International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Human genetic susceptibility to tuberculosis

Marwah S. Kadhim[1], Osama Mohammed Ghazi[2], Salam Hussein Alwan[3]*

[1,2,3] *College of Dentistry, University of Al-Qadisiyah, Diwaniya, Iraq*

Abstract

Recent research has shown that genetic variables, including those discovered in the TB genes NRAMP1 (Natural-Resistance-Associated Macrophage Protein 1) and VDR (Vitamin D Receptor) candidate gene, are one of the causes of the development of tuberculosis. The NRAMP1 gene is called the SLC11A1 gene and is placed long arm of chromosome 2 (2q35). The current study aimed to determine human genetic susceptibility to tuberculosis. 35 blood samples were collected in EDTA tubes from patients with TB from Al-Diwanyiah Teaching Hospital, samples were collected and placed in a refrigerator and then used for blood DNA extraction. Demographic characteristics of patients: Age range, Mean ± SD (41.74±8.014), gender: males (16) and female (19), resident: rural (20) and urban (15). Correlation between room number in house and family history of TB cases number in each room's total (35), positive family history of TB (16), and negative family history of TB (19). Relationship between the SNP genotypes of NRAMP1 and males/females with a Family history of TB is statically Significant (P<0.05).

Keywords: Mycobacterium tuberculosis, Genomic ancestry, Polymorphism, NRAMP1-3′UTR.

Full length article **Corresponding Author*, e-mail: salam.hussein@qu.edu.iq

1. Introduction

Tuberculosis (TB) is one of the most dangerous diseases that affect the lungs and it is called pulmonary tuberculosis (PTB). It occurs through coughing and sneezing based on the transmission of bacteria from one infected person to another. Tuberculosis is one of the causes of death worldwide and is due to many causes that may be environmental and hosts genetic factors [1-2]. The World Health Organization (WHO) reports for 2014 on tuberculosis proved that it is one of the most dangerous infectious diseases that led to the death of many people and become a global health problem. In 2013 the number of people infected with tuberculosis reached 9.0 million, and the number of deaths reached 1.5 million, the percentage divided into 1.1 million HIV-negative cases and 0.4 of HIV-positive cases [3-4]. Despite the fact that Mycobacterium tuberculosis (M. TB) is thought to have infected nearly one-third of the world's population, only 10% of those infected go on to acquire an active TB disease [5-7]. According to TB research, genetic factors significantly influence how the disease develops. Natural-Resistance-Associated Macrophage Protein 1 (NRAMP1) and VDR (Vitamin D Receptor) are two of the genes reported to be connected to PTB. Potential genes SLC11A1 (Solute Carrier Family 11 proton-coupled divalent metal ion transporter) membrane1 is the formal name for the NRAMP1 gene. It is situated on Chromosome 2 long arm (2q35), and exon number 16.
NRAMP1 is crucial for the early innate response to mycobacterial infection because it promotes microbicidal responses in infected macrophages. Iron is a crucial ingredient for mycobacteria; the cell also needs it to produce reactive oxygen and nitrogen intermediates. By aggressively removing iron or other divalent cations from the phagosome space, NRAMP1 may be able to regulate intracellular microbial proliferation [8-10]. A review of twin studies revealed a moderate genetic predisposition to tuberculosis in humans [11-13].

2. Materials and Methods

2.1. Samples collections

EDTA (Ethylenediaminetetraacetic acid) tubes were held there until blood DNA was collected from 35 TB patients at Al-Diwaniya Teaching Hospital. Blood samples were drawn into extracted from the samples.

2.2. Genomic DNA extraction

Using frozen blood samples, genomic DNA was extracted (Genomic DNA Mini Kit, Geneaid. USA). The frozen Blood extraction Protocol technique with Proteinase K was used to perform the extraction in accordance with the manufacturer's instructions. The extracted gDNA was then examined using a Nanodrop spectrophotometer before being chilled at -20 °C in preparation for PCR amplification.

