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Original Research Article

Relationship of TNFα-Gene Polymorphisms with TNFα Serum Level in Liver and Pancras Disorders in Sample of Beta Thalasemia Major Iraqi Patients

Hawraa Allawi Luaibi[1*], Bushra Jasim Mohammed[2]

[1]Department of Medical Rehabilitation and Physiotherapy Techniques, Technical Institute of Al-Diwaniyah, AL– Furat Al-Awsat Technical University (ATU), Al-Diwaniyah, Iraq
[2]Department of Genetic Engineering, Institution of Genetic Engineering and Biotechnology for postgraduate Studies, University of Baghdad, Baghdad, Iraq

***Corresponding Author**
Hawraa Allawi Luaibi
Department of Medical Rehabilitation and Physiotherapy Techniques, Technical Institute of Al-Diwaniyah, AL– Furat Al-Awsat Technical University (ATU), Al-Diwaniyah, Iraq

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Abstract: ***Background****:* Thalassemia is one of the genetic diseases in Iraq, where thousands of Iraqis suffer from this chronic disease. Tumor necrosis factor alpha (TNFα) is once of a powerful inflammatories cytokine that increases cytokine and has vital influence on immune responses. ***Objective****:* The study was aimed to investigate the relationship serum level of (*TNFα*- 308) correlated with pancreatic and liver disease. disorders in a subset of beta thalassemia major (BTM) people in general. ***Methods****:* Patients with pancreatic issues with thalassemia major received forty blood samples obtained, forty patients who suffering from thalassemia together with liver disorder, and Thalassemia was discovered in 40 people individuals were not diagnosed with liver or pancreatic issues, and forty samples as control were taken from people are the same age and gender. For the purpose of detecting and genotyping of TNFα-308 in all samples, the method was used T-ARMS-PCR. TNF-α serum level was detection with use ELISA. ***Results****:* The findings of estimation TNF α serum level showed that group B had higher level (137.894 ± 4.216 pg/ml) and group D had the lowest level (89.870 ±3.644 pg/ml). In addition, the findings of effect *TNFα*-308 genotypes on TNF α serum level of β- thalassemia major patients recorded that highest TNF α serum level in GA genotype which was (136.560±2.694 pg/ml) as compared to the GG genotype which was (106.746±2.235 pg/ml). Also the result show correlation between TNFα- 308 genotypes with TNF α serum level in all disease groups. ***Conclusion:*** Through the results we obtained, we concluded that there is relation between TNFα- 308 genotypes with TNF α serum level in A, B and C groups as well as show high TNF α serum level (pg/ml) in BTM patients as compared with control group.

Keywords: TNFα 308 G\A, ELISA, Beta thalassemia major, T-ARMS-PCR.

1- INTRODUCTION

BTM is the generality common genetic disease worldwide, according to estimates that 3% of world population carries one of β-thalassemia phenotype properties [1]. Tumor necrosis factor-α, have a wide range of biological activities and recognized to be an important mediator of systemic inflammation, and immune response, TNF-α can be

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utilization as a predictor of liver inflammation [2] due to double properties of pro- and anti- inflammatory cytokines, TNF-α act a role in defensive responses, but cause damage to organ when the balance is broken [3]. Many TNF-α polymorphisms have been recognition within the *TNF-α* promoter at the positions, relative to the transcription start location one of these sites is -308 (G/A), Moreover, A single base variations in the coding sequence of DNA of a percentage of the individuals in a population is recognized as a single nucleotide polymorphism (SNP). In the human genome, they are the most frequent types of variation [4].

There are three single- nucleotide polymorphisms (SNPs) in the TNF-α gene's promoter region. By regulating the creation of TNF-α, these SNPs modify the concentration of the hormonal in the circulatory system. among these SNPs include, 308 (rs1800629*)* polymorphism. TNF-α 308G/A polymorphism is a alteration G-to-A SNP at position 308, affecting *TNF-α* gene organization and related to variable transcriptiona activities in several diseases, also act important job in the etiology of numerous diseases [5].

