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Research paper

The Role of IL-6, TGF-β, and Genetic Variants in The Pathophysiology of Thalassemia

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Abstract

This study investigates the role of inflammatory cytokines and genetic factors in thalassemia, a genetic blood disorder characterized by chronic anemia and systemic complications. The study compared levels of interleukin-6 (IL-6) and transforming growth factor-beta (TGF-β) between thalassemia patients and healthy controls, as well as analyzed genotype and allele frequencies to explore potential genetic associations. Results showed significantly elevated levels of IL-6 (25.88 ± 8.85 pg/ml vs. 14.32 ± 12.14 pg/ml, $p < 0.001$) and TGF-β (1590.91 ± 413.31 pg/ml . 30.23 ± 16.19 pg/ml, $p < 0.001$) in thalassemia patients, indicating a strong association with chronic inflammation and fibrosis. Genetic analysis revealed that the AG genotype and G allele were more frequent in controls (60% and 75%, respectively) compared to patients (50% and 65%, $p = 0.002$ and $p = 0.02$), suggesting a potential protective effect. These findings highlight the importance of cytokine dysregulation and genetic factors in the pathophysiology of thalassemia. Elevated IL-6 and TGF-β levels may serve as biomarkers for disease progression and complications, while genetic profiling could aid in risk stratification and personalized treatment strategies. Future research with larger cohorts is needed to validate these findings and explore their clinical applications. This study contributes to the growing understanding of thalassemia and underscores the potential for targeted therapies to improve patient outcomes.

Keywords: *Thalassemia; Inflammatory Cytokines; IL-6; TGF-B; Genetic Factors.*

1. Introduction

Genetic disorders in human hemoglobin that fall underneath the category of single-gene disorders are among the greatest common disorders. affecting approximately 5% of the total world population who carry one or additional mutations in the genes responsible for hemoglobin creation [1], [2]. One of the most shared genetic blood disorders affecting humans is thalassemia (Mediterranean anemia). This is because hemoglobin is primarily composed of two types of protein, alpha-globin and beta-globin, which bind with the heme pigment to form the complete hemoglobin molecule. The portion of the hemoglobin molecule is made up of two distinct chains, alpha and beta, and whichever can be impacted. In excess of 200 different mutations can cause thalassemia [3], [4]. Because each is illustrious by the attendance of a small number of common mutations and a large number of rare mutations, these mutations are not evenly distributed, but rather must have a geographic and ethnic origin. Thalassemia is a hereditary disorder, which means that at least one of your parents must be a carrier. It is caused by a genetic mutation or the deletion of exact key genetic components. Thalassemia is classified into two types: alpha and beta-thalassemia. Every of them is classified into several types based on the degree of the mutation. Alpha and beta thalassemia are produced by reduced or inattentive production of Beta-globin chains [5]. Beta-thalassemia is more common in people of Mediterranean, African, and Southeast Asian ancestry, whereas alpha thalassemia is more common in people of African and Southeast Asian ancestry [6]. Mutations in the HBB gene on chromosome 11 result in autosomal receding beta thalassemia. The nature of the mutation determines the severity of the illness [7]. The cornerstones of treatment for thalassemia are red blood cell transfusions and iron chelation therapy, with allogeneic hematopoietic stem cell transplantation and gene therapy providing additional disease-management options for qualified patients. With up to 90% of severe cases of Beta-thalassemia happening in resource-constrained countries, and estimates indicating that 22,500 deaths occur each year as a direct result of insufficient transfusion. β-Thalassemia is a genetic disorder with decreased synthesis of beta-globin chains, produced by point mutations in the HBB gene, which are essential components of hemoglobin. This mutation, along with the capture of maturation arbitrated by the TGF β superfamily, leads to ineffective erythropoiesis, a high rate of proliferation and apoptosis, extra medullary hematopoiesis, and plain anemia [8]. Inflammatory signals play a significant role in enhancing NF-κB activity and promote the synthesis of pro-inflammatory cytokines, interferons type I (IFNα and IFNβ), type II (IFNγ), and tumor necrosis factor α (TNFα) with a direct impact on early differentiation and maturation of HSCs [9]. A crucial role in boosting self-renewal and differentiation properties of HSCs is attributed to mesenchymal stem cells (MSCs) by IL-6 and IL-1 cytokines relief as a response to contagion and inflammation. Therefore,

this study aimed to investigate the association of pro-inflammatory cytokines, as well as interleukin-6 (IL-6) and transforming growth factor β (TGF-β), with Thalasemia.