2.3. PCR amplification

Using specific primers, forward primer (5'-GCATCTCCCCAATTCATGGT-3) and reverse primer (5'-CAGGATA GAGTGGGACAGTT-3), a PCR assay was conducted to detect Natural resistance-associated macrophage protein (NRAMP-1) on loci 3'UTR polymorphism. The prime movers were given by (Bioneer company. Korea). The AccuPower® PCR PreMix kit, manufactured by Bioneer in Korea, was then used to create the PCR master mix. A freeze-dried pellet of Taq DNA polymerase 1U, Tris-HCl (pH 9.0) 10mM, tracking dye, KCl 30mM, $MgCl_2$ 1.5mM, stabilizer, and dNTPs 250µM are included in the PCR premix tube, 10mM, KCl, 30mM, $MgCl_2$, and 1.5mM. After adding 5µl of purified genomic DNA, 1.5µl of 10pmole forwarding primer, and 1.5µl of 10pmole reverse primer to the 20µl total volume of the PCR master mix reaction as directed by the kit's instructions, the PCR premix tube was completed by deionizing PCR water into 20 l and was briefly mixed using an Exispin vortex centrifuge (Bioneer. Korea). The following thermocycler conditions were set up as the protocol for the reaction, which was carried out in a thermocycler (T100 Thermal cycler Biorad. USA): initial denaturation temperature of 94 °C for 5 min; followed by 35 cycles at denaturation 94 °C for 30 s, annealing 55 °C for 30 s, extension 72 °C for 1 min s, and final extension at 72 °C for 5 min. The PCR results were conducted through an electrophoresis process in a 2 percent agarose gel, stained with ethidium bromide, and then exposed to ultraviolet light to be seen.

2.4. PCR analysis

PCR products were separated by a 2 percent agarose gel electrophoresis containing ethidium bromide and viewed under a UV transilluminator in order to detect NRAMP-1.

2.5. Statistical Analysis

Microsoft Excel 2010 and the Statistical Package for Social Sciences (SPSS) version 20 software were used for all calculations. Results from every study were presented as the mean value plus standard deviation (SD). Only results with a p-value of 0.05 were deemed statistically significant, and one-way analysis of variance was used to check for variations between mean values that were statistically significant.

3. Results and Discussion

3.1. Demographic Characteristics of Patients: Age range, Mean ± SD (41.74±8.014), gender: males (16) and female (19), resident: rural (20) and urban (15), room number in house total (35).

3.2. Cases Clinical Characteristics: Family history of TB: positive 46% and negative 54%, History of DM positive 49% and negative 51%, Pulmonary TB positive 54% and negative 46% Extra pulmonary TB site Miliary TB 3%, as show in Table 2. Pulmonary TB (n=19) P = (0.009), history of DM (n=17) P value (0.0081), Steroid (n=7) P = 0.0411, and other immune compromised agents (n=7) P =0.0033, as show in Table 3.

3.3. Room Number in House: Cases number in each rooms total (35), two room p= 0.0488, Three p=0.0113, Four p=0.0151, Five p=<0.0001 and Six p= <0.0001, as show in Table 4 and Figure 1, 2. To prevent erroneous conclusions due to population substructure in genetic association research in admixed populations, genomic ancestry should be examined. This has been carried out in numerous investigations involving various illnesses [14-16]. The prevalence of the variation TGTG and TGTGdel alleles in the NRAMP1-3′UTR gene was similar in this analysis to that reported in earlier research in other populations [17-18]. The outcomes of the research demonstrated that all NRAMP1-3′UTR polymorphism investigations were connected to an increased susceptibility to TB in the group under study, as seen in Table 6. In comparison to the existence of the other genotypes of these polymorphisms (TGTG p =0.0131 or 0.0166 or and TGTGdel p =0.006 or 0.031), the presence of reference homozygous of TGTG and TGTGdel appears to raise the chance of acquiring TB by almost two-fold as displayed in Table 5,6. These outcomes contrast with those found in a study done on Iranian and Indian populations, where these reference homozygous genotypes were used [19-21]. Furthermore, the conclusion of research on the Turkish population, where the reference allele (G) was linked to a roughly 1.5-fold increased risk of contracting TB, is supported by our data for NRAMP1-3′UTR [22-23].

3.4. SNP Genotypes of NRAMP13'UTR: (TGTG p =0.0131 or 0.0166 and TGTG del p =0.006 or 0.031) as shown in Table 5,6 and Figure 3,4.

