Journal of Applied and Natural Science
17(2), 486 - 498 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Correlation of (IL-10) gene rs 1800896 polymorphism and tuberculosis risk in Iraq's Nineveh city

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Article Info
https://doi.org/10.31018/jans.v17i2.6381
Received: November 11, 2024
Revised: April 25, 2025
Accepted: May 06, 2025

How to Cite
Mahmood, K. I. *et al.* (2025). Correlation of (IL-10) gene rs 1800896 polymorphism and tuberculosis risk in Iraq's Nineveh city. *Journal of Applied and Natural Science*, 17(2), 486 - 498. https://doi.org/10.31018/jans.v17i2.6381

Abstract
The equilibrium between host immune factors and bacterial evasion methods is critical for tuberculosis (TB) control. One immunomodulatory factor that has been suggested to affect a person's vulnerability to tuberculosis is interleukin-10 (IL-10).The study aimed to Identify: (1) Association of (IL-10) gene rs 1800896 polymorphism and the hazard of TB infection, (2) a novel variation of this gene that may be influencing its activity, (3) Association of some haematology tests and tuberculosis. The study included 334 patients with tuberculosis and control. DNA was extracted from blood samples, (IL-10) gene rs 180096 polymorphism was detected by Tetra ARMS PCR and gene sequencing method. (IL-10) levels were detected by Elisa test. Sequencing results for amplifying of the (IL-10) gene in tuberculosis patients revealed the existence of several nucleotide sequence variations that have not been reported before. Both a new phenotype of the (IL-10) protein and a new genotype for the (IL-10) gene were discovered in Nineveh, Iraq, at the National Center for Biotechnology Information. The ARMS PCR reaction results indicated a correlation between the genomic variation of the (IL-10) gene at the site (rs 180096) and tuberculosis patients. The genetic variation was observed in three different genotypes with varying proportions. The native allele C had a higher allelic frequency (65%) than the mutant allele T (35%). The study concluded that individuals with the (IL-10) gene rs180096 TT genotype under the dominant model were more likely to be at risk of TB infection.

Keywords: IL-10 gene, Immune genes, *Mycobacterium tuberculosis,* Polymorphism, Tuberculosis

INTRODUCTION

When *Mycobacterium tuberculosis* (Mtb) comes into contact with host cells, the immune system develops a complex and diversified defense mechanism, resulting in one of three outcomes: latent infection in 90% of individuals, active tuberculosis in 5-10% or the pathogen's total removal in 5% (WHO, 2022; Zhuang *et al*., 2024). In the fight against Mtb, innate and adaptive immune defense play a role in the struggle, and the bacteria's virulence and the human's genetic vulnerability decide the outcome. (Scriba *et al*., 2017). Traditionally, macrophages, and CD4+ T cells and granuloma formation have been regarded as the cornerstones of immunological response against Mtb, and their significance is evident (De Martino *et al.,* 2019; Carabalí-Isajar *et al*., 2023)

Mycobacterium tuberculosis enters the lungs and reaches the alveoli. The first cells encountered by *Mycobacterium tuberculosis* are the alveolar macrophages and other cells. These cells become activated and engulf the bacteria, leading to the secretion of anti-TB cytokines. There is compelling evidence that human hereditary factors are the most important danger factors for TB infection. Numerous genes responsible for encoding different cytokines are crucial for determining human TB susceptibility. This susceptibility varies among humans and is determined by single nucleotide polymorphisms (SNPs) that affect an individual's ability to produce these cytokines (García-Elorriaga *et al.,* 2013; Van Tong *et al.,* 2017; Harishankar *et al*., 2018 and Gao *et al*., 2024).

Single nucleotide polymorphisms are defined as changes that influence a single nucleotide of a genome, are the most prevalent kind of genetic variation in humans, are found in 1% of a population, and it is a kind of mu-

tation (He *et al.,* 2018, Liu *et al.,* 2022). SNPS can change immunity and result in genetic vulnerability to tuberculosis. (Aravindan, 2019; Wodelo *et al.,* 2024)

IL-10, an essential anti-inflammatory and immune regulatory cytokine, plays a central part in maintaining the equilibrium of immune response, minimizing the human immune response to Mtb and preventing human cell harm (Iyer and Cheng 2012). IL-10 is produced due to the activation of various immune cells such as monocytes, macrophages, mast cells, neutrophils and eosinophils. Most hematopoietic cells express IL-10, and the receptor is made up of two chains named IL-10R1 and IL-10R2 . Deficiency expression of IL-10 can lead to more severe response and increase mortality. (Namaei *et al.,* 2019; Wong *et al.,* 2020 and Ferreira *et al.,* 2021).

The human IL-10 gene is found on chromosome 1. It contains five exons and four introns, and spans 5.1 kb pairs (Ma *et al*., 2019). The IL-10 gene promoter contains many polymorphisms, mostly single nucleotide polymorphisms (SNPs). There is some evidence that certain of these polymorphisms are connected with altered IL-10 expression in vitro. There is some evidence linking IL-10 gene variation to sickness severity.(Trifunović *et al.,* 2015; Salman *et al*., 2017 and Wu *et al.,* 2019).

Whole genome sequencing methods (WGS) can help detect previously unknown epidemics. This method involves sequencing the complete genome, allowing for the identification of rare mutations, SNPs and the definition of heteroresistance. Whole Genome Sequencing (WGS) also facilitates the design of oligonucleotide primers and assesses the genotypic sensitivity to most essential drugs for treating Multi-Drug-Resistant TB. It should be emphasized that WGS can incur significant expenses, representing one of its drawbacks (Saati *et al.,* 2021; Wang *et al.,* 2022).

This research aimed to identify the human hereditary factors that are significant elements of Mtb susceptibility because 1-Cytokine genes, particularly the (IL-10) gene, are important in modulating the human immune response to TB, 2- These factors play an important role in the treatment and outcome of TB, 3- Polymorphisms in these hereditary factors have a variety of effects on susceptibility to TB among specific ethnicities, 4- There have been few studies conducted in Iraq in this area: the present study analyzed the connection between (IL-10) gene polymorphisms and the hazards of tuberculosis infection in the Nineveh population.

MATERIALS AND METHODS

Patients

The present case-control- study was conducted at the chest and respiratory diseases clinic (CRDC) in Mosul city in Nineveh governorate from January 2023 until January 2024. Three hundred thirty-four patients were diagnosed with TB who were attended (CRDC) . Diagnosis was performed by Tuberculin skin test, Ziehl Neelsen stain and GeneXpert MTB-RIF assay G4, USA. GeneXpert assay is used for two purposes: i) to diagnose DNA sequences of Mtb and ii) to conduct a rapid rifampicin sensitivity test. Data on TB patients were obtained from a questionnaire form collected from each TB patient. The participants were divided into tuberculosis (n=334) and control (n=334).