2. Materials and Methods

This study was conducted by collecting (30) blood samples of Thalassemia diagnosed and (20) subjects as Control patients study agreed out in Ibn Albaladi Hospital in Baghdad governorate from October 2023 to April 2024 on the study population.

3. Serum Samples Treatment

Approximately 5 ml of abstention human blood was collected from each subject (patients and control) and transferred into treated test tubes and allowable for 30 minute to mass at room temperature, the sample was centrifuged for 15 minutes at 3000 rotations per minute and the serum was immediately detached and stored at (- 20 0 C) till cast-off for interleukin 6 (IL-6) and Tumor Growth factor-beta (TGF-β).

4. Determination of Interleukin 6 (IL-6) and TGF-β

IL-6 and TGF-β concentrations were estimated in human serum using an ELISA kit from Koma BIOTICH, Company (Korea)[10].

5. DNA Extraction and Polymorphism Genotyping

Genomic DNA was extracted from whole blood using the G-spin™ DNA Extraction Kit (Intron Biotechnology, Korea). DNA concentration and purity were assessed using a NanoDrop spectrophotometer. DNA integrity was confirmed by electrophoresis on a 2% agarose gel at 100 volts for 90 minutes. Samples were stored at –20°C for future use. The polymerase chain reaction (PCR) was performed in a 25µl reaction mixture, pre-mix 5µl (Intron, Korea), 2µl DNA, 2µl of each primer, and 16µl of distilled water as presented in Table 1).

Table 1: PCR Amplification Program TGF-B and IL-6 Gene (Rs1800629 and Rs2736100)

Steps	Predesigned SNP Temp.	Duration	Cycles
Enzyme activation	95°C	10 minutes	HOLD
Denaturation	95°C	15 seconds	40
Annealing/ Extension	60°C	1 minute (scanning)	

6. Statistical Analysis

Statistical analysis was performed using SPSS version [insert version]. Continuous variables were compared using the unpaired Student's t-test. Genotype and allele frequencies were analyzed using Pearson's chi-square test. A p-value of less than 0.05 was considered statistically significant. No correction for multiple comparisons was applied due to the exploratory nature of the study. Power analysis was not conducted, which is acknowledged as a limitation.

7. Results Discussion

A total of 50 subjects participated in the present study, distributed as follows:-
Group 1: (20) apparently healthy subjects as controls.
Group 2 Group 2: (30) Patients with Thalassemia.
Cytokine Levels (IL-6 and TGF-β)
IL-6 (Interleukin-6):
Table 2) showed that the control group had an average IL-6 level of 14.32 ± 12.14 pg/ml. The patient group had a significantly higher average IL-6 level of 25.88 ± 8.85 pg/ml.
The p-value is <0.001, which indicates that this difference is statistically significant. This suggests that IL-6 levels are elevated in patients compared to the control group.
TGF-β (Transforming Growth Factor-beta)
The control group had an average TGF-β level in Table 2) of 30.23 ± 16.19pg/ml. The patient group had a much higher average TGF-β level of 1590.91 ± 413.31 pg/ml.
The p-value is <0.001, which is also statistically significant. This indicates a substantial increase in TGF-β levels in patients compared to the control group.

Table 2: The Mean Values and SD Of IL-6 and TGF-B in Thalassemia Patients and Control Subjects.

Parameters	Control Mean ± SD	Patients Mean ± SD	P value
IL-6 pg/ml	14.32±12.14	25.88±8.85	<0.001[S]
TGF-β pg/ml	30.23±16.19	1590.91 ± 413.31	<0.001[S]

T-test was used *: significant at $p \leq 0.05$.
SD: standard deviation; S: significant; NS: Non-significant.