2 METHODS AND MATERIALS

Samples that contained blood were obtained from overall of 120 patients from Ibn Al-Baldi Hospital in Baghdad, All three disease groups include patients suffering from thalassemia, and each group includes 40 patients and is divided as follows: Group A include patients with pancreatic disorder, Individuals with dysfunctional livers are in group B, while those with pancreas or liver disorders are in group C. D is the reference group, included healthy individuals over the age of 18 . After analyzing the medical records of each individual at Ibn Al-Balad Hospital, the type of thalassemia was determined. B-thalassemia major patients were identified. Both sexes took part in each group. To find liver and pancreatic abnormalities, ferritin, which ALT, which is along with enzyme levels have been determined in each among the four separate groups.

Each participant had a two-milliliter amount of blood obtained. After getting carefully mixed and maintained at -20°C, the samples were placed in anticoagulant tubes using EDTA. In complying with protocol [6], genomic DNA was taken from cold samples of blood using a DNA isolation kit (Geneaid/Taiwan). Using Tetra-ARMS-PCR, the TNF (G/A) nucleotide polymorphism (rs1800629) identified. Bioneer/Korea produced TNF gene-specific primers based on NCBI Gene Bank material base as illustrated in Table 1. Protocol arms pcr of TNF-at the place 308 G/A can be found in Table 2. For serum separation, two ml of blood was put into a sterile gel vaccum tube, left to coagulation for two hours at temperature (25°C), then centrifuged at 3000 rpm for a period 5 min, then serum was separated and transferred to Eppendr of tube, after that stored freezing at (-20°C) until use for evaluation of TNF-α serum level by ELISA. Serum level of TNF-α was assessed by utilize human *TNF-α* (hTNF-α) kit ELISA, and done according to company instruction (Elabscience). SPSS was utilized to analyze the investigation's data [7].

Table 1: T-ARMS PCR Primers of *TNFα* at *308* G/A gene polymorphism with amplicon size and sequence

Primer	Sequence (5'-3')	Product size
TNFα -308 (G)	GGCAATAGGTTTTGAGGGGCGTGG	251bp
TNFα -308 (A)	ACCCTGGAGGCTGAACCCCGGCCT	202bp
TNFα -308 (OT-F)	GCCCCTCCCAGTTCTAGTTCTATC	400bp
TNFα -308 (OT-R)	AAGCGGTAGTGGGCCCTGCACCTTC	

Table 2: PCR ARMS Program of *TNF-α* at locus *308* G/A

PCR step	Temperature	Time	Repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	30 sec.	35cycle
Annealing	66°C	30 sec.	
Extension	72°C	1min.	
Final extension	72°C	5min	1
Hold	4°C	Forever	-

3 - RESULTS

The findings for estimation TNF α serum level in BTM patients and control groups have seen high TNF α serum level (pg/ml) in BTM patients as compared with D group with (P<0.01), This Interferon alpha serum level was 89.870 ±3.644 pg/ml in group D (Control), while it was 137.894 ± 4.216 pg./ml in group B, 136.722 ± 4.72 pg/ml in group A, and 115.076 ± 3.975 pg/ml in group C as shown in table (3).

Table 3: Compare TNF α serum level in A –B -C and D groups

Groups	TNFα serum (pg/ml) Mean ± SE
Group A	136.722 ± 4.727 a
Group B	137.894 ± 4.216 a
Group C	115.076± 3.975 b
Group D	89.870 ±3.644 c
L.S.D=0.01=13.287**	
** Different limited letters in a single column suggest significantly distinct outcomes (P≤0.01).	

The findings of effect *TNFα*-308 (rs1800629) genotypes on TNF α serum level of BTM patients recorded that highest level of TNF α serum level in GA and AA genotypes which (136.560±2.694 pg/ml and 120.660±4.633 pg/ml) respectively as compared to the GG genotype which was (106.746±2.235 pg/ml) with (P ≤ 0.05) as explained in table (4).