Blood sample collection

Six milliliters of blood was collected from every patient with active TB . Blood sample was divided into three parts , EDTA tube for DNA extraction and CBC test, gel tube for (IL-10), (IL-6) and CRP measurements and Tri sodium citrate tube for ESR test.

DNA extraction

DNA was extracted from blood samples using a kit supplied by Qiagen company, and then the DNA purity and concentration were measured by Biodrop device (Ibrahim and Faisal, 2024).

Detection of the hereditary variation of the IL-10 gene at the locus (rs1800896) of tuberculosis patients by the ARMS-PCR technique

The genetic variation of the IL-10 gene was detected at the site (rs1800896), when 4 microliters (100 ng) of template DNA and 1 microliter (10 picompl) of every primer particular for the gene were added to the substances of the pre-mix, as showen in Table 1.

Then, the reaction tubes were put into the thermocycler to carry out the double reaction, which was programmed as indicated in Table 2.

Determination of the nucleotide sequence of the amplified fragments depending on the DNA Sequencing method

The sequences of the nitrogenous bases of the IL-10 gene were firm at the site (rs1800896). The gene sequences were analyzed using the Hitachi 3130 Gene Analyzer equipped by the Japanese company. The gene sequences were compared to those documented in the National Center for Biotechnology Information (NCBI). The data were aligned using BLAST software.

Determination of the correlation of Inflammatory parameters associated with TB and the activity of (IL-10) gene in studied samples based on genotypes of the genetic variation of the locus (rs 180096) in patient samples

Inflammatory parameters tests related to active TB patients were measured for tuberculosis and healthy control groups. Serum levels (IL-10) were assessed via ELISA test using ELISA BioRAD (model 680), Japan.

Table 1. Illustrating the primer design for the (IL-10) gene's (rs180096) polymorphism using the PCR technique.

Primer	Sequence	Band size	Annealing
IF96	TTTCCTCTTACCTATCCCTACTTCCACT	240 bp	68 °C
IR96	AAGACAACACTACTAAGGCTTCTTTGGTAG	190 bp	
OF96	GAATTTGGTTTCCTCACCCTACTG	390 bp	
OR96	CTGAAGAAGTCCTGATGTCACTGC		

Table 2. Depicting the multiplication reaction and its Unique program.

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	6 min.	1
2.	Denaturation	95	45 sec.	
3.	Annealing	61	1 min.	35
4.	Extension	72	1 min.	
5.	Final extension	72	5 min.	1

Serum levels of (IL-6) were assessed using the Immunoassay analyzer Cobas e 411 (Roche, Germany). CRP and ESR were measured according to manufactures' instructions.

CBC test was carried out when EDTA blood samples were analyzed by Auto hematology Analyzer Mindary (BC- 10) Germany.

Ethics committee approval

The protocol was approved by the Medical Research Ethics Committee, College of Medicine University of Mosul (UOM / COM / MREC / 21-22 (75).

Statistical Analysis

The extracted data were investigated using SPSS (version 26), ANOVA with the Dunkan test and T- tests, which were used to demonstrate the differences between the data.

RESULTS AND DISCUSSION

Features of TB patients

The result of GeneXpert assay showed that 334 patients were diagnosed with tuberculosis including (168; 50.3%) females and (166; 49.7%) males.The median age of TB patients was 38.58± 20.019 with a range from 1 to 79 years, female to male ratio was 1.012 . The majority of cases 324(97%) were rifampicin- sensitive tuberculosis (RST) and 10(3%) were rifampicin resistant- tuberculosis (RRT). High rate of TB in age group (15-24) because of this age group is more motility and have more economic activities compared to older persons in addition to social factors such as poverty, overcrowding, and poor houses, this agrees with Aljanaby *et al.* (2022) in Baghdad city. Table 3 shows features of tuberculosis patients in Nineveh governorate, Iraq, in the year 2023 (n=334).

Detection of the hereditary variation of the IL-10 gene on the locus (rs1800896) of tuberculosis patients using the ARMS-PCR technique.

Results of the ARMS PCR reaction (Fig. 1) indicated a correlation between the hereditary variation of the (IL-10) gene at the site (rs 180096) and tuberculosis patients. The genetic variation was observed in three different genotypes, with varying proportions as shown in (Table 4).

The results of PCR reaction of rifampicin- resistant tuberculosis samples in fig. 1 consisted of three bands as follows:

For the primary gene section, the first band measured 390 bp in size.

For the normal allele, the second band measured 250 bp.

The mutant allele's third band measured 190 base pairs.

Therefore, sample no. 5 had a normal genotype (CC). The genotypes of the samples (3 and 6) were heterogeneous (CT). The samples (1, 2, 4, 7, 8, 9, 10) also showed a mutant genotype (TT).

According to Fig. 2, the interaction results of rifampicin -sensitive tuberculosis samples showed that the genotype of sample 10 is normal (CC), While the genotype of samples (1, 2, 3, 4, 5, 6, 7, 8 and 9) are TT mutant.

In (Table 4.) results for patients with tuberculosis revealed that the normal genotype (CC) had a frequency value of 60%, the highest percentage compared to the 10% heterogeneous genotype (CT) and the 30% mutant genotype (TT). In contrast to the control group, 80% of the individuals had the normal genotype, 10% had the heterogeneous genotype, and 10% had the mutant genotype. Additionally, it was discovered that the Odd Ratio was 4. This value is higher than 1 at the probability level P = 0.2371 and is regarded as a risk factor for tuberculosis development.

