24–26] Out of three SNPs in the promoter region, the -592C allele was associated with a high IL-10 level in previous studies.[27,28] In the present study, although there was no significant difference in IL-10 serum levels between different genotypes, the frequency of A allele and AA genotype was higher in the DR-TB group. This increment in A allele frequency was also found in Sudan[28], and Korea[29]; however, other studies conducted in Tunisian[27], and Turkish[30] populations did not report the same results. Comparison of IL-10 serum levels among the three groups showed a difference in the values of this cytokine, thus the IL-10 levels in PTB group (DR and DS-TB) were higher than that of the healthy subjects and DR-TB group had the highest IL-10 levels. In contrast to the present study, Butov et al. demonstrated that IL-10 level was lower in the TB group (MDR or non-MDR) at the beginning of treatment and despite its increase during the first two months of the treatment in both TB groups, it was still lower in the MDR-TB group compare to the controls.[31] Increased IL-10 downregulates the secretion of IFN-γ production by T cells[32] and this downregulation might be the reason for the highest IL-10 levels and the lowest IFN-γ levels in the DR-TB group.

Furthermore, it is important to consider some interfering factors such as age and gender differences and their influence on cytokine levels. Larson et al. studied 33 healthy donors and measured the serum levels of 92 cytokines in both sexes and different ages.[33] Although the levels of some cytokines were different at different ages and between men and women, interleukin 10 was not included in these cytokines.[33] Also, Alvarez-Rodrigues et al. analyzed 73 healthy individuals in their research and divided them into three different groups according to age. In their study, they found a positive correlation between IL-10 circulation and age; however, there was no significant difference in IFN-γ serum levels.[34]

IFN-γ is necessary for the protective response to TB.[22] The serum levels of IFN-γ were significantly higher in DS-TB patients than HCs. Elevated IFN-γ serum levels in patients with TB have been reported in previous studies.[12,35] The difference between DR-TB and DS-TB in IFN-γ levels may also reflect the difference in the severity of TB disease in the studied patients, given that in patients with more severe disease, the reduced level of IFN-γ would be expected.[36] Our results showed no association between *IFN-γ* gene polymorphism (+874A/T)) and susceptibility to TB. There are similar studies reported from African American[37], and Indian[25] populations. However, some studies showed positive association and it was indicated that IFN-γ+874 A allele was significantly associated with increased susceptibility to TB, and AA genotype was strongly associated with TB severity at the time of the disease diagnosis.[38,21] Our results indicated that the frequencies of the IFN-γ+874A/T polymorphisms were not different between DS-TB and DR-TB patients. Possibly, the presence of this polymorphism +874A/T by itself is not a certain factor for the development of DR-TB. In previous studies, an association was found between *ABCB1*[39], *HLA-DR*, and *HLA-DQ*[40] gene polymorphisms and DR-TB. IFN-γ variant genotypes have been shown to associate with a changed level of IFN-γ production.

In this study, IFN-γ levels were statistically significantly different between the patients with AA and the one with TT genotype in DS TB group. Similarly, the TT genotype of +874 (A/T) polymorphism was associated with a raised IFN-γ production in response to Mtb antigens in Spanish and Turkish populations.[38,41] IFN-γ/IL-10 ratio might provide a useful indicator of disease severity in patients with TB.[42] Our results showed that the ratio of IFN-γ/IL-10 was significantly lower in the DR-TB patients compared with that DS-TB group. Similar to the study of Shahiratmadja et al. IFN-γ/IL-10 ratio in peripheral blood mononuclear cells was significantly increased in patients with TB before, during, and after the completion of treatment compared to HCs[43], our data are aligned

with suggestions that the bacterial load of the patients in the DR-TB group may be higher due to treatment failure and also protective Th1 responses are more likely disrupted in some patients. However, with a small sample size and because only a few reports are available regarding the role of host genetics in the development of DR-TB; these data must be interpreted more cautiously. Moreover, the difference in results between this study and others could be due to the various haplotype structures associated with specific ethnic groups.

Conclusion

In brief, the results presented here demonstrated that single nucleotide polymorphisms in the cytokine genes may have a role in variation of certain serum cytokine levels and may be counted as the risk factors for tuberculosis, especially for DR-TB. Since various factors, such as cytokine measuring methods and the inclusion criteria could possible affect the results caution must be paid to interpret the results. So further studies with a larger sample size and special attention to the synergistic role of cytokines (such as the synergistic function of IFN-γ and TNF-α) are warranted. Also, the study of other upstream and downstream proteins involved in the secretion of IFN-γ and IL-10 may improve our insight into the mechanism of such a complex disease.

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