Table 1: Demographic characteristics of patients

Cases Demographic Characteristics		
Age range	**Mean ± SD.**	**SE.**
6-87 year	41.74±8.014	1.355
Age groups (year)	N.	%
6-15	4	11.4
16-30	8	23
31-50	11	31.4
>50	12	34.2
Gender	N.	%
Males	16	46
Females	19	54
Resident	N.	%
Rural	20	57
Urban	15	43
Room number in house	N.	%
Two	14	40
Three	13	37
Four	5	14
Five	1	3
Six	2	6
Total	**35**	**100**

N=number

Table 2: Clinical characteristics of cases

Cases Clinical Characteristics	**N.**	**%**
Family history of TB		
Positive	16	46
Negative	19	54
History of DM		
Positive	17	49
Negative	18	51
Pulmonary TB		
Positive	19	54
Negative	16	46
Extra pulmonary TB site		
Miliary TB	1	3

Table 3: Distribution of Family history of TB according to Clinical characteristics

Cases Clinical Characteristics	Positive Family history of TB	Negative Family history of TB	P-value
	N (%)	N (%)	
Pulmonary TB (n=19)	5(26)	14(74)	0.009
History of DM (n=17)	14(82)	3(18)	0.0081
Steroid (n=7)	4(57)	3(43)	0.0411
Other immune-compromised agents (n=7)	2(29)	5((71)	0.0033

Table 4: Correlation between room number in house and family history of TB

Room number in a house	Cases number in each room	Positive Family history of TB	Negative Family history of TB	P-value
		N (%)	N (%)	
Two	14	8 (57)	6 (43)	0.0488
Three	13	4 (31)	9 (69)	0.0113
Four	5	3(60)	2(40)	0.0151
Five	1	1(100)	0(0)	<0.0001
Six	2	0 (0)	2 (100)	<0.0001
Total	35	16	19	