The present results in Table 5 showed that the frequency of the normal allele C was high at 65% compared to the mutant allele T at 35%. It was also shown to us from the table that the value of the OR level is 3.0513, and thus, it was higher than 1 at the probability

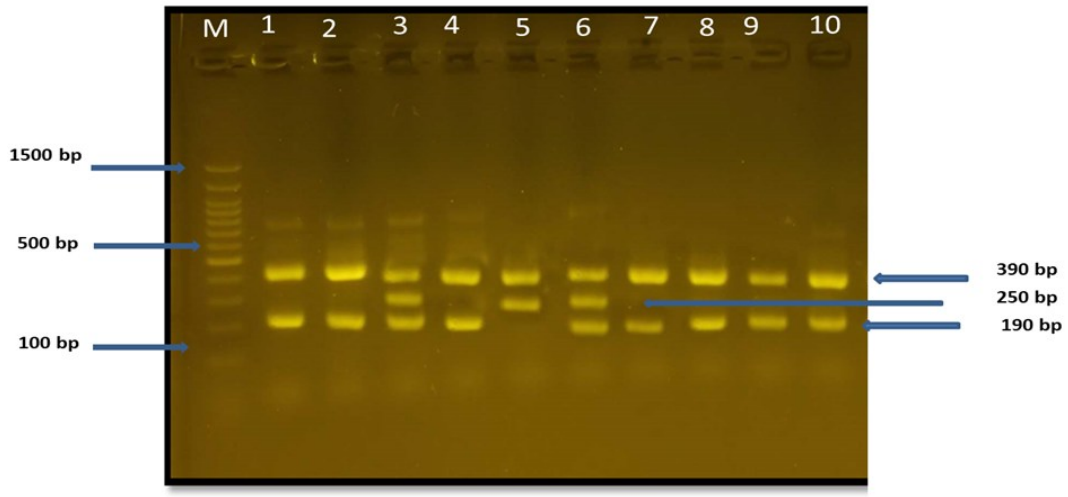


Fig. 1. *Outcomes of the T-ARMS-PCR test for the IL-10 gene's genetic variant at the location (rs180096) of the rifampicin - resistant TB samples.*

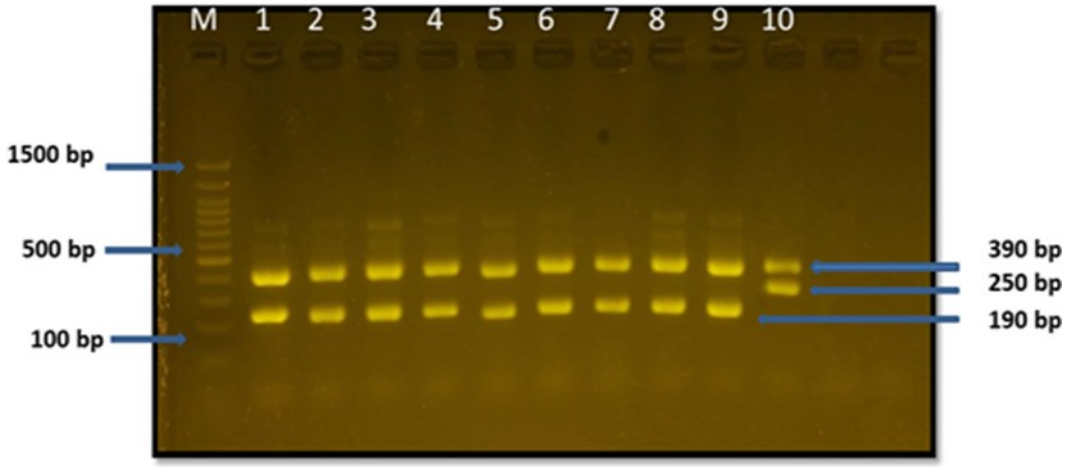


Fig. 2. *Displaying the outcomes of the T-ARMS-PCR reaction for the IL-10 gene genetic variant at the location (rs1800896) of the rifampicin-sensitive TB samples*

level P = 0.0115, and it is recognized as a risk factor for the enhancement of tuberculosis. This difference in frequency was thought to be one of the primary causes of the (IL-10) protein's loss of biological function, as it suggests a malfunction in the gene's expression process.

When comparing the results obtained in Table 6, it was found that the percentage of observation obtained with the percentage of observation expected resulting from Hardy-Weinberg equilibrium has a value of P = 0.000484, less than P = 0.05. This indicates the presence of variation within the study groups that they are subject to the law of equilibrium, and that the influence is environmental. It is necessary to search for and identify the causes and the variation within the proportions of the recorded and expected genotype. This indicates the effect of this variation on the disease condition, and it is considered one of the hazard factors causing tuberculosis and its progression. This agrees with Liu *et al.* (2015); He *et al.* (2018), who proved the association of IL-10 polymorphism and tuberculosis risk.

Verifying that the inheritance of this variation in the study groups is recessive, this means that the variant pattern gives a similar appearance to the normal pattern, as revealed in Table 7.

Verifying that the inheritance of this variation in the study groups is dominant, the variant produces a protein as it is in the mutant, which depends on the P value, as illustrated in Table 8.

When comparing the difference in the proportions of genotypes between patients and the control group within the recessive distribution test, we noticed that the P value = 0.2452, which is greater than P>0.05, which means that there is a major dissimilarity between the study groups in addition to the difference in the recorded percentages. However, when comparing the results within the dominance distribution, it was found that the P value = 0.2826, which is greater than P> 0.5. This means that there is a major dissimilarity in the proportions of genotypes between the study groups. Therefore, the mutant genotype TT is considered to have a dominant effect and not a recessive one on the tubercu-

Table 3. Showing features of TB patients (n=334) in the Nineveh Governorate in the year 2023.

Characters	Frequency	Percentage %
Mean age (SD)	38.58	
Age range (years) :		
0-4	13	3.9
5-14	21	6.3
15-24	69	20.7
25-34	51	15.3
35-44	51	15.3
45-54	38	11.4
55-64	37	11.1
< 65	54	16
Gender :		
Female	168	50.3
Male	166	49.7
Rifampicin resistance status:		
Rifampicin resistant TB	10	3
Rifampicin sensitive TB	324	97
Site of TB :		
Pulmonary TB (PTB)	231	69.2
Extrapulmonary TB (EPTB)	103	30.8
Extrapulmonary TB include :		
Lymph nodes	46	44.7
Pleural	21	20.4
Bone and joints	14	13.6
Meningitis	4	3.9
Gastrointestinal	2	1.9
Endocardium	4	3.9
Skin TB	1	1
Others	11	10.6
Comorbidity :		
Diabetes mellitus	83	24.9
Contact with active TB patients	13	3.9
Chronic Kidney disease	40	12
Liver disease	20	6
Cancer	11	3
Smoking	52	15.6
Prison inmates	12	3.6
Malnutrition	54	16
Sectors :		
Left sector	142	42.51
Right sector	96	28.74
Qayyarah	26	7.78
Hamdania	22	6.59
Talafar	17	5
Telkif	12	3.59
Baag	13	3.89
Singar	3	1
Shikhan	1	0.3
Makhmor	2	0.6

Table 4. Dispersion of the frequency of genotypes of (IL-10) at position (rs 180096) between tuberculosis and control groups.

