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Relationship Between Common Polymorphisms of NRAMP1 Gene and Pulmonary Tuberculosis in Iranian Lur Population

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ABSTRACT

This study aimed to investigate the relationship between the common polymorphisms of the NRAMP1 gene and the genetic susceptibility to pulmonary tuberculosis in the Lur Population of Lorestan province of Iran. In this case-control study, 100 patients with pulmonary tuberculosis were studied as a case group, and 100 healthy controls were studied as controls; and three common polymorphisms of the NRAMP1 gene (3'UTR, INT4, and D543N) were genotyped using PCR-RFLP. The GG genotype of D543N polymorphism (OR=2.042, 95%CI=1.024-4.071) and G allele of D543N polymorphism (OR=2.043, 95%CI=1.140-3.663) were significantly associated with increased susceptibility to pulmonary tuberculosis. On the other hand, the frequency of allele A of D543N polymorphism was significantly lower in patients with pulmonary tuberculosis (OR=0.489, 95%CI=0.273-0.878). The GG genotype and G allele of D543N polymorphism significantly increase the genetic susceptibility to pulmonary tuberculosis in the Lur Population of Lorestan province. Also, allele A of D543N polymorphism significantly affects resistance to pulmonary tuberculosis in this population.

Keywords: NRAMP1; Pulmonary tuberculosis; Lur Population; Lorestan province

Introduction

Pulmonary tuberculosis (TB) is a chronic infectious disease that is one of the most important causes of death in developing countries. TB is caused by Mycobacterium tuberculosis, which affects the lungs. The WHO has reported that 1.5 million people died from pulmonary tuberculosis in 2020. Pulmonary tuberculosis, with 8.8 million new infections each year after COVID-19 and even more than HIV/AIDS, is the second leading infectious cause of death and the 13th leading cause of death in the world. About one-third of the world's people have been infected with Mycobacterium tuberculosis, but only 5-10% of them will develop active pulmonary tuberculosis [1]. According to the WHO report, 5.6 million men, 3.3 million women, and 1.1 million children have been diagnosed with pulmonary tuberculosis in 2020. Although pulmonary tuberculosis has been observed in all age groups and all countries of the world, on the other hand, it is preventable and curable. In various studies, it has been reported that most people who have contracted tuberculosis reside in China, Pakistan, South Africa, India, the Philippines, and Nigeria. This suggests that genetic and ethnic differences may influence

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susceptibility to pulmonary tuberculosis. The most important risk factors for pulmonary tuberculosis include alcohol abuse, diabetes, HIV, chronic corticosteroid treatment, malnutrition, advanced age, weakened immunity, socioeconomic status, and genetic factors [2].

The role of genetic factors in susceptibility to pulmonary tuberculosis is still inconclusive. Exposure to these risk factors was not a definite reason for pulmonary tuberculosis. Therefore, genetic factors such as single nucleotide polymorphisms may play an effective role in pulmonary tuberculosis. Also, some studies have shown that individuals' genetic susceptibility and environmental factors may play a role in the mechanism of pulmonary tuberculosis infection [3].

In several studies, the role of different genes in susceptibility to pulmonary tuberculosis has been investigated, indicating the role of ethnicity in the disease. It has also been reported that these genetic factors are associated with the severity of the disease and concurrent infection with various diseases (especially HIV). Most of these genes are localized in the loci involved in the immune response, which shows that people's genetic background is effective in the immune response and the potential for pulmonary tuberculosis [4]. The KIR3DS1 gene combines with HLA-B Bw4 and Ile80 ligand [5, 6]. Also, other studies showed Toll-like Receptors (TLRs) [7-11], Mannose Binding Lectin (MBL) [12], Purinergic receptor P2X, ligand-gated ion channel 7 (P2X7) [13], Interlukin-10 (IL-10) [14] and Tumor Necrosis Factor-α (TNF-α) [15], vitamin D receptor (VDR) [16] has roles in the genetic susceptibility or resistance to TB. When infected with Mycobacterium tuberculosis, the patient's immune system attempts to eliminate the pathogen by producing defensive protein molecules and recruiting phagocytic cells.