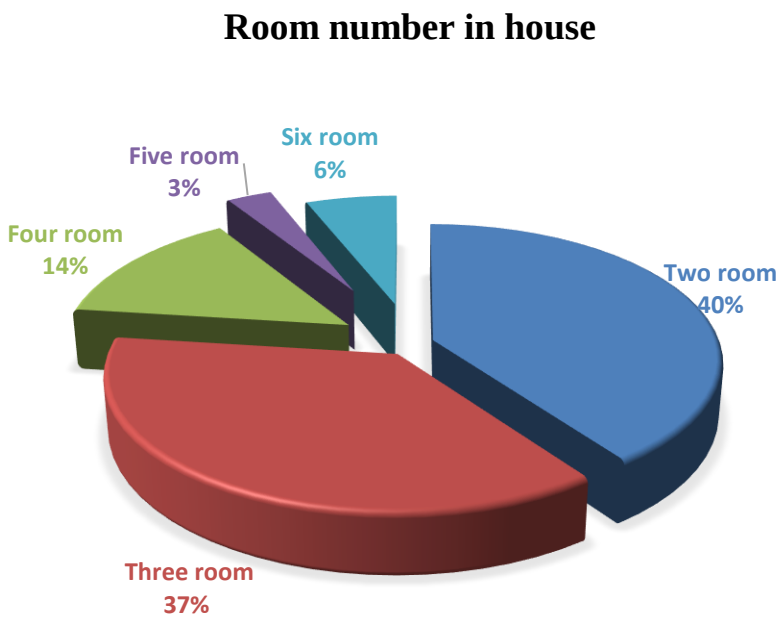

Fig. 1: Room number in patient's house

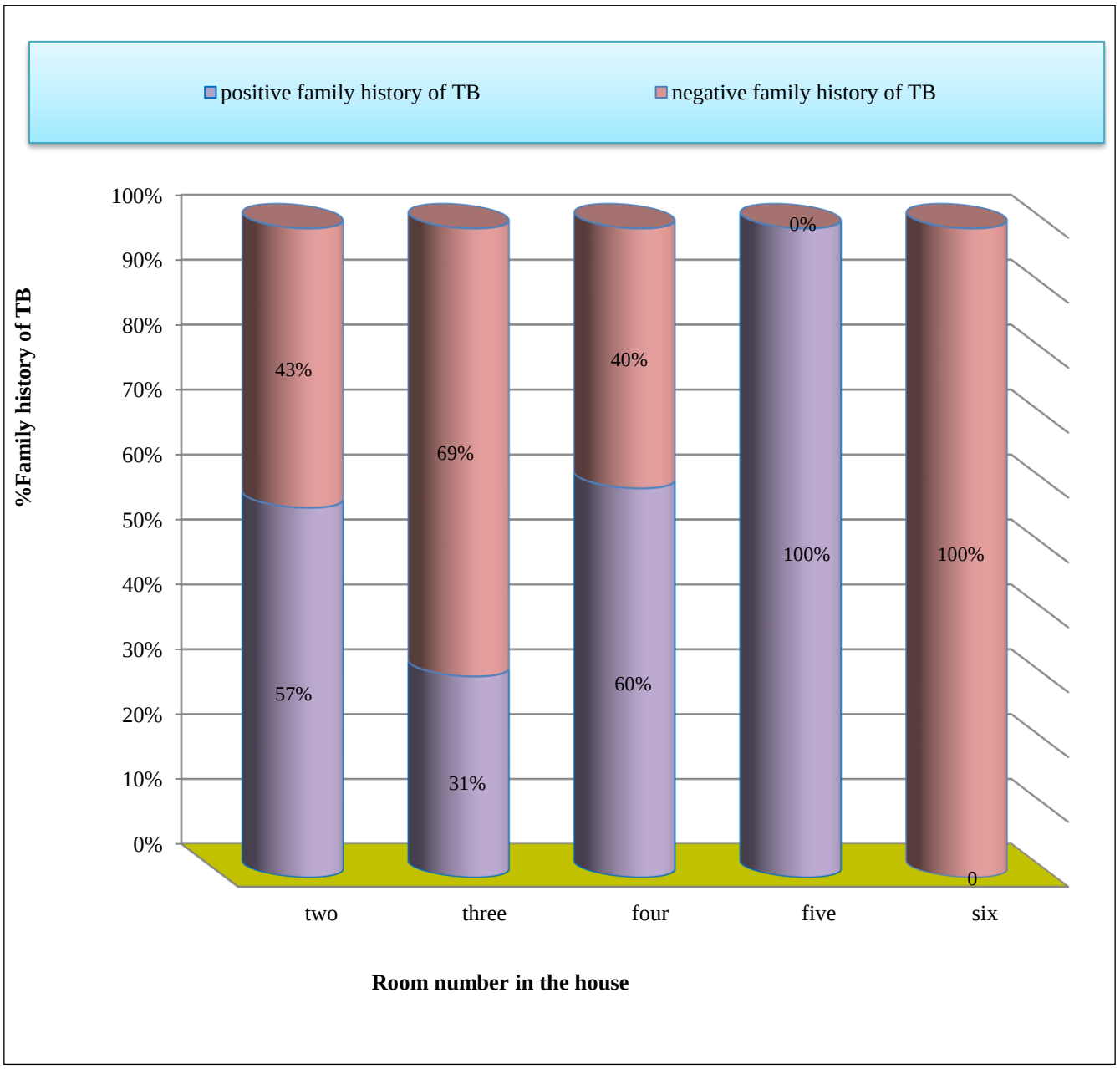

Fig. 2: Distribution of family history of TB according to room number in the house

Table 5: Relationship between the SNP genotypes of NRAMP1 and the risk of a Family history of TB

Table 6: Relationship between the SNP genotypes of NRAMP1 and males/females with Family history of TB
*=Statically Significant (P<0.05), OR=odd ratio, X^2= chi square

SNP of Gene	polymorphism	Type	Females with a Family history of TB	Males with a Family history of TB	X^2	OR	95% C. I	P value
			N (%)	N (%)				
NRAMP1-3'UTR	**Genotypes**							
	TGTG++	Wild homozygous	1 (11)	2(29)	7.77	5.20	5.222 to 95.810	0.037*
	TGTG+/del	Heterozgous	3 (33)	2 (29)	1.044	0.222	0.01 to 2.811	0.399
	TGTGdel/ TGTGdel	Mutant homozygous	5 (56)	3 (42)	3.190	2.911	2.999 to 9.917	0.049*
	Alleles							
	TGTG	Wild	5 (28)	6 (46)	8	6.944	9.053 to 107.7	0.0166*
	TGTGdel	Mutant	13 (72)	7 (54)	9.55	4.118	2.03 to 88.111	0.031*

