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The Association Between Cytokines (IFN-Γ and IL-4) Gene Polymorphisms and Clinical Manifestations of B-Thalassemia in Iraqi Patients

Bashar Abd Alkadhim Naji

Al-Diwaniyah Education Directorate, Ministry of Education, Iraq
Email: sci.bio.phd.22.2@qu.edu.iq

Abstract: This study examined the relationship between genetic polymorphisms in two cytokine genes, IFN-γ and IL-4, and β-thalassemia in the Iraqi population. The study included 50 patients with β-thalassemia and 30 healthy controls. Most companies have not shown any significant variations in age or gender distribution. Scientists examined the specific location (A/T UTR 5644) within the IFN-γ gene. Individuals with the heterozygous (AT) and homozygous (AA) genotypes for this genetic variant were more common than the control group. The genotypes were associated with an increased prevalence of β-thalassemia. The allele A of the IFN-γ gene became more prevalent in patients, suggesting it as a risk factor. The study examined a polymorphism in the IL-4 gene referred to as IL-4 (T/C). Patients having the homozygous genotype (TT) for this polymorphism were more numerous than the control group. This genotype is closely associated with an increased incidence of β-thalassemia. The prevalence of the IL-4 gene allele T increased in patients, raising the risk of β-thalassemia. The heterozygous genotype (TC) showed a trend towards improved hazard but did not reach statistical significance. Research indicates that specific genetic variations (polymorphisms) in the IFN-γ and IL-4 genes are associated with an increased risk of β-thalassemia in Iraqi patients. The insights could be a valuable resource in understanding disease progression and shaping future personalized treatment strategies.

Keywords: β-thalassemia, Cytokine Gene Polymorphism

1. Introduction

β-thalassemia is a genetic blood circumstance this is common in several areas, consisting of Iraq. The illness results in a choppy manufacturing of hemoglobin, ensuing in an excessive case of anemia [1]. Recent studies imply that genetic changes in cytokine-controlling genes may impact the severity of sicknesses in people with β-thalassemia. This observe seeks to explore the relationship among unique cytokine gene versions and the clinical signs of β-thalassemia in Iraqi patients [2]. Cytokines are critical signaling molecules that play an essential role in regulating the immune device. Interferon-gamma (IFN-γ) and interleukin-4 (IL-four) are two crucial cytokines. IFN-γ is related to the T helper 1 (Th1) cell pathway, which stimulates inflammation to fight infections. IL-four is linked to the Th2 pathway, which opposes infection and complements tissue recuperation. Research has indicated a choppy manufacturing of cytokines in individuals with β-thalassemia, which may also influence the severity of the situation [3]. Gene polymorphisms are genetic differences inside the DNA collection of a gene. Particular genetic versions can impact the quantity or performance of cytokine production [4]. Studies imply that genetic versions in cytokine genes which includes IFN-γ and IL-4 may want to effect the likelihood of growing several issues, consisting of β-thalassemia. It is vital to recognize the impact of cytokine gene polymorphisms on β-thalassemia to create tailor-made therapeutic procedures [5]. This examine centers on the Iraqi affected person network, in which β-thalassemia is a extraordinary fitness problem. Studying this unique population is crucial because of the possible genetic variations whilst as compared to previously examined populations.

2. Materials and Methods

Samples collection: A total of 30 healthy individuals and 50 Iraqi patients with β-thalassemia were chosen at random for the bone marrow transplantation study. DNA was extracted from whole blood that had been collected using EDTA as an anticoagulant. The cytokine typing was carried out using a PCR-SSP assay, which allows for

the effective and quick investigation of polymorphisms by using the same amplification and detection conditions. The genotype and allele frequencies of the following cytokine genes: IL-4 (C/T) (F-CAAGTTACTGACAATCTGGTGT; R-CGGCACATGCTAGCAGGAA; 223bp), and IFN-γ (A/T) (F-TCAACAAAGCTGATACTCCA; R-TTCTTACAACACAAAATCAAATCA; 261 bp).

Statistical analysis: By comparing all the genotype frequencies using the chi-square test, we were able to test for Hardy-Weinberg equilibrium.

3. Results

Demographic characteristics of patients and control subjects

Fifty people diagnosed with β-thalassemia and thirty individuals who appeared to be in good health participated in this study. In table (1), you can see the demographic information about the patients and the control group. No significant difference was found in the mean age (P = 0.514) between the control subjects and patients, with the former having an average age of 10.04 ± 13.77 years and the latter having an average age of 11.31 ± 15.89 years. The age distribution of patients and control subjects did not differ significantly from one another (P = 0.854). The gender distribution of the subjects in the study was not significantly different between the two groups (P = 0.324), with the patients' group consisting of 24 men and 26 females (48.00%) and the control group 16 men and 14 females (46.66%).