Natural resistance-associated macrophage protein 1 (NRAMP1) is also called the solute carrier family 11 proton-coupled divalent metal ion transporter membrane1 (SLC11A1). It plays an important role in creating an immune response in PULMONARY TB[10, 17]. NRAMP1 encodes a divalent transition metal (Fe and Mn) transporter on the lysosomal membrane [18]. In addition, Iron has a crucial role in producing reactive oxygen and nitrogen intermediates in macrophages and is an important mycobacterial nutrient [19].

Studies have shown that NRAMP1 is associated with various inflammatory and infectious diseases. They include Leishmania, Salmonella, and Mycobacteria. Immune cytochemical studies show that NRAMP1 protein exhibits pleiotropic effects on macrophage activation, including up-regulation of MHCII molecules, increased production of ROS, increased expression of iNOS (inducible NO synthase), reactive nitrogen intermediates involved in the oxidative burst, increased production of the proinflammatory cytokines, such as IL1-β and TNFα, regulation of chemokines and increased NO release [20], regulation of intracellular membrane vesicle trafficking of [21, 22], regulation of cytoplasmic cation levels that promotes the generation of toxic hydroxyl radicals, which significantly contribute to the killing of intracellular pathogens[23], Although it is not yet known exactly the function of human NRAMP1 protein.

NRMAP1 polymorphisms contains rs17235416 (3'UTR), rs17235409 (D543N), rs3731865 (INT4), and rs34448891 (5 = (GT) n). In several studies, the relationship between NRAMP1 polymorphisms and the risk of pulmonary tuberculosis (PTB) has been investigated; however, due to the low sample size in some studies and different ethnicities, the results of these studies could be more consistent and conclusive [24, 25]. In a study conducted by Medapati et al. [25] in the Indian population, the results showed a significant relationship between NRAMP1 3'UTR polymorphism and genetic susceptibility to pulmonary tuberculosis. However, no significant relationship was observed in the study of Jafari et al. [26].

Studies conducted in West African populations have shown a significant relationship between NRAMP1 gene

polymorphisms (3'UTR, INT4, and D543N) and genetic susceptibility to pulmonary tuberculosis in some populations, including the Gambian population [27]. However, there was no significant relationship between some populations, including Thais [10], Brazilians [28], Taiwanese [29], and Moroccans [30].

The present study aimed to investigate three polymorphisms in the NRAMP gene: INT4 (469+14G>C) rs3731865, D543N (codon 543 Asp to Asn) rs17235409, and 3'UTR (1729 + 55del4) rs17235416, about the genetic susceptibility to TB in Lur population residents of Lorestan Province of Iran. Identification of host genes in different ethnicities can effectively identify the role of ethnic differences in genetic susceptibility to some infectious diseases. It may also play an effective role in our knowledge of pathogenesis, prevention, and treatment of pulmonary tuberculosis. This article is based on the STROBE statement guidelines for reporting observational studies [31]. The impact of this study is that the present project's results provide information for this ethnicity to be used in the future after the development of personalized medicine.

Materials and Methods

Study design

In this case-control study, the number of patients in the case group and the control group were determined using the statistical formula, and based on previous studies, 100 subjects were assigned to each group. The statistical population of the patient group included all patients with pulmonary TB referred to Khorramabad Clinic of Health, whose disease was confirmed by histological and sputum culture. The case group was 100 Iranian unrelated Lur patients with Pulmonary Tuberculosis who were referred to Khorramabad Health Center. All patients received standard tuberculosis treatment, and none had drug resistance. The statistical population of the control group was selected from healthy individuals of the Lur population in the Lorestan province. The study included the control group by simple sampling (accessible) and based on age and gender matched with the patient population. The control group had no history of pulmonary tuberculosis and no evidence of previous radiographic changes of the chest associated with tuberculosis. All subjects under study had parents of the same race. Individuals who had any of the exclusion criteria were excluded from the study.