Genotypes SNP1	Patients		Control		P- Value	OR	(95%CI)
	NO.	%	NO.	%			
CC	12	60	8	80			
CT	2	10	1	10	P= 0.2371	4.000	0.4017 to 39.8291
TT	6	30	1	10			

CC: normal genotype, CT: heterogeneous genotype, TT: mutant genotype

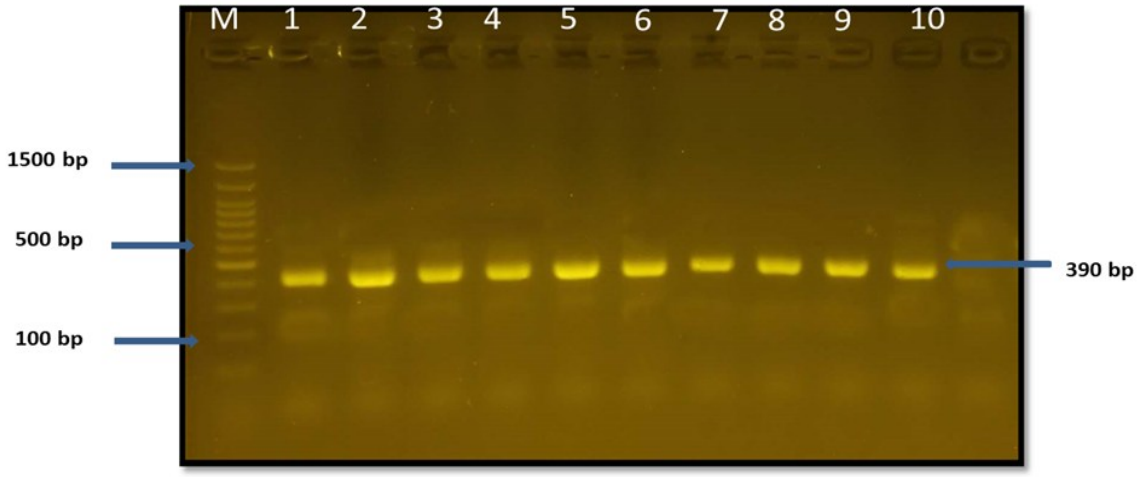


Fig. 3. *IL-10 gene PCR having a reaction yield of 390 bp and a size index M of 100 bp prepared by Biolabs; Agarose gel was used for separation, and it was generated at a concentration of 2%*

losis condition.

Determine the nucleotide sequence of the amplified fragments established on the DNA Sequencing procedure (Fig. 3).

The aim of conducting nucleotide sequencing tests is to identify new variations or variances in this gene that might have a direct or indirect impact on its function and which are one of the main reasons for the development of tuberculosis, in addition to confirming with certainty that the primers used in this study belong to the IL-10 gene, as the results of the sequencing tests to amplify the IL-10 gene showed previously unrecorded differences in the sequences of a number of nucleotides.

In the current study, the results of matching the nucleotide sequences of the IL-10 gene to the models revealed that the matching rate reached 100% in some samples with the nucleotide sequences on the NCBI site, which indicates the accuracy of the primer design, which was used for the head time, as shown in Fig. 4, 5 and 6.

It was seen that there are many different genetic variations, such as transversion, transduction, deletion or addition, and their locations depend on the different or variable bases (Table 9). These variations may lead to a decrease in the effectiveness of the IL-10 gene in protein construction and thus increase the risk factors for tuberculosis. One of the results mentioned above is the occurrence of many and different mutations in the IL-10 gene, which may be one of the causes leading to tuberculosis.

The present study aligns with several studies that

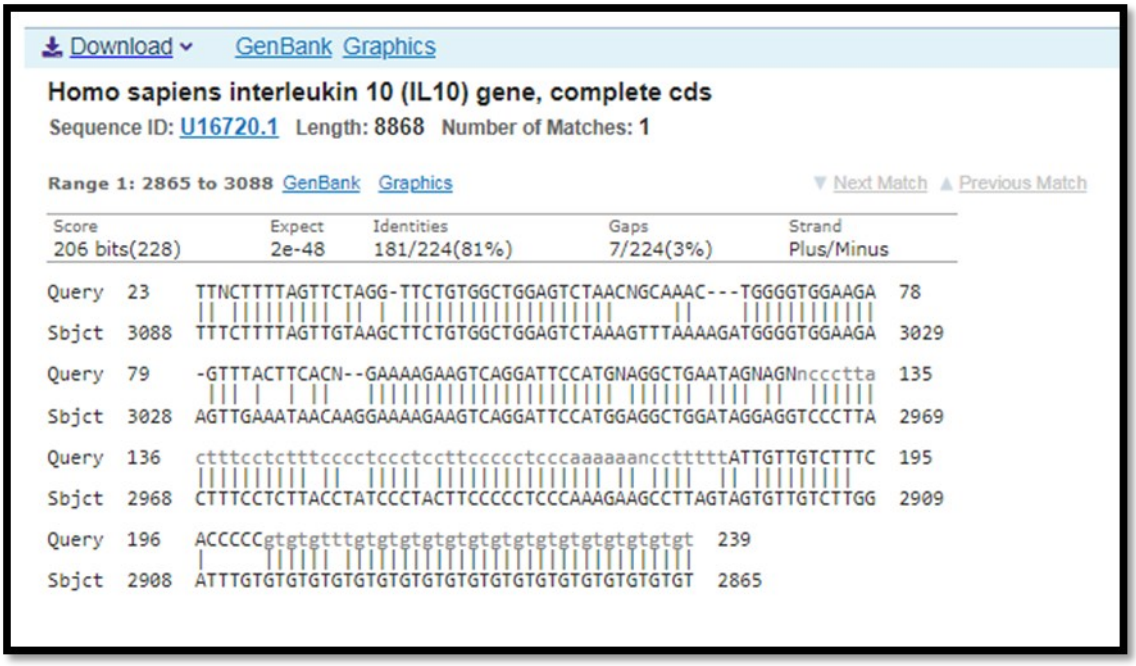


Fig. 4. *Displaying the results of comparing the (IL-10) gene's nucleotide sequence of rifampicin- resistant TB samples to the NCBI site's nucleotide sequences*