Table (1): Demographic characteristics of patients and control subjects

Characteristic	**Patients** ***n*** **= 50**	**Control** ***n*** **=30**	***P***
Age (years)			
Mean ±SD	14.31 ± 15.89	16.04 ± 13.77	**0.514 † NS**
Range	10-33	12-32	
< 11, *n* (%)	17 (34.00%)	11 (36.66 %)	**0.854 ¥ NS**
12-20, *n* (%)	13 (26.00%)	9 (30.00 %)	
25-35, *n* (%)	11 (22.00%)	6 (20.00%)	
≥ 40, *n* (%)	9 (18.00%)	4 (13.33 %)	
Gender			
Male, *n* (%)	24 (48.00 %)	16 (53.33 %)	**0.324 ¥ NS**
Female, *n* (%)	26 (52.00 %)	14 (46.66 %)	

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P > 0.05$; HS: highly significant at $P \leq 0.05$.

Detection of IFN-γ (A/T) Polymorphism

The distribution of *IFN-γ (A/T)* Polymorphism was detected by ARMS-PCR technique. At this locus there are three genotypes; AT, AA and TT. figure (1).

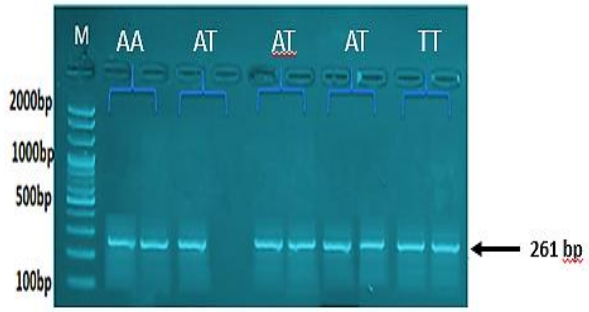


Figure (1): Agarose gel electrophoresis image that showed the ARMS-PCR product analysis of *IFN-γ (A/T)* gene polymorphism. Where M: marker (2000-100bp).

Genotypic and Alleles Analysis for studied genes in Patients with β-thalassemia and Control groups

Table (2) displays the correlation between the risk of β-thalassemia and the IFN-γ (A/T UTR 5644) POLY gene polymorphism. The frequency of the heterozygous genotype AT was significantly higher in the sick group (24 versus 11 in the control group) with a p-value of 0.000020. Hence, the β-thalassemia risk factor genotype CG was associated with an odds ratio of 4.1758 (95% CI: 1.3722-12.7079). Conversely, there was a statistically significant difference between the sick group and the control group in terms of the frequency of the homozygous genotype AA (19 vs. 7, respectively) (P = 0.0099). Hence, having genotype AA increased the likelihood of developing β-thalassemia by 4.0336 (95% CI: 1.3953-11.6608).

Table (2): *IFN-γ (A/T)* POLY genotype frequency in patients with β-thalassemia and control group.

IFN-γ (A/T UTR 5644)	**Patients n = 50**	**Control n =30**	**P1**	**P2**	**OR**	**95% CI**
AA	19	7	0.0099¥S	0.0020 ¥S	4.1758	1.3722-12.7079
AT	24	11		0.253 ¥NS	4.0336	1.3953-11.6608
TT	7	12		Reference	Reference	Reference

***P*1: overall comparison; *P*2: Individual genotype comparison versus reference; n: number of cases; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; NS: non-significant.**

Table (3) shows the relationship between the risk of β-thalassemia and the IFN-γ (A/T) poly allele polymorphism. There was a highly significant difference (P = 0.0071) between the frequency of allele A in the sick group and the control group, with 62 and 25, respectively. The odds ratio for β-thalassemia was 2.1628 (95% confidence interval of 0.2627-0.8139) due to genotype A.

Table (3): *IFN-γ (A/T)* POLY allele frequency in patients with β-thalassemia and control group

IFN-γ (A/T)	**Patients *n* = 50**	**Control *n* = 30**	***P***	**OR**	**95%CI**
A	62	25	0.0071 ¥ HS	2.1628	1.2286- 3.8072
T	38	35		0.4624	0.2627- 0.8139