Inclusion and exclusion criteria

The Inclusion criteria were: patients with newly diagnosed PTB, having Lur ethnicity, having PTB symptoms, Sputum smear-positive, a chest radiograph of active disease, and satisfaction to participate in the study. Also, people with diabetes mellitus, ischemic heart disease, chronic renal failure, any chronic inflammatory disease, jaundice, hepatitis C, HIV, any autoimmune disease, immunosuppressive drugs, HBsAg positive, and People who did not agree to participate in the study were excluded.

DNA Extraction

After obtaining informed written consent from 100 patients with PTB and 100 healthy controls who entered the study, 2ml of peripheral blood was taken in the EDTA anticoagulant tubes. After transferring the samples to the Laboratory of Immunogenetics, using the kit QIAmp (Qiagen-Germany) according to the manufacturer's protocol, DNA was extracted from genomic samples of peripheral blood leukocytes. After quantitative and qualitative measurements, DNA samples were stored at -70 ° C until testing time.

Genotyping by PCR-RFLP

In this study, the most common polymorphisms of the NRAMP1 gene, INT4 (469 + 14G/C) (rs3731865), 3'UTR (1729 + 55del4) (rs17235416), and D543N (codon 543, Asp to Asn) (rs17235409) were examined in the patients and control groups. The temperature conditions of the PCR reaction are listed in Table 1. PCR amplification was performed using pure Mastercycler DNA (BioRad, USA) in a 20µl reaction volume.

The PCR-RFLP technique, previously used by Medapati et al. in 2008 [34], was used to determine the common polymorphisms of the NRAMP1 gene. Forward and reverse primer sequences of NRAMP1 gene polymorphisms designed in previous studies were used. These sequences are listed in Table 2.

In order to perform RFLP for 3'UTR polymorphism, 20µl of amplicon was digested with five units of Fok1 digesting enzyme at 37°C for 2 hours. For the TGTG + allele, the length of the fragments was 33+211 bp, and for the TGTGdel allele, the length was 240 bp [25, 34].

In order to perform RFLP for INT4 polymorphism, 20µl of amplicon was digested with five units of ApaI digesting enzyme at 37°C for 2 hours. For the G allele, the length of the fragments was 455 bp; for the C allele, the length was 169 bp [33, 34].

In order to perform RFLP for D543N polymorphism, 20µl of amplicon was digested with five units of AvaII digesting enzyme at 37°C for 2 hours. For the G allele, the length of the fragments was 126+79+39 bp; for the A allele, the length was 211+33 bp [33, 34].

The proliferated PCR product was electrophoresed on a 2% agarose gel containing ethidium bromide. Bands appeared under ultraviolet light. Genotypes were determined according to the pattern of the produced part. Finally, to ensure the correct result of the PCR, we randomly sent 5% of the samples to determine the DNA sequence.

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Table 1. Thermal conditions of PCR reaction [25]

Number of cycles	Steps	Duration	Temperature
1	Initial denaturation	5 minutes	94 °C
35	denaturation	30 seconds	95 °C
	Annealing	30 seconds	52 °C
	Extension	30 seconds	72 °C
1	Final elongation	10 minutes	72 °C