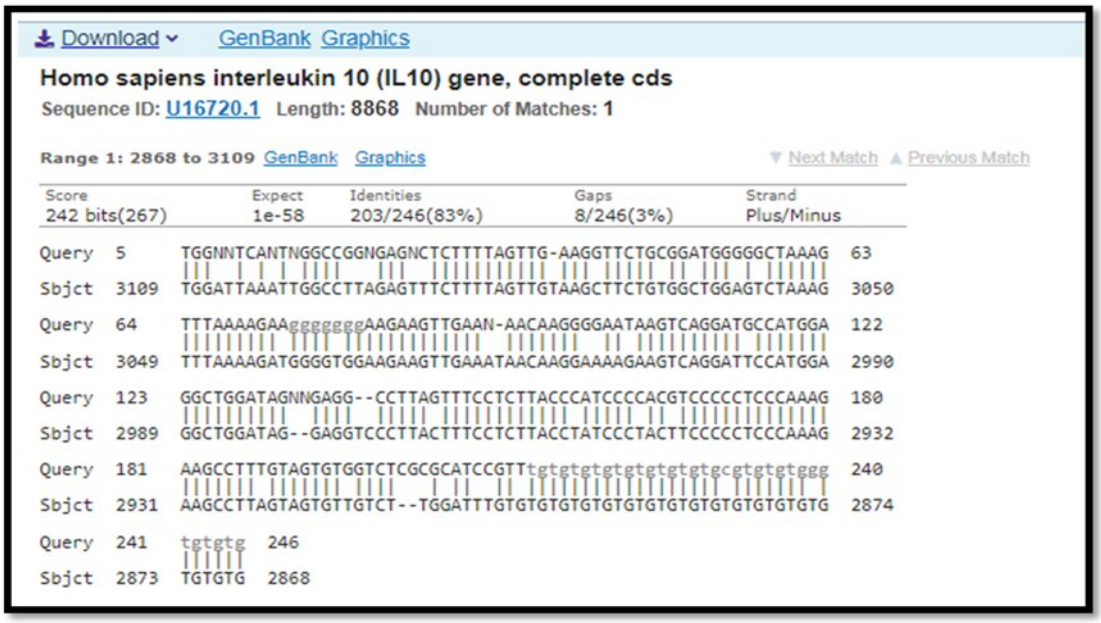


Fig. 5. *Displaying the results of comparing the (IL-10) gene's nucleotide sequence of rifampicin-resistant TB samples to the NCBI site's nucleotide sequences*

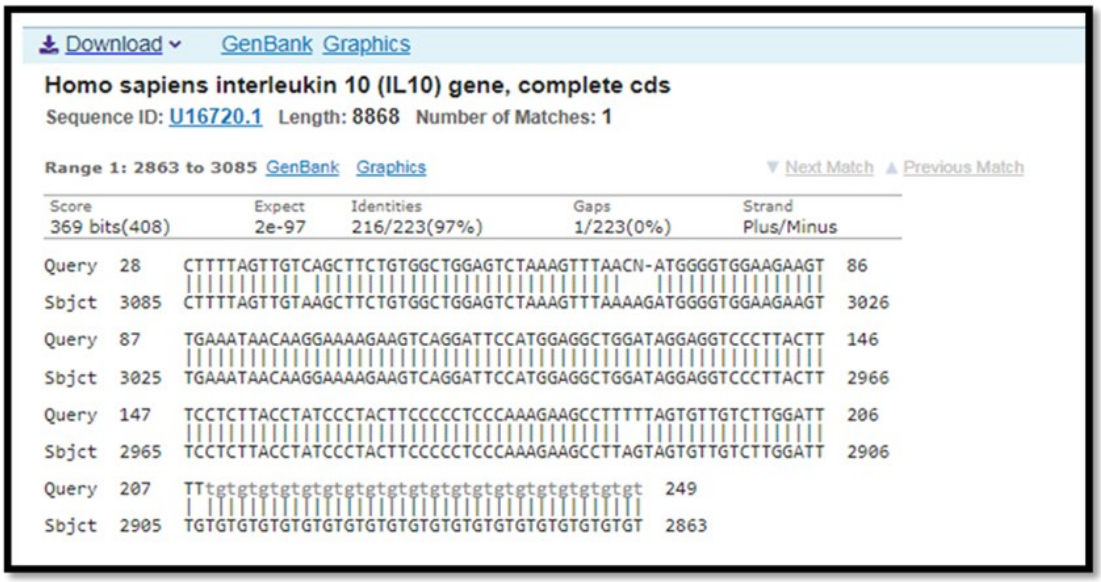


Fig. 6. *Displaying the results of comparing the (IL-10) gene's nucleotide sequence of rifampicin-sensitive TB samples to the NCBI site's nucleotide sequences*

Table 5. Distribution of frequency alleles between tuberculosis and control groups.

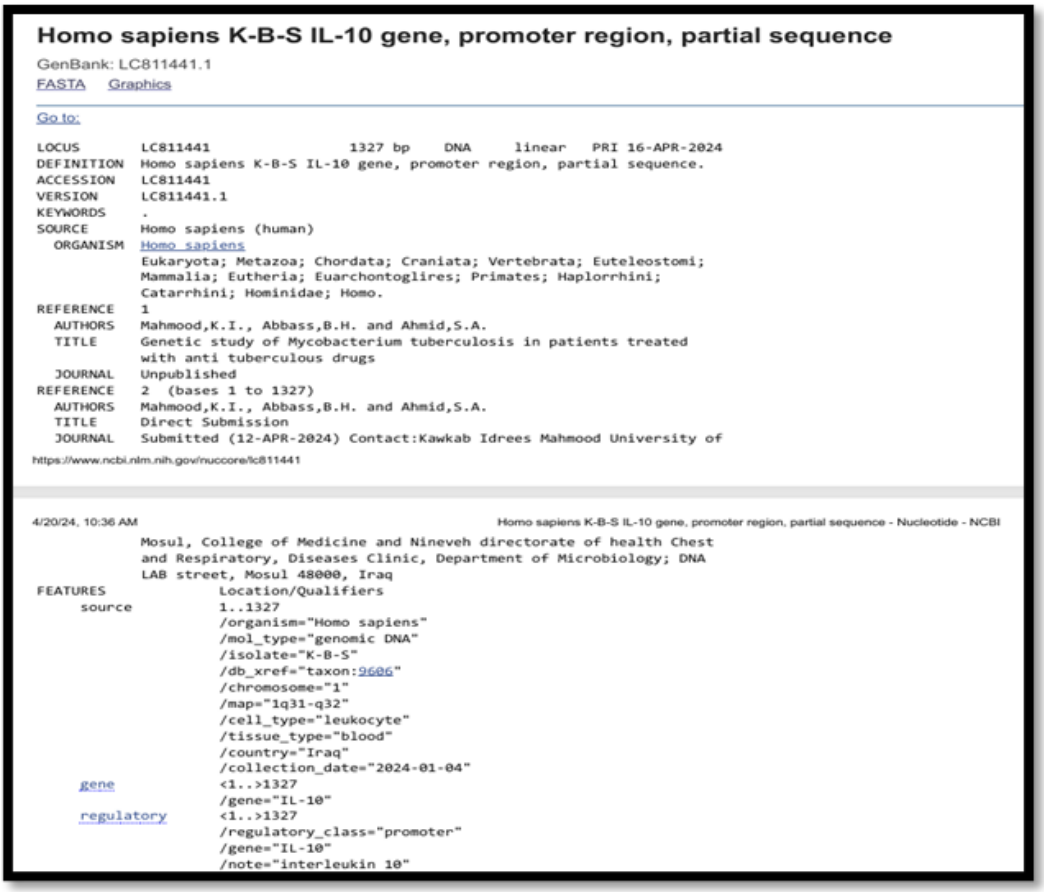
Genotypes Alleles	Patients		Control		P- Value	OR	(95%CI)
	No	%	No	%			
C	26	65	17	85	P = 0.0115	3.0513	0.7609 to 12.2353
T	14	35	3	15			

