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Investigating The Impact of Catalase Gene Polymorphism Rs7943316 on Beta-Thalassemia Major Susceptibility in An Iraqi Patient

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Keywords: Beta-Thalassemia Major, CAT rs7943316, Oxidative Stress, Genotype.

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Abstract:

Beta-thalassemia major (β-TM) is a severe genetic blood disorder prevalent in regions including the Mediterranean, Middle East, and parts of Asia, marked by impaired synthesis of the beta-globin chain of hemoglobin. This leads to significant health complications such as chronic anemia, iron overload, and increased oxidative stress in patients. This study investigated the potential role of the rs7943316 polymorphism in the catalase gene in the pathophysiology of β-thalassemia major in Iraq. Between October 2023-January 2024, 105 blood samples were collected from 60 β-thalassemia patients on iron therapy for at least two years and 45 healthy controls. This study compared the demographic characteristics of the control and β-TM patients. The study found significant differences in hemoglobin and ferritin levels between the groups and significant variation in human catalase. Patients with β-TM exhibited elevated levels (225 ± 121 KU/L) compared to the control group (85.2 ± 18.6 KU/L), with a p-value of less than 0.0001. The study found no significant correlation between the rs7943316 polymorphism and an increased risk or severity of β-thalassemia in the studied population. Catalase serum levels were significantly higher in β-thalassemia patients than in controls, suggesting that this polymorphism does not significantly contribute to clinical variability or risk.

Keywords: Beta-thalassemia major, CAT rs7943316, Oxidative stress, Genotype

التحقيق في تأثير تعدد أشكال جين الكاتالاز *rs7943316* على قابلية الإصابة بفقر الدم البحري (البيتا ثلاسيميا) بيتا في المرضى العراقيين

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الخلاصة:

الثلاسيميا بيتا الكبرى هي اضطراب دموي وراثي شديد منتشر في مناطق تشمل البحر الأبيض المتوسط والشرق الأوسط وأجزاء من آسيا، يتميز بضعف تخليق سلسلة بيتا–غلوبين الهيموغلوبين. هذا يؤدي إلى مضاعفات صحية كبيرة مثل فقر الدم المزمن، وزيادة الحديد، وزيادة الإجهاد التأكسدي لدى المرضى. تدرس الدراسة الدور المحتمل لتعدد الأشكال rs7943316 في جين الكاتالاز في الفيزيولوجيا المرضية لفقر الدم المنجلي الشديد في السكان العراقيين. بين أكتوبر ٢٠٢٣ ويناير ٢٠٢٤، تم جمع ١٠٥ عينات دم من ٦٠ مريضًا بمرض الثلاسيميا بيتا الذين يتلقون علاج الحديد لمدة لا تقل عن عامين و٤٥ شخصًا سليمًا كضوابط. قامت الدراسة بمقارنة الخصائص الديموغرافية لمجموعة التحكم ومرضى β–TM. وجدت الدراسة اختلافات كبيرة في مستويات الهيموغلوبين ومستويات الفيريتين بين المجموعتين، كما وجدت الدراسة تباينًا كبيرًا في الكاتالاز البشري. أظهر المرضى المصابون بمرض الثلاسيميا بيتا مستويات مرتفعة (٢٢٥ ± ١٢١ KU/L) مقارنة بمجموعة التحكم (٨٥.٢ ± ١٨.٦ KU/L)، مع قيمة p أقل من ٠.٠٠٠٠١. لم تجد الدراسة أي ارتباط كبير بين تعدد أشكال rs7943316 وزيادة خطر أو شدة β–thalassemia في السكان الذين تم دراستهم. كانت مستويات الكاتالاز في المصل أعلى بشكل ملحوظ في مرضى بيتا–ثلاسيميا. بالمقارنة مع المجموعة الضابطة، مما يشير إلى أن هذا التعدد الشكلي لا يساهم بشكل كبير في التباين السريري أو الخطر.