SNP of Gene	polymorphism	Type	Positive Family history of TB	Negative Family history of TB	X^2	OR	95% CI OR	P value
			N (%)	N (%)				
NRAMP1-3'UTR	**Genotypes**							
	TGTG++	Wild homozygous	4 (25)	9 (47)	6.05	2.899	0.78 to 4.111	0.007*
	TGTG+/del	Heterozgous	1 (6)	6 (32)	52.22	12.32	33.15 to 225.20	0.0002*
	TGTGdel/TGTGdel	Mutant homozygous	11 (69)	4 (21)	14.26	6.711	1.721 to 57.933	0.0145*
	Alleles							
	TGTG	Wild	9 (28)	24 (63)	8	11.04	5.011 to 77.610	0.0131*
	TGTGdel	Mutant	23 (72)	14 (37)	24.61	6.996	2.820 to 72.030	0.006*

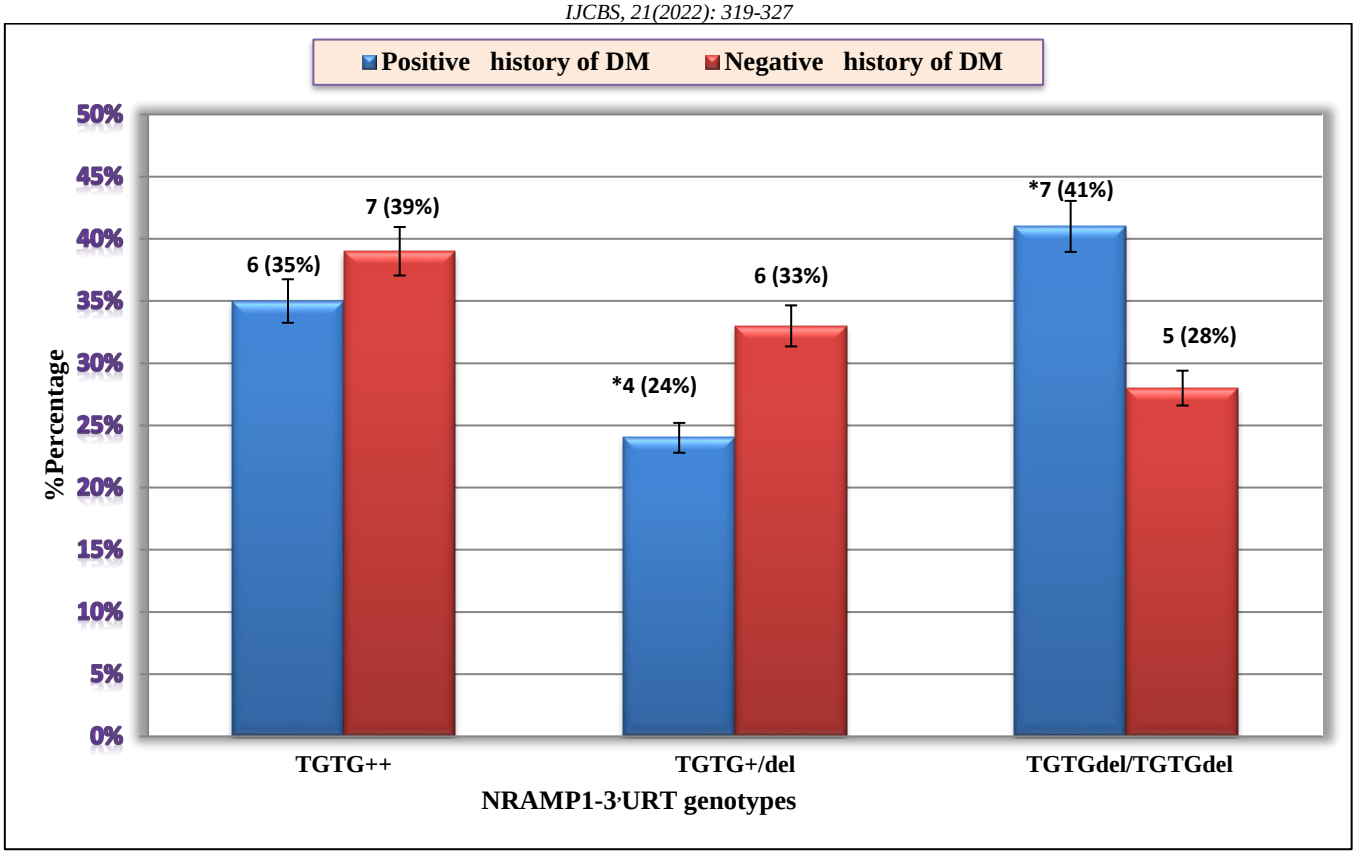

Fig. 3: Relationship between the SNP genotypes of NRAMP1 and the risk of a history of DM. *= statically significant ($p<0.05$) is compared with negative history of DM