Table 2: Primer sequences of NRAMP1 gene polymorphisms

Common gene polymorphisms of NRAMP1	Primer sequences	Digestive Enzyme	Fragment Length (bp)	Reference
3′UTR (1729 + 55del4) (rs17235416)	F: 5'-GCA TCT CCC CAA TTC ATG GT-3' R: 5'-AAC TGT CCC ACT CTA TCC TG-3'	*Fok1*	244 TGTG+ (211+33) TGTGdel (240)	(38,58)
INT4 (469 +14G/C) (rs3731865)	F: 5'- TCTCTGGCTGAAGGCTCTCC -3' R: 5'- TGTGCTATCAGTTGAGCCTC -3'	*Apa I*	624 G (455) C (169)	(38,59)
D543N (codon 543, Asp to Asn) (1627G/A) (rs17235409)	F: 5'- GCA TCT CCC CAA TTC ATG GT -3' R: 5'- AAC TGT CCC ACT CTA TCC TG -3'	*Ava II*	244 G (126+79+39) A(211+33)	(38,59)

Statistical Analysis

After data collection, the data were entered into SPSS 18 software, and statistical analysis was performed. Genotypic and allelic frequencies of common polymorphisms of NRAMP1 gene (INT4 (469+ 14G/C) (rs3731865), 3'UTR (1729 + 55del4) (rs17235416) and D543N (codon 543, Asp to Asn) (rs17235409) in the patient group and control group was determined by direct counting. Departure from Hardy-Weinberg equilibrium and frequency of all polymorphisms in the patient and control groups were determined. Using descriptive statistics (frequency and percentages), the results were presented in statistical tables, and analytical statistics (t-test and chi-square) were used to measure the association and effect of the variables. The Chi-Square and Fisher's exact test calculated differences in genotypic and allelic distribution of NRAMP1 gene polymorphisms between the patient and control groups. Finally, $P<0.05$ was considered statistically significant. Also, the information analysis used odds ratio (OR) and 95% confidence interval (CI).

Ethical considerations

The protocol of this study was approved by the Ethics Committee in the research of Lorestan University of Medical Sciences with the code of ethics IR.LUMS.REC.1395.114. Entry into the study was voluntary. Before the beginning of the study, informed consent was obtained from each patient for using patient information in relevant research. Also, all patient information was considered a secret, and any disclosure was refused. Identification codes were used to prevent the registration of patients' names and last names. The data were confidential and recorded as SPSS software by numerical codes.

Results

In this case-control study, the case group comprised 46 males and 54 females, and the control group comprised 52 males and 48 females. The mean age was 34.73 ± 3.21(in the age range of 26 to 57 years old) in the case group, and 31.12 ± 2.25 (in the age range of 23 to 61 years old) in the control group. Genotypic and allelic distribution of NRAMP1 polymorphisms were not diverted from Hardy-Weinberg equilibrium in all samples. The genotypic frequency of 3'UTR, INT4, and D543N polymorphisms and the association of each with PTB genetic susceptibility are presented in Table 3.

As shown in Table 3, for the 3'UTR polymorphism, the frequency of TGTG +/+, TGTG +/del, and TGTG del/del genotypes in the patient group was 91%, 3%, and 6%, respectively, and in the control group, was 89%, 8%, and 3%, respectively. For the INT4 polymorphism, the frequency of GG, GC, and CC genotypes in the patient group was 69%, 29%, and 2%, respectively, and in the control group, was 71%, 26%, and 3%, respectively. For the D543N polymorphism, the frequency of GG, GA, and AA genotypes in the patient group was 84%, 12%, and 4%, respectively, and in the control group, was 72%, 19%, and 9%, respectively.

GG genotype of D543N polymorphism was significantly associated with increased susceptibility to pulmonary tuberculosis (84% in the case group vs. 72% in the control group, 95%CI= 1.024-4.071, OR=2.042, P=0.0405). This genotype almost doubles the chance of having PTB in the population under study (OR=2.042). The genotypic frequency of 3'UTR and INT4 polymorphisms was not significantly different between the patients and the control group.

Table 4 presents the allelic frequency of 3'UTR, INT4, and D543N polymorphisms and their association with PTB genetic susceptibility. As shown in Table 4, for the 3'UTR polymorphism, the frequency of TGTG + and TGTG del alleles in the patient group was 92.5% and 7.5%, respectively, and in the control group was 93% and 7%, respectively. For the INT4 polymorphism, the frequency of G and C alleles in the patient

group was 83.5% and 16.5%, respectively, and in the control group, was 84% and 16%, respectively. For the D543N polymorphism, the frequency of G and an allele in the patient group was 90% and 10%, respectively, and in the control group, was 81.5% and 18.5%, respectively.