Table 6. Results of the Hardy-Weinberg Equilibrium test

Genotype	CC	CT	TT
Observed genotype	12	2	6
Expected genotype	8.5	9.1	2.5
P-value = 0.000484		Chi squared value X2=12.174858	

Table 7. Distribution of (IL-10) gene polymorphism under the recessive model in studied groups

Genotype	Patients	Control	OR	CI	P-value
CC+CT	14 (70%)	9 (90%)	3.8571	0.3959 to 37.5837	P = 0.2452
TT	6 (30%)	1 (10%)			



Homo sapiens K-B-S IL-10 gene, promoter region, partial sequence

GenBank: LC811441.1
FASTA Graphics

Go to:

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VERSION     LC811441.1
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  AUTHORS   Mahmood,K.I., Abbass,B.H. and Ahmid,S.A.
  TITLE     Genetic study of Mycobacterium tuberculosis in patients treated
            with anti tuberculous drugs
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1327)
  AUTHORS   Mahmood,K.I., Abbass,B.H. and Ahmid,S.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-APR-2024) Contact:Kawkab Idrees Mahmood University of
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https://www.ncbi.nlm.nih.gov/nuccore/lc811441

4/20/24, 10:36 AM Homo sapiens K-B-S IL-10 gene, promoter region, partial sequence - Nucleotide - NCBI

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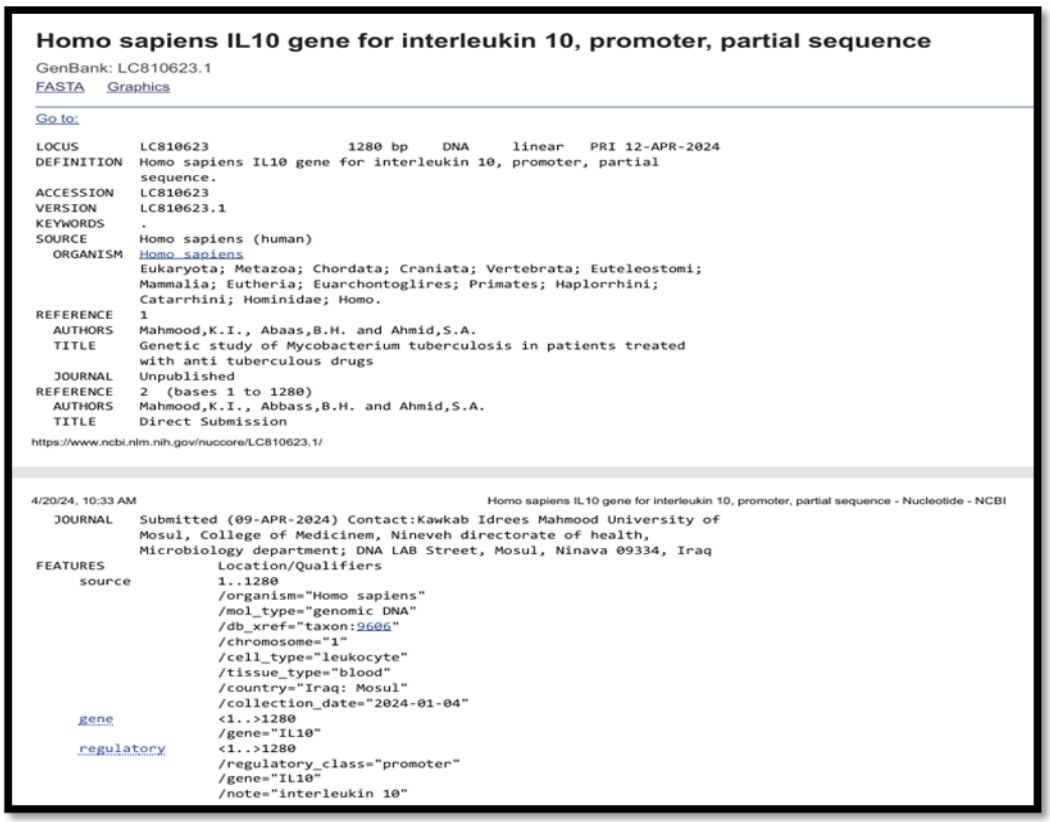
Fig. 7. *Registration of a new genotype for the (IL-10) gene in Nineveh Governorate within the NCBI, and the identification number GeneBank: LC811441.1.*



Homo sapiens IL10 gene for interleukin 10, promoter, partial sequence

GenBank: LC810623.1
FASTA Graphics

Go to:

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  AUTHORS   Mahmood,K.I., Abaas,B.H. and Ahmid,S.A.
  TITLE     Genetic study of Mycobacterium tuberculosis in patients treated
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  JOURNAL   Unpublished
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  AUTHORS   Mahmood,K.I., Abbass,B.H. and Ahmid,S.A.
  TITLE     Direct Submission
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https://www.ncbi.nlm.nih.gov/nuccore/LC810623.1/

4/20/24, 10:33 AM Homo sapiens IL10 gene for interleukin 10, promoter, partial sequence - Nucleotide - NCBI

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  JOURNAL   Submitted (09-APR-2024) Contact:Kawkab Idrees Mahmood University of
            Mosul, College of Medicinem, Nineveh directorate of health,
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Fig. 8. *Registration of a new phenotype for the (IL-10) protein which was encoded by (IL-10) gene in Nineveh Governorate within NCBI, and the identification number GeneBank: LC810623.1*

Table 8. Distribution of (IL-10) gene polymorphism under dominant model in studied groups