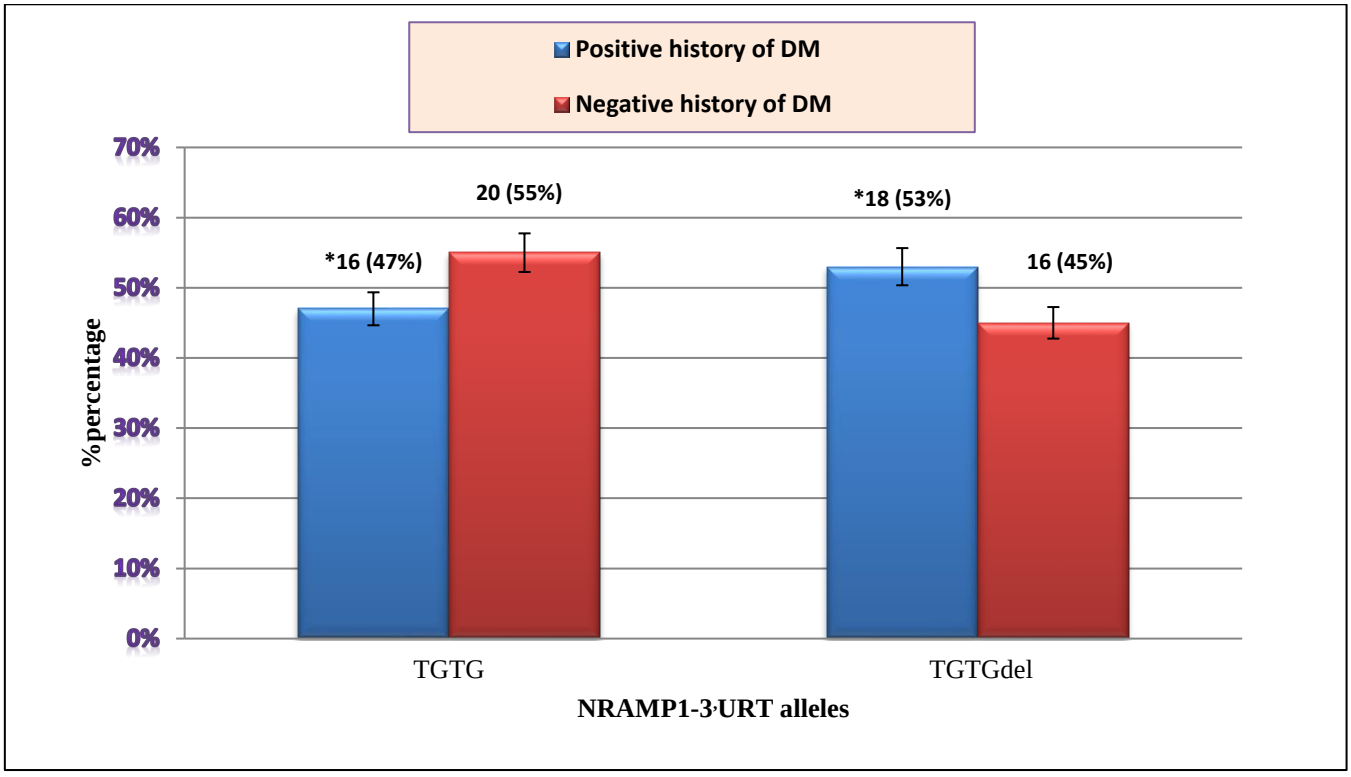

Fig. 4: Relationship between the SNP alleles of NRAMP1 and the risk of a history of DM. *= statically significant ($p<0.05$) is compared with negative history of DM

4. Conclusion

The presence of M. tuberculosis infection in the Iraqi population may be associated with elevated risk of TB, which can be protected by the I genotypes of these polymorphisms (TGTG p =0.0131 or 0.0166 or and TGTGdel p =0.006 or 0.031), the presence of reference homozygous of TGTG and TGTGdel appears to raise the chance of acquiring TB by almost two-fold. The results showed that the relationship between the SNP genotypes of NRAMP1 and males/females with a Family history of TB is statically significant (P<0.05).

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