G-allele of D543N polymorphism was statistically significantly associated with increased susceptibility to pulmonary tuberculosis (90% in the case group vs. 81.5% in the control group, 95%CI= 1.140-3.663, OR=2.043, P=0.015). This allele almost doubles the chance of having pulmonary tuberculosis in the population under study (OR=2.043).

The frequency of allele A of the D543N polymorphism was significantly lower in patients than in the control group (10% in the case group vs. 18.5% in the control group, 95%CI= 0.273-0.878, OR=0.489, P=0.015). This allele, 51%, reduces the chance of having pulmonary tuberculosis in the population under study (OR=0.489).

The allelic frequency of 3'UTR and INT4 polymorphisms was not significantly different between the patients and the control group.

Table 3. Genotype distribution of NRAMP1 polymorphisms in patients and controls

NRAMP1 polymorphisms	Genotypes	Percentage of patients group	Percentage of control group	OR	95%CI	P Value
3′UTR (rs17235416)	TGTG +/+	91	89	1.250	0.494-3.162	0.6374
	TGTG +/del	3	8	0.356	0.092-1.382	0.1209
	TGTG del/del	6	3	2.064	0.502-8.493	0.3062
INT4 (rs3731865)	GG	69	71	0.909	0.496-1.665	0.7576
	GC	29	26	1.163	0.624-2.164	0.6347
	CC	2	3	0.660	0.108-4.037	0.6506
D543N (rs17235409)	GG	84	72	2.042	1.024-4.071	**0.0405***
	GA	12	19	0.581	0.266-1.272	0.1714
	AA	4	9	0.421	0.125-1.416	0.1515

Table 4. Allelic distribution of NRAMP1 polymorphisms in patients and controls

NRAMP1 polymorphisms	Alleles	Percentage of patients group	Percentage of control group	OR	95%CI	P Value
3′UTR (rs17235416)	TGTG +	92.5	93	0.928	0.436-1.978	0.8471
	TGTG del	7.5	7	1.077	0.506-2.295	0.8471
INT4 (rs3731865)	G	83.5	84	0.964	0.567-1.640	0.8922
	C	16.5	16	1.037	0.610-1.765	0.8922
D543N (rs17235409)	G	90	81.5	2.043	1.140-3.663	**0.015***
	A	10	18.5	0.489	0.273-0.878	**0.015***

Discussion

Pulmonary tuberculosis is one of the common infectious diseases that annually causes more than three million deaths worldwide. The causative agent of this disease is Mycobacterium tuberculosis. Despite the implementation of extensive control programs over the past two decades, PTB remains a high-risk infectious disease. On the other hand, the emergence and expansion of drug-resistant strains has created new concerns globally. Studies have shown that the sensitivity to the disease, and even its course and its trends among different people, is different, and any person exposed to this bacterium does not necessarily have tuberculosis. These differences can be due to host factors, especially the genetic susceptibility of different individuals to the disease [35].

So far, no study has been done to investigate the role of common NRAMP1 gene polymorphisms with genetic susceptibility to PTB in the Lur population of Lorestan province.

In the present study, we observed that the GG genotype of D543N polymorphism was statistically significant associated with increased genetic susceptibility to PTB. This genotype almost doubles the chance of having PTB in the population under study. G allele of D543N polymorphism was significantly associated with increased genetic susceptibility to PTB. This allele almost doubles the chance of having PTB in the population under study. The frequency of allele A of D543N polymorphism was significantly lower in patients than in the control group. This allele, 51%, reduces the chance of having PTB in the population under study. The genotypic and allelic frequency of 3'UTR and INT4 polymorphisms was not significantly different between the patients and the control group.