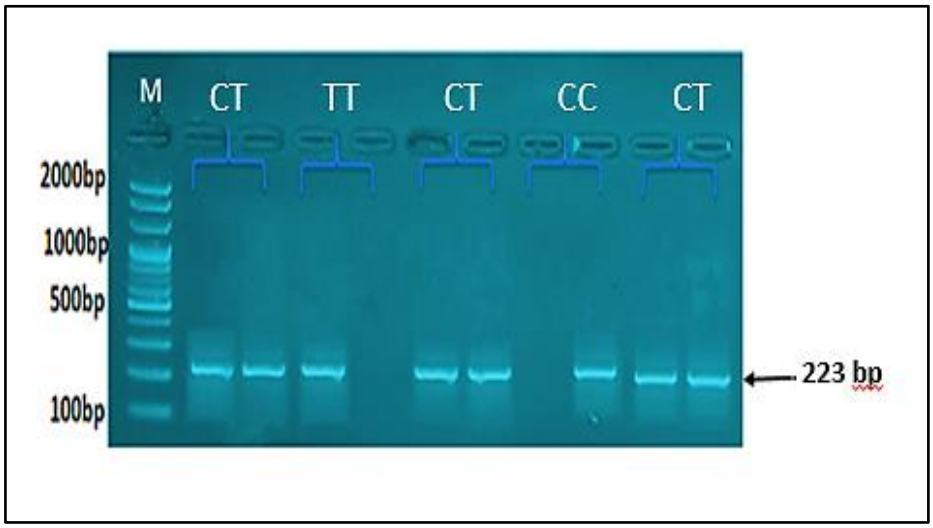
***n*: number of alleles; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; EF: etiologic fraction; PF: preventive fraction; HS: highly significant at *P* ≤ 0.01**

Detection of IL-4 Polymorphism

The distribution of *IL-4 (C/T)* Polymorphism was detected by ARMS-PCR technique. At this locus there are three genotypes; TC, TT and CC. figure (2).

Figure (2): Agarose gel electrophoresis image that showed the ARMS-PCR product analysis of *IL-4 (C/T)* gene polymorphism. Where M: marker (2000-100bp).

Genotypic and Alleles Analysis for studied genes in Patients with β-thalassemia and Control groups



In table (4), we can see the correlation between the β-thalassemia risk and the IL-4 (C/T) POLY gene polymorphism. There was a non-significant difference (P=0.0023) between the sick group and the control group with respect to the frequency of the heterozygous genotype CT (26 vs. 13). Thus, the β-thalassemia risk factor genotype CT was associated with an odds ratio of 2.333 (95% CI of 0.539 -10.1021). However, there was a statistically significant difference between the sick group and the control group in terms of the frequency of the homozygous genotype TT; 11 patients in the former group and 6 in the latter (P = 0.0041). Hence, having the TT genotype increased the likelihood of developing β-thalassemia by 7.210 (95% CI: 1.821-27.074).

Table (4): *IL-4 POLY* genotype frequency in patients with β-thalassemia and control group.

IL-4 (C/T)	**Patients *n* = 50**	**Control *n* =30**	***P*1**	***P*2**	**OR**	**95% CI**
TT	11	6	0.0023 ¥S	0.0041 ¥S	2.333	0.539-10.1021
CT	26	13		0.236 ¥NS	7.210	1.821-27.074
CC	13	11		Reference	Reference	Reference

***P*1: overall comparison; *P*2: Individual genotype comparison versus reference; n: number of cases; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; NS: non-significant.**

Table (5) displays the correlation between the risk of β-thalassemia and the IL-4 (C/T) poly allele polymorphism. The frequency of allele C was significantly higher in the sick group (48 versus 25 in the control group) with a p-value of 0.0034. Type G was associated with an increased risk of β-thalassemia, as indicated by an odds ratio of 4.214 (95% CI: 1.863 -8.591).

Table (5): *IL-4* POLY allele frequency in patients with β-thalassemia and control group

IL-4 (C/T)	**Patients *n* = 100**	**Control *n* = 60**	***P***	**OR**	**95%CI**
T	48	25	0.0034 ¥S	4.214	1.863-8.591
C	52	35		0.861	0.214-1.024

***n*: number of alleles; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; EF: etiologic fraction; PF: preventive fraction; HS: highly significant at *P* ≤ 0.01**

4. Discussion

People with β-thalassemia who have had numerous blood transfusions are more likely to experience unfavorable effects on hematopoiesis due to elevated cytokine levels. A link between cytokine genotype and the physiological production of certain cytokines. It is not uncommon for healthy people to produce cytokines at high, intermediate, or low amounts [6]. Natural selection tends to favour the most advantageous genotype over time, which can explain why a genotype associated with a specific production rate tends to be more common. According to our analysis, our patients had a higher frequency of the A allele at position UTR 5644 IFN-γ and the C allele at position -590 IL-4 compared to the control subjects [7]. The cytokine interleukin 4 (IL-4) is secreted by mast cells and activated T cells. The pleiotropic effects on several cell lineages are due to its membership in the T helper 2 (Th2) class. Th2 immune responses rely on IL-4, which helps Th2 lymphocytes and mast cells mature and become more specialised [8]. By preventing the production of Th1 cytokines, IL-4 reduces Th1 activity and increases tolerance. There is evidence that the IL-4 gene's -590 (CJT) variant is associated with increased IgE levels in asthmatic families [9]. Based on our findings, patients are more likely to have the C allele at IL-4 gene location -590. This provides more evidence that thalassemia patients may have an enhanced IgE response and elevated IL-4 levels [9]. Two common alleles, 2 and 3, make up the IFN-γ microsatellite. In peripheral blood mononuclear cells activated by mitogens, allele 2 is associated with higher levels of IFN-γ production than the other alleles [10]. People who are physically and mentally well tend to produce more intermediate goods. There is evidence linking the IFN-γ Intron 1 microsatellite to several autoimmune and immunological disorders, such as renal transplant rejection and lung transplant fibrosis [11]. IFN-γ differs from other interferons in that it has immunomodulatory effects rather than antiviral ones [12]. The regulation of T cell development and functional differentiation is influenced by IFN-γ, and it also impacts cell-mediated cytotoxicity mechanisms. According to the research, patients show a significant decrease in the A allele at position UTR 5644 IFN-γ [13].