الكلمات المفتاحية: الثلاسيميا الكبرى، *CAT rs7943316*، الإجهاد التأكسدي، النمط الجيني

1. Introduction:

Beta-thalassemia (β-TM) is a group of hereditary hemoglobinopathies characterized by the synthesis of an insufficient beta-globin chain, splicing mutation, or unstable transcript and α-globin overproduction imbalance [1]. The number of defective genes determines the clinical framework, which ranges from the asymptomatic form to transfusion-dependent thalassemic forms[2]. In compound syndromes, the lack or presence of a specific mutation may accentuate the imbalance of chains, influencing the clinical picture, such as the association with an α-globin chain mutation [3]. β-TM is a genetic disorder primarily found in the Mediterranean,

Middle East, Indian subcontinent, tropical and subtropical regions, Central and South America, Southeast Asia, Indonesia, Malaysia, and South China [4]. It causes structural abnormalities in the β-globin chain and is a significant global health issue. Annually, 9000-40000 children are born with transfusion-dependent β-thalassemia, with a 1% carrier frequency in late pregnancies [5].

Different types of beta-thalassemia can be characterized into two basic forms: transfusion-dependent and non-transfusion-dependent, which can be classified as β-TM or Cooley's anemia as a more severe form that requires regular blood transfusion and iron chelation therapy following it, and beta-thalassemia intermedia (TI) as the non-transfusion-dependent type with a milder form of the disease, in which patients are diagnosed later with more intermediate signs and symptoms [6]. Beta-thalassemia minor (beta-thalassemia carriers) is a minor form of the disease that is heterozygous and is known as the thalassemia trait in some parts of the world .[7] The severity of the disease depends on several factors, such as the nature of the genetic mutation and the genetic factors between individuals and families that are different in each region owing to geographical and environmental bases [8].

The Middle East has the highest carrier rate (2–15%), resulting in an elevated prevalence of β-TM. In North Africa, a carrier rate as high as one in seven has been reported for some tribal groups. In Europe, the Mediterranean belt has a dual prevalence varying from 1 to 15% in the south to 0–1% in northern operative European countries, while in non-operative, pre-, and postoperative donors, the carrier rate is almost the same, that is, 0–1% [9]. The Indian subcontinent reveals significant regional variation in the carrier frequency with an increased rate reported from the central region, i.e., 0.3–17%. South-East Asians have the highest carrier frequencies of 8% and 10% in the Malay Peninsula and Indonesian archipelago, respectively [10]. Severe anemia can cause symptoms such as pale skin, bone pain, abdominal swelling, fatigue, irritability, and increased shortness of breath, with some individuals experiencing severe symptoms, whereas others only experience mild symptoms [11]. Late complications can have long-term consequences. Approximately half of the individuals with β-TM can live beyond 40 years on regular transfusions, proper iron chelation, and careful hygiene [12].

Patients with β-thalassemia may experience excessive reactive oxygen species (ROS) due to repeated blood transfusions, resulting in altered oxidative status. This is higher than that in other chronic transfusion patients who also experience ROS damage [13]. Catalase (CAT) is a vital enzyme in cellular homeostasis and is involved in the degradation of hydrogen peroxide, a

reactive oxygen species (ROS) that can cause oxidative stress when present in excess. When hydrogen peroxide levels are high, body defense mechanisms increase catalase production, reduce stress, and mitigate damage [14]. Catalase activity can be influenced by genetic mutations that alter its structure or function, or by environmental factors, such as exposure to toxins, drugs, or radiation. These factors can inhibit catalase activity and contribute to oxidative stress, highlighting the importance of understanding and managing these factors to ensure optimal catalase function [15]. Catalase gene polymorphism rs7943316 has been associated with an increased risk of beta-thalassemia major in certain populations. This polymorphism, located in the promoter region of the catalase gene, affects the A-21T (rs7943316) site near the transcription start point [16]. This study aimed to investigate the potential contribution of the rs7943316 polymorphism in the catalase gene to the pathophysiology of β-thalassemia major in an Iraqi population. Analysis of the genotypic and allelic frequencies of this polymorphism in patients with beta-thalassemia