Genotype	Patients	Control	OR	CI	P-value
GC/GC	12 (60%)	8 (80%)	2.66	0.4456 to 15.9595	0.2826
GC/AT +AT/AT	8 (40%)	2 (20%)			

Table 9. Showing a comparison of the matching result between the nucleotide sequence of the IL-10 gene in the study samples as in fig.5 matched to the unique gene sequence on the NCBI

Sequence ID	Nucleotide	Location	Mutation type	Identity	Gaps
U16720.1	G → T	3056	Transversion	83%	3%
U16720.1	G → A	3058	Transition	83%	3%
U16720.1	A → C	3062	Transversion	83%	3%
U16720.1	C → T	3065	Transition	83%	3%
U16720.1	G → C	3071	Transversion	83%	3%
U16720.1	- →T	3075	Deletion	83%	3%
U16720.1	C→T	3087	Transition	83%	3%
U16720.1	G → T	3093	Transversion	83%	3%
U16720.1	G →T	3094	Transversion	83%	3%
U16720.1	C→A	3103	Transversion	83%	3%
U16720.1	G →T	2997	Transversion	83%	3%
U16720.1	T →G	3008	Transversion	83%	3%
U16720.1	G→ A	3011	Transition	83%	3%
U16720.1	G→A	3012	Transition	83%	3%
U16720.1	- →T	3020	Deletion	83%	3%
U16720.1	G→T	3035	Transversion	83%	3%
U16720.1	A→ T	3040	Transversion	83%	3%
U16720.1	- →C	2974	Deletion	83%	3%
U16720.1	G → -	2912	Addition	83%	3%
U16720.1	C → -	2913	Addition	83%	3%

Table 10. Comparison of laboratory parameters of tuberculosis patients and control groups

Laboratory parameters	Normal range	Control group (n=90)	TB group (n=90)	T- value	P-value
(IL-10),(ng/ml)	2-600	100.00±00.00	80.00±00.00	424.00	0.001
(IL-6), (pg/ml)	0-7	3.14±0.11	51.4±2.2	301.23	0.001
CRP ,(mg/ml)	0-6	3.00+0.09	41.8±1.5	291.91	0.001
ESR ,(mm/h)	0-15	5.51±0.16	59.0±1.5	393.86	0.001
WBC count ,($\times10^9$ /L)	4-11 $\times10^9$	6.95±0.13	8.2±0.18	21.29	0.001
RBC,($\times10^{12}$ /L)	3.5-5.5 $\times10^{12}$	4.49±0.11	4±0.18	8.63	0.001
Hemoglobin , (g/dL)	11-16	11.82±0.81	7.1±0.33	41.94	0.001
Platelets , ($\times10^9$/L)	100-400$\times10^{12}$	210.12±4.07	140.2±2.1	276.04	0.001

proved an important connection between (IL-10) gene rs 180069 polymorphism and the risk of infection with pulmonary TB disease in Indonesia (Anggraini *et al.,* 2023), Extra pulmonary TB in the Kashmiri population (Hu *et al.,* 2015) and latent TB in China (Wani *et al.,* 2021). A study conducted in Mexico (García-Elorriaga *et al.,* 2013) found that 592 (IL-10) polymorphism at a higher rate in pulmonary TB patients with kind 2 diabetes than in the control healthy group. However, a study conducted in Iran found that SPNs at 592 locations of IL-10 gene may be linked with vulnerability to Drug Resistant -Tuberculosis (Shermeh *et al*., 2020). Meta-analysis studies conducted by Liu *et al.,* (2015), Gao *et al.* (2015), and Areeshi *et al.* (2017) showed a link between (IL-10) - 1082, 819, and 592 polymorphisms and TB risk in particular ethnic groups.

Iraqi genetic studies on tuberculosis patients proved that polymorphism in some cytokine genes was related to TB susceptibility, including: (IL-17A) (Ameen *et al.,* 2018), (IFN- γ) (Al-Azawi and Al-Saeedi, 2018), (IL-37) (Ali *et al.,* 2022), (HLA-G) (AL- Tamimi *et al.,* 2022) and (TNF-α) (AL- Gawwam and Hassan, 2024).

Recording the new genotype of the IL-10 gene and its encoding protein on the global NCBI website for the Iraqi community

Through the current revision, a novel genotype of the IL -10 gene was documented in the city of Mosul at the National Center for Genetic Information (NCBI), as shown in Fig. 8 and was given the GenBank identification number LC811441.1 (Fig. 7).

Table 11. Association between rs 180096 genotypes in IL-10 gene and inflammatory parameters of tuberculosis patients

Laboratory parameters	Normal range	Genotypes SNP			F- value	P-value
		CC	CT	TT		
(IL-10),(ng/ml)	2-600	100+0.01 a	90+0.00 b	80+0.2	132.11	0.001
(IL-6), (pg/ml)	0-7	4.±1.76 c	50.±1.4 b	60.±3.27 a	1295.660	0.001
CRP ,(mg/ml)	0-6	3.1±1.20 c	40.±2.8 b	50.±3.27 a	946.490	0.001
ESR ,(mm/h)	0-15	7 ±1.41 c	60.±2.8 b	77.±1.33 a	7622.550	0.001
WBC count ,($\times10^9$ /L)	4-11 $\times10^9$	6. ±1.15 a	8.±0.00 a	10.±4.43 a	4.037	0.001
RBC,($\times10^{12}$ /L)	3.5-5.5 $\times10^{12}$	4.4 ±0.84 a	4.±1.41 a	3.50±0.42 a	6.237	0.001
Hemoglobin , (g/dL)	11-16	11.±1.05 a	7±1.4 b	6.33±1.75 b	29.925	0.001
Platelets , ($\times10^9$/L)	100-400$\times10^9$	150.±0.00a	130±2.9 b	154.73±33 c	2415.261	0.001

The difference in letters (a,b,c) indicates that there is a momentous variance at (P≤0.05)