Various studies have investigated the role of common NRAMP1 gene polymorphisms in genetic susceptibility or resistance to PTB in different populations, and they have reported different results.

3'UTR Polymorphism

As it was observed, there was no statistically significant relationship between any of the genotypes and alleles of TGTG +/+, TGTG +/ Del, TGTG del/del, TGTG + and TGTG del with genetic susceptibility to or resistance to PTB in the studied population. These results were consistent with the results of some similar studies. For example, in 2010, a study by Hatta et al. was conducted on 58 patients with PTB and 198 healthy individuals in Indonesia using PCR-RFLP techniques. In their study, there was no significant correlation between genotypes and alleles of 3'UTR polymorphism with genetic susceptibility to or resistance to PTB [36], also, in the study of Stagas et al. In 2011, no significant correlation was found between genotypes and alleles of 3'UTR polymorphism with genetic susceptibility to PTB [37]. In 2016, a case-control study was performed by Jafari et al. On 94 patients with PTB and 122 healthy individuals from the Iranian population using ARMS- the PCR technique. In their study, there was no statistically significant correlation between genotypes and alleles of 3'UTR Polymorphism with genetic susceptibility to PTB [26]. Also, in 2015, a study by Trifunovic et al. was conducted on 110 patients with pulmonary TB and 67 healthy people from the Serbian population using the PCR-RFLP technique. They observed in their study that there was no significant correlation between genotypes and alleles of 3'UTR polymorphism with genetic susceptibility to pulmonary disease [38]

In 2017, Medapati et al. conducted a case-control study in the Indian population using the PCR-RFLP technique. In their study, there was a statistically significant correlation between genotypes and alleles of 3'UTR polymorphism with increasing genetic susceptibility to pulmonary TB in the Indian population [25].

In 2011, a study by Nugraha et al. was conducted on 69 patients with pulmonary TB and 43 healthy individuals from the Indonesian population. Their study

observed a significant correlation between TGTG del/del genotype 3'UTR polymorphism and the genetic susceptibility to pulmonary tuberculosis (29% in the patient group vs. 5% in the control group) [32]. The results of a meta-analysis study that was conducted in 2012 by Meilang and colleagues found that, in general, in all races studied, 3'UTR polymorphism was significantly associated with increased genetic susceptibility to pulmonary tuberculosis [39].

INT4 Polymorphism

As it was observed, there was no statistically significant relationship between any of the genotypes and alleles of GG, GC, CC, G, and C with genetic susceptibility to or resistance to PTB in the studied population. These results were consistent with the results of similar studies, such as the study of Nugraha et al. In 2011, there was no statistically significant relationship between the genotypes and alleles of INT4 polymorphism with genetic susceptibility to PTB [32]. It was also reported by Trifunovic et al. Study, which there was no statistically significant correlation between genotypes and alleles of INT4 polymorphism in genetic susceptibility to PTB [38]. In the study of Jafari et al. In 2016, no significant association was found between genotypes and alleles of INT4 Polymorphism and genetic susceptibility to PTB in Iranian population [26]. Also, in the study of Hatta et al. in 2010, there was no significant correlation between genotypes and INT4 polymorphism alleles with genetic susceptibility to PTB [36].

On the other hand, some studies have reported a significant association between INT4 polymorphism and PTB, for example, in a meta-analysis study conducted by Meilang et al. In 2012, it was reported that, in general, the INT4 polymorphism was significantly associated with increasing the genetic susceptibility to PTB [39]. Also, in the study of Ghozali et al. in 2016, was observed a significant correlation between the C allele of INT4 polymorphism and PTB (10% in the patient group vs. 2% in the control group) [40]. In 2011, another study by Stagas et al. was conducted on 142 patients with PTB and 144 healthy people from the Egyptian population. Their study observed that the CC genotype of INT4 polymorphism had a statistically significant correlation with the Egyptian population's increased risk of PTB [37]. Also 2017, Yuan et al. conducted a systematic review and meta-analysis. They reported that, in general, and in the comprehensive analysis of studies conducted in different races, INT4 polymorphism significantly increased the genetic susceptibility to PTB. It was also observed that in the Africans, there is a significant correlation between GC genotype and C allele of INT4 polymorphism, with increasing genetic susceptibility to PTB [41].