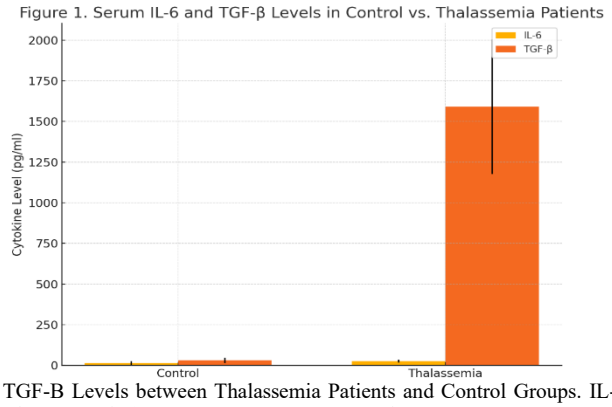

Fig. 1: Comparison of Serum IL-6 And TGF-B Levels between Thalassemia Patients and Control Groups. IL-6 and TGF-B Levels Were Significantly Elevated in Thalassemia Patients Compared to Controls ($P < 0.001$). Data are presented as Mean ± SD.

Both IL-6 and TGF-β levels are significantly higher in patients compared to the control group, suggesting that these cytokines may play a role in the disease process of thalassemia.

The study found that IL-6 levels were significantly higher in thalassemia patients compared to the control group (25.88 ± 8.85 pg/ml, 14.32 ± 12.14 pg/ml, $p < 0.001$). IL-6 is a pro-inflammatory cytokine known to play a role in immune regulation and inflammation. Elevated IL-6 levels have been previously reported in thalassemia patients, particularly in those with iron overload due to frequent blood transfusions {11}. The increased IL-6 levels may contribute to chronic inflammation, which is a common complication in thalassemia patients, leading to tissue damage and other comorbidities [12]. The significant elevation of IL-6 levels in thalassemia patients compared to controls aligns with previous studies that have identified chronic inflammation as a hallmark of thalassemia. IL-6 is a key pro-inflammatory cytokine that promotes the production of acute-phase proteins and contributes to the inflammatory cascade [11]. Chronic inflammation in thalassemia is thought to arise from multiple factors, including ineffective erythropoiesis, hemolysis, and iron overload [13]. Elevated IL-6 levels have been linked to complications such as osteoporosis, cardiovascular disease, and liver fibrosis in thalassemia patients [14].

The study also found a dramatic increase in TGF-β levels in thalassemia patients (1590.91 ± 413.31 pg/ml) compared to controls (30.23 ± 16.19 pg/ml, $p < 0.001$). TGF-β is a multifunctional cytokine involved in fibrosis, immune regulation, and tissue repair. Elevated TGF-β levels have been associated with complications such as liver fibrosis and cardiac dysfunction in thalassemia patients [15]. The high levels of TGF-β observed in this study may reflect ongoing tissue damage and fibrotic processes, which are common in thalassemia due to chronic anemia and iron overload [16]. The studied increase in TGF-β levels in thalassemia patients compared to controls underscores the role of this cytokine in fibrosis and tissue remodeling. TGF-β promotes fibrosis primarily through activation of the SMAD signaling pathway, which stimulates transcription of genes encoding extracellular matrix proteins and profibrotic mediators. TGF-β is a potent profibrotic cytokine that stimulates the production of extracellular matrix components, leading to fibrosis in organs such as the liver, heart, and spleen [17]. Iron overload, a common complication in thalassemia due to frequent blood transfusions, exacerbates TGF-β-mediated fibrosis by promoting oxidative stress and tissue damage [18]

The elevated levels of IL-6 and TGF-β suggest that chronic inflammation and fibrosis may be key pathological processes in thalassemia. These cytokines could serve as potential biomarkers for disease progression and complications, such as organ damage. Targeting these cytokines with anti-inflammatory or anti-fibrotic therapies could be a promising approach to managing thalassemia-related complications [19].