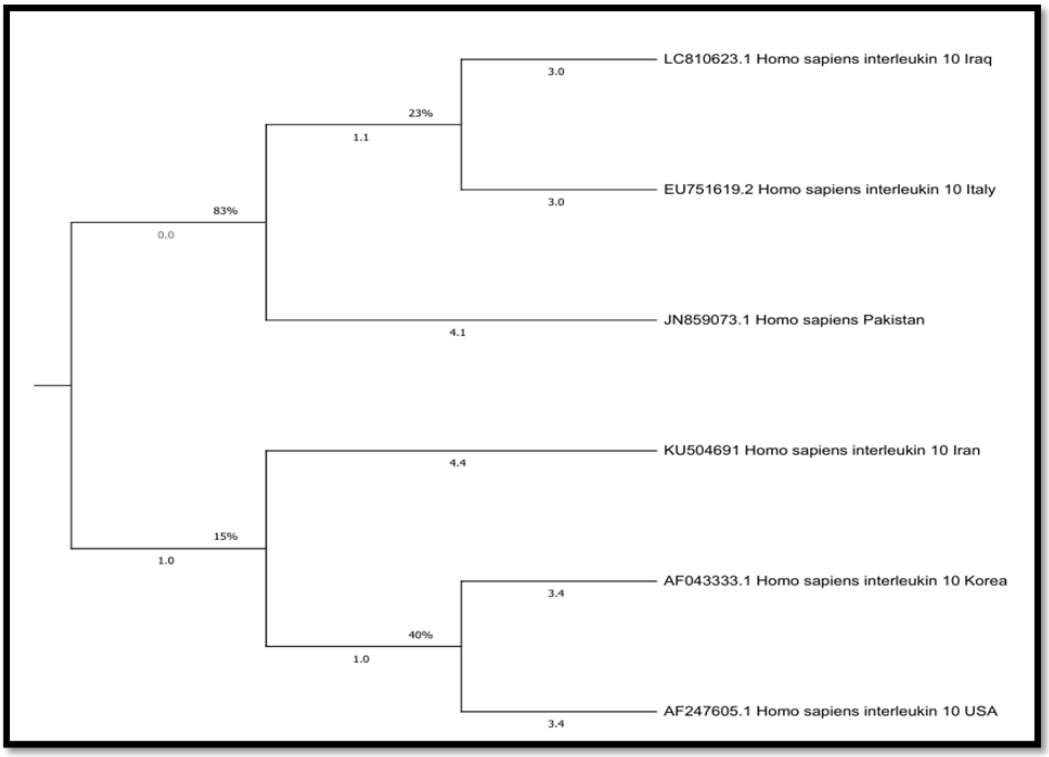

Fig. 9. *Genetic proximity and distance tree of (IL-10) genotype*

The sequences of the different nitrogenous bases of the genotype were determined, and another phenotype of the IL-10 protein encoded by the IL-10 gene was recorded in the study (NCBI), and the identification number Gene Bank: LC810623.1 as in Fig. 8.

Preparing the dimension tree and the genetic evolutionary tree for the recorded genotype of the IL-10 gene

The evolutionary tree was also prepared to determine the percentage of distance and genetic proximity of the recorded genotype in this study for the IL-10 gene with the rest of the different genetic patterns. It is clear from the shape of the genetic dimension and based on the evolutionary tree that the recorded genotype of the IL-10 gene has a large variation and is genetically distant from the rest of the globally recorded genotypes, as in Fig. 9.

It is clear from Fig. 10. of the evolutionary tree that the recorded genotype in Nineveh, Iraq is close to the isolation of the Italian community and far from the rest of the recorded isolations worldwide.

Association of tuberculosis with inflammatory parameters

Table 10 compares laboratory parameters of tuberculosis patients and control groups . The tuberculosis patients had higher levels of IL-6, CRP, ESR and WBC and lower levels of (Il-10), RBC, Hb and platelets compared with those of the healthy group. The variances between the two groups were substantial (P< 0.001) .

Association of genotypes with inflammatory indicators

Table 11 shows an association between rs 180096 genotypes in IL-10 gene and inflammatory parameters

of tuberculosis patients. It was found that (IL-6), CRB, ESR and WBC count were increased and (IL-10), RBC, Hb and platelets counts were decreased in TT genotype compared with those of the CT genotype and the CC genotype . The variance between the groups was substantial at P= 0.001.

In this study, the result of CBC showed a significant decrease in hemoglobin level, RBCs and platelet counts, as well as a substantial rise in WBC count in TB patients matched to the control group. This result reflects anemia in TB patients that is attributed to chronic inflammation, increased IL-6, decreased production of erythropoietin and change in iron metabolism. The reason for the increased WBCs in TB patients is the raised macrophage and polymorphonuclear WBCs, which participate in immune defense pathways against invading MTB . The present findings agree with many studies on tuberculosis conducted in India (Rohini *et al.,* 2016), Ethiopia (Abay *et al.,* 2018) and Korea (Kang *et al.,* 2021).

The result of inflammatory parameters showed an important rise in IL-6, CRP and ESR levels in TB patients matched to the regulator group. Decreased production of IL-10 protein in TB patients may be due to single nucleotide polymorphisms in (IL-10) genes, which can modify the quantities and functions of secreted (IL-10). This might profoundly influence illness results, pathogenicity, severity, and human immune guidelines. The present finding agrees with several researches on TB carried out in various locations including (Abakay *et al.,* 2015) in Turkey, (Yoon *et al.,* 2017) in USA, (Ma *et al.,* 2019) in China, (Naim, 2019) in Indonesia, (Stefanescu *et al.,*2021) in Romania, (Saripalli and Ramapuram, 2022) in Southern India and (Sulochana *et al.,* 2022) in Tamil Nadu, India.

Mortazavi Moghaddam *et al.* (2020) found that the concurrent rise in IL-10 and IL-13 levels through pulmonary tuberculosis therapy, along with a favorable correlation between the two interleukins throughout and after therapy in patients who return to regular radiography, likely indicates the function and collaboration of these two interleukins in removing radiographs and avoiding lung scarring.

Regarding the association of CC, CT and TT genotypes with inflammatory parameters in TB patients, the present study found a significant association of IL-10 gene rs 180096 TT genotypes with the risk of TB infection (p= 0.001).

Conclusion

Mycobacterium tuberculosis causes TB, an infectious disease, and genomic polymorphism is the mechanism that pointers to the development from infection to TB sickness because hereditary factors influence Individuals' immunological responses. Polymorphisms in different genes can alter immune responses, so all *M. tuberculosis*-infected persons do not get tuberculosis. Small Nuclear Polymorphisms in the IL-10 gene act as the risk elements for tuberculosis and have a role in differences in serum (IL-10) levels. The discovery of host genetic markers will aid in predicting TB development and knowledge of the disease's immune pathogenesis. This study entered a new genotype in the NCBI database for the IL-10 protein and the IL-10 gene for Iraq's Nineveh community with accession numbers LC810623.1 and LC811441.1, respectively.

ACKNOWLEDGEMENTS

The author thanks the College of Medicine, University of Mosul for documenting this work.

Conflict of interests

The authors declare that they have no conflict of interest.

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