D543N Polymorphism

As it was observed, there was a significant correlation between GG genotype and G allele with increasing genetic susceptibility to PTB. Also, there was a significant association between allele A and resistance to PTB in the studied population. However, there was no significant relationship between GA and AA genotypes with genetic susceptibility to or resistance to PTB in the studied population. These results were consistent with similar studies, such as a meta-analysis by Meilang et al. In 2012, it was reported that in general, in all of the studied races, D543N polymorphism has a significant correlation with the increased genetic susceptibility to PTB [39]. On the other hand, some studies have reported no significant relationship between D543N polymorphism and PTB. For example, in the study of Ghozali et al. in 2016, no significant association was found between allele A of D543N polymorphism and genetic susceptibility to PTB (16.3% in the patient group vs. 15% in the control group). Also, their study showed no significant correlation between the GA genotype of D543N polymorphism and genetic susceptibility to PTB [40]. Also, in the study of Stagas et al. In 2011, no significant correlation was found between

genotypes and alleles of D543N polymorphism with genetic susceptibility to PTB [37]. In a systematic and meta-analysis study by Yuan et al. in 2017, there was no significant relationship between D543N polymorphism and genetic susceptibility to pulmonary tuberculosis in general and in a comprehensive analysis of studies in different races. Although in the subgroup analysis based on race, there was a significant correlation between GA genotype and allele A of D543N polymorphism in American race, with an increased risk of PTB [41]. Also, in the study of Jafari et al. In 2016, no significant association was found between genotypes and alleles of D543N polymorphism and genetic susceptibility to pulmonary tuberculosis [26], in the study of Trifunovic et al. In 2015, there was no statistically significant relationship between genotypes and alleles of D543N polymorphism in genetic susceptibility to pulmonary disease [38]. In a study by Hatta et al. in 2010, there was no significant correlation between genotypes and alleles of D543N polymorphism with genetic susceptibility or resistance to pulmonary tuberculosis [36], also, in the study of Nugraha et al. In 2011, there was no statistically significant relationship between genotypes and alleles of D543N polymorphism with genetic susceptibility to PTB [32].

Limitations

The limitations and problems of this study can be pointed to as problems with sampling from healthy individuals.

Conclusion

The present study observed that the GG genotype and allele G of the D543N polymorphism of the NRAMP1 gene significantly increased genetic susceptibility to PTB in Lur population residents in the Lorestan province of Iran. Also, allele A of the D543N polymorphism of the NRAMP1 gene is effective in resistance to pulmonary tuberculosis in this population. No significant correlation was found between the genotypes and alleles of the 3'UTR and INT4 polymorphisms of the NRAMP1 gene and the genetic susceptibility to or resistance to pulmonary tuberculosis in this population. These results were in line with the results of some studies carried out for this purpose on other populations, but they differed from the results of others. The contradictory results reported by various studies are not unpredictable because factors such as variation in sample size, differences in the intensity of the relationship in different studies, different genotyping techniques, and different races can lead to differences in results.

In future studies, this study should be done with a larger sample size and with different genotyping techniques on the Lur and other Iranian ethnicities. Similar studies are also suggested to investigate the association of polymorphisms of other genes involved in genetic susceptibility or resistance to pulmonary tuberculosis. So far, their role in genetic susceptibility or resistance to tuberculosis in different ethnic groups has had conflicting results. Clinical studies are suggested for the future in the field of personalized medicine.

Conflict of Interests

Authors declare that they do not have any conflict interests.

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