2. Material and methods:

Design and Sampling: This case-control study was conducted from October 2023 to January 2024. Blood samples (105) were collected from patients visiting the Thalassemia Center in Al-Diwaniyah City, Iraq. They were labelled 60 as study cases, which were diagnosed with β-thalassemia and were referred to as iron therapy; their ages ranged from 2 to 36 years. The remaining 45 subjects were healthy and used as controls, with no history of β-thalassemia or other related blood disorders.

2-1 Processing of blood sample: Under sterile conditions and in duplicate, 5 ml of venous blood was collected from each participant. Three milliliters of blood in the tube was used to produce serum, to subsequently measure the activity of CAT. Serum was obtained by centrifugation for 3 min at 5000 rpm and stored at room temperature (25°C) for 30 min prior to CAT activity analysis. The remaining 2 ml of blood was collected in EDTA tubes for DNA extraction and molecular biology examination.

2-2 Catalase Concentration: Catalase levels in the serum were determined using an ELISA kit purchased from BT Lab China. Catalase standards were prepared, and a standard curve was created to calculate CAT abundance in the samples, following the manufacturer's instructions for preparing wash buffer and distilled water.

2-3 Extraction of genomic DNA: Genomic DNA was isolated from the collected blood samples following the manufacturer's protocol using a Bosphore® Genomic DNA Extraction Spin Kit and measured for both quality and quantity using a NanoDrop UV spectrophotometer. The absorbance ratio at 260/280 nm was recorded and used as an indicator of DNA purity.

2-4 Genotyping of rs7943316 SNP: A single nucleotide polymorphism (SNP) in the catalase gene (rs7943316) was genotyped using an allele-specific PCR assay. Primer sequences specific for each allele (A and T) for subjects identified by the SNP were inferred using the Primer-BLAST program from the National Center for Biotechnology Information (NCBI). The sequences are as follows:

F: GATTGGCTGAGCCTGAAGTCGCCACCGA,
R: CAGGCAAATCTGCCTGTTGCCCCGTGA,
F: TCTCCGGTCTTCAGGCCTCCTTCGGAGAGC,
and R: GCTCGGGGAGCACAGAGTGTACCTGCGC

Primers to amplify the two target regions of the catalase gene were designed and used in PCR experiments. The PCR products were electrophoresed on an agarose gel. The DNA fragments migrated into the agarose matrix. After applying a voltage, they were compared to the DNA ladder to identify specific alleles at the rs7943316 SNP.

3.Statistical analysis

Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23. Genotypic and allelic frequencies of the proportion of genotype variants of rs7943316 in the patient and control groups were analyzed and evaluated. The $\chi 2$ test was used to compare genotypic and allelic frequencies among patients and controls. Odds ratios and 95% confidence intervals provided an estimation of the odds for the association of the rs7943316 polymorphism with the risk of β-thalassemia. Statistical significance was set by 0.05.

4. Results

This study compared the demographic characteristics of control subjects and those with β-TM, a form of thalassemia. The control group had 45 individuals with an average age of 23.4 ± 8.2 years, while the β-TM group had 60 individuals with an average age of 20.3 ± 6.2 years. The control group had a higher percentage of males (61.7%) than did the β-TM group (46.7%). The study found a notable difference ($p < 0.001$) in β-TM and controls in hemoglobin levels (Hb), that showed an average Hb of 7.91 g/dL in β-TM as opposed to 11.7 g/dL in controls.

There was a statistically significant difference between the study and control groups regarding the PCV ($P < 0.001$). The mean PCV in the study group was lower than that in the control group at 26.8%, signifying reduced red blood cell mass and a chronic anemic state related to β-thalassemia major. There was a significantly higher average ferritin level (2718 ng/mL) in the β-thalassemia major group than in the control group, which had an average of 91.6 ng/Ml. There was a highly significant difference in the serum ferritin levels between the groups ($p < 0.001$).