Table 4: impact of *TNF-α 308* genotypes on TNFα level serum in *B* -TM patients

Genotype *TNFα-308*	TNFα serum (pg/ml) Mean ± SE
GG	106.746±2.235 c
GA	136.560±2.694 a
AA	120.660±4.633 b
L.S.D(0.05)=13.54*	
* (P≤0.05), different small letters in same column include significantly different	

The relationship between TNF α serum level with *TNFα- 308* genotypes in pancrease and liver disorders thalassemia patients as A, B and C groups, in detail detected a great increase at genotype G A in patients (P ≤ 0.05) in TNF α serum level, followed by A A genotypes patients and finally G G genotypes patients. Furthermore, in patients with liver and the pancreas problems with thalassemia, there was a relationship between the presence of the mutant (A) allele heterozygous (GA) and homozygous (AA) and TNFα serum level, illustrated in table (5).

Table 5: Relationship between *TNFα- 308* Interferon alpha levels in the blood and genotype in all three types

Groups	Genotypes *TNFα- 308*			L.S.D (0.05
	G G	G A	A A	
Group A	81.953±13.111	139.691±4.842	118.407±5.353	35.808*
Group B	93.283±4.146	140.181±4.542	132.334±5.863	
Group C	76.461±8.583	124.218±5.677	106.403±4.635	
* (P≤0.05)				

4 -DISCUSSION

Overexpression of *TNF-α* is considered to contribute to different diseases, such as inflammatory bowel disease, rheumatoid arthritis, as well as sepsis. raised TNF serum has been discovered to be indication for activation of immune in patients with thalassemia, as concentrations of this cytokin appeared a clear connections with blood transfusion needs [8]. In a previous research by Meliconi *et al.,* [9] revealed, about 50% of blood transfusion-dependent patients with homozygous *β*-thalassmia had increased TNF levels. Also Talsania *et al*. [10] suggested that abnormally increase serum concentrations of TNF they found in another hemolytic conditions, like sickle cell anemia. The increased serum level may be due to chronic iron increase which responsible for most of the disorders that occur during the course of *β* -thalassemia major.

Our results was agreement with the results of Malallah *et al*. [11] when found serum level of TNF to be significantly (P < 0.05) higher in thalassemic patients than that in controls. As well as Szuster-Ciesielska *et al*. [12] observed increased TNF in liver cirrhosis and chronic pancreatitis. Tumor necrosis factor-α, act as important role by making hepatocyte apoptosis, which mediates hepatotoxicity in lipo-poly-saccharide (LPS)- [13]. T macrophages and lymphocytes generate tumor necrosis factor-α, this has been demonstrated to possess pro-inflammatory qualities that involve endothelial cell activation, coagulation cascade motivation, acute phase formation of proteins, and others. As stated by Shimizu *et al.,* larger the blood TNF levels in thalassemia patients therefore indicate to a persisting inflammation reaction [14] when reported

that the increase in TNF levels is because chronic inflammatory processes.

The results we obtained are consistent with study of Darweesh and Kadhem [15] who suggested that levels of TNF-α were significantly higher different ($p < 0.05$) among the patients with *TNF-α* -308 genotypes AA with mean serum levels (58.2) and GA with mean serum levels, (49.5pg/mL). while GG genotype exhibited a little TNF- serum level 24.06pg/mL. The *TNF-α* -308 G/A promoter polymorphism appears to be closely related with the growth of many diseases, however, some conflicting results have been documented as mentioned by Laddha *et al*., [16].

The results of correlation between *TNFα-308* genotypes with TNF α serum level can be explained by *TNF-α* (rs1800629) gene polymorphism that led to increase the *TNF α* gene expression which leads to increase production of the TNF α protein that was present as a TNF- α serum level. The polymorphism in the *TNF* -α 308 position lies inside the promoter area for the gene for *TNF* and might change the binding of transcription factors, so leading to increased *TNF-* mRNA synthesis [17, 18] As well as , the G>A change polymorphism at 308 locations of the *TNF-α* gene is significant for its expression since it is located inside the binding site of AP-2 repressive transcription factor [19]. who discovered that the mean TNF-α serum level in patients had AA genotype thalassemia was approximately 12.3 pg/mL, which was far greater ($P < 0.05$) than mean levels in patients had GA genotype thalassemia (5 pg/mL) and GG genotype thalassemia (2.5 pg/mL) [20].

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