Table 3: Prognostic Association of Genotypes with Allele Frequencies in Control and Patient Groups (Pearson's Chi-Square Test)

Genotyping	Control n=20	Patients n=20	Pearson's chi-square	P- value
GG	4(20%)	8(40%)	0.91NS	0.34
AG	12(60%)	10(50%)	12.01*	0.002
AA	4(20%)	2(10%)	0.83 NS	0.36
Total	20	20		
Allele frequency				
G	30(75%)	26(65%)	5.37*	0.02
A	10(25%)	14(35%)	2.14 NS	0.14

* (P<0.05): significant, ** (P<0.01): highly significant, NS: non-significant.
n= number of cases.

8. Genotyping Results

The study also looked at genetic variations (genotypes) in the groups, specifically focusing on the frequencies of different alleles (G and A) and their combinations (GG, AG, AA). Table 3) showed.

GG genotype: 20% of the control group had this genotype, compared to 40% of the patient group. The difference is not statistically significant (p=0.34).

AG genotype: 60% of the control group had this genotype, compared to 50% of the patient group. The difference is statistically significant (p=0.002).

AA genotype: 20% of the control group had this genotype, compared to 10% of the patient group. The difference is not statistically significant (p=0.36).

Allele Frequencies:

G allele: 75% of the control group had this allele, compared to 65% of the patient group. The difference is statistically significant (p=0.02).

An allele: 25% of the control group had this allele, compared to 35% of the patient group. The difference is not statistically significant (p=0.14).

The study examined the frequencies of specific genotypes (GG, AG, AA) and alleles (G, A) in both thalassemia patients and controls. The AG genotype was significantly more frequent in the control group (60%) compared to the patient group (50%, $p = 0.002$). Additionally, the G allele was more frequent in the control group (75%) than in the patient group (65%, $p = 0.02$). These findings suggest a potential

protective effect of the G allele against thalassemia, as it is more common in healthy individuals. However, the GG and AA genotypes did not show significant differences between the groups, indicating that the AG genotype may play a more complex role in disease susceptibility.

The association between the AG genotype and the G allele with lower disease prevalence aligns with previous studies that have explored genetic polymorphisms in thalassemia. For example, certain genetic variants have been linked to milder forms of thalassemia or reduced disease severity [20]. The findings from this study suggest that genetic factors may influence the risk of developing thalassemia, and further research is needed to explore the specific mechanisms by which these genetic variations confer protection or susceptibility.

The study found that the AG genotype and G allele were more frequent in the control group compared to the thalassemia patients, suggesting a potential protective effect. This finding is consistent with research showing that genetic polymorphisms can influence the severity and clinical presentation of thalassemia [21]. For example, certain genetic variants have been associated with milder forms of thalassemia or reduced disease severity, possibly by modulating hemoglobin production or reducing oxidative stress [22]. The identification of genetic markers associated with thalassemia susceptibility has important implications for genetic screening and counseling. Understanding the genetic basis of thalassemia could help identify individuals at risk of developing severe disease and guide personalized treatment strategies [23].

Note: Genotyping analysis was conducted on a subset of 20 thalassemia patients due to the limited availability of high-quality DNA samples. All 30 patients were included in the cytokine level analysis.

For example, patients with certain genotypes may benefit from early intervention with therapies that target inflammation or iron overload. Sympathetic, the genetic basis of thalassemia could lead to better risk stratification and personalized treatment strategies. For instance, individuals with certain genotypes may benefit from early intervention or targeted therapies to prevent disease progression [24].

One limitation of this study is the relatively small sample size, which may reduce the statistical power and limit the generalizability of the findings. Future studies with larger, multi-center cohorts are needed to validate these results.

Future studies should explore the use of IL-6 and TGF-β inhibitors in thalassemia patients with elevated cytokine levels to evaluate their effect on inflammation, fibrosis, and clinical outcomes. Additionally, investigating genotype-specific responses to such therapies may support personalized treatment strategies.

9. Conclusion

In conclusion, this study highlights the potential role of IL-6 and TGF-β as biomarkers for inflammation and fibrosis in thalassemia patients. The findings also suggest that genetic factors, particularly the AG genotype and G allele, may influence disease susceptibility. These results contribute to the growing body of evidence on the pathophysiology of thalassemia and underscore the need for further research to explore the therapeutic potential of targeting these pathways.

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