Table 1 provides a comprehensive comparative analysis of the biomarkers between the control group and individuals with β-TM. The concentration of human catalase exhibited significant variation. Patients with β-TM exhibited elevated levels (225 ± 121 KU/L) compared to the control group (85.2 ± 18.6 KU/L), with a p-value of less than 0.0001.

Table 1: Comparison between β-TM and control group for all study biomarkers

Characteristic	Control *n* = 45	β-TM *n* = 60	*p*
Human Catalase (KU/L)			
Mean ±SD	85.2 ± 18.6	225 ± 121	<0.0001 I****
Range	45.8 – 124.8	102 – 490	

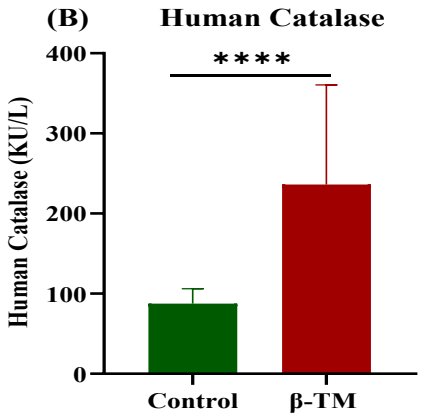

Figure 1: The distribution of Human Catalase in the form of a bar chart

In the present study, the distribution of the A and T SNP (rs7943316) in the catalase gene was analyzed by PCR. The distribution of genotypes and allele frequencies between β-thalassemia cases and healthy controls was compared **(Figure 1** and **2** and **Table 1)**.

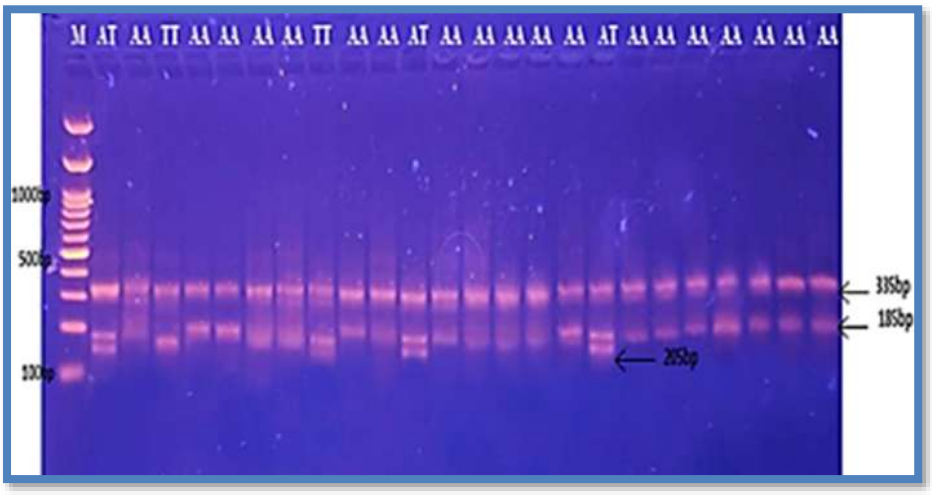

Figure 2: Agarose gel electrophoresis image of the Tetra-ARMS-PCR of the rs7943316 polymorphism in β-thalassemia patients. A. M: DNA marker; the study identifies three genotypes: TT (335bp, 185bp), AT (111bp, 205bp), and AA (335bp, 205bp) in different locations.

Table 2 presents the frequencies of three genotypes in thalassemia patients and healthy controls: AA, AT, and TT genotypes. There were no significant differences between the patient and control groups.

Table 2: Distribution of CAT SNP-21 A/T (rs7943316) genotypes and allele frequency in thalassemia patients and healthy control.

Genotype	Observed		Expected		P-value	O