5. Conclusion

Ultimately, researching cytokine gene polymorphisms genetically is expected to offer insights into illness vulnerability across many ethnicities. Researching correlations between cytokine genotypes and immunological phenomena might help explain fundamental biological processes and suggest clinical methods for predicting, preventing, or managing detrimental conditions in diseases. Additionally, it may expand the donor pool through haplo-identical transplants.

6. References

1. Al-Dedah R M, Al-wazni W S, Al-Ghanimi H H, Abduallah F. (2018). Biochemical and Hematological Study with the Appreciation of some Immunological Parameters in Thalassemia Patients at Kerbala Province. Journal of Pure and Applied Microbiology, 12(4), 1965-1974.
2. Al-Hakeim H K, Najm A H, Al-Dujaili A H, Maes M (2020). Major Depression in Children with Transfusion-Dependent Thalassemia Is Strongly Associated with the Combined Effects of Blood Transfusion Rate, Iron Overload, and Increased Pro-inflammatory Cytokines. Neurotoxicity research, 38(1), 228-241.
3. Ratha R, Altaei T (2013). Therapeutic Drug Monitoring of Chelating Agent Deferoxamine for β-Thalassemia Major Patients. International Journal of Clinical Medicine, 4(08), 331.
4. Tari K, Valizadeh Ardalan P, Abbaszadehdibavar M, Atashi A., Jalili, A, Gheidishahran M. (2018). Thalassemia an update: molecular basis, clinical features and treatment. International journal of biomedicine and public health, 1(1), 48-58.
5. Shanaki M, Ehteram H, Nasiri H, Azad M, Kouhkan F, Pakzad R, Mobarra N. (2016). Assessment of Liver and Kidney Functional Parameters along with oxidative Stress and Inflammatory Biomarker in Patients with β-Thalassemia major. Iranian Journal of Pediatric Hematology and Oncology, 6(4), 249-260.
6. Banchereau J, Briere F, Galizzi JP, et al. Human interleukin 4. J Lipid Mediat Cell Signal 1994; 9(1):43-53.
7. Noguchi E, Shibasaki M, Arinami T, et al. Association of asthma and the interleukin-4 promoter gene in Japanese. Clin Exp Allergy 1998; 28(4):449-53.
8. Ali E.Y.and Abdulla. M. M .(2020) . Epidemiological and clinical profile of Iraqi patients with B-thalassemia magor. European Journal of Biology and Medical Science.,8(3):1-15 .

9. Amin ,S. S.; Jalal, S. D.; Ali, K. M.; Mohammed, A. I.; Rasool, L. K. and Osman, T. J.(2020). Beta-Thalassemia Intermedia, A Single Thalassemia Center Experience from Northeastern Iraq. BioMed Research International., 2020 :11.
10. Aminianfar, M.; Khani, F. and Ghasemzadeh, I.(2017) . Evaluation of hepatitis C, hepatitis B, and HIV virus Serology pandemic in thalassemia patients of Shahid Mohammadi Hospital of Bandar Abbas, Iran. Electron Physician.,9(3):4014-4019.
11. Bagheri, M.; Amirzargar, A. A.; Ghavamzadeh, A.; Alimoghadam, K.; Khosravi, F.; Ansaripour, B.and Nikbin, B. (2005). Cytokine gene polymorphisms in iranian patients with beta-thalassemia major. Iranian Journal of Immunology., 2(1): 43-49.
12. Riaz, M.; Abbas, M.; Rasool, G.; Baig, I. S.; Mahmood, Z.; Munir, N. And Akram, M. (2022). Prevalence of transfusion-transmitted infections in multiple blood transfusion-dependent thalassemic patients in Asia: A systemic review. International Journal of Immunopathology and Pharmacology., 36.
13. Cavet J, Dickinson AM, Norden J, et al. Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. Blood 2001; 98(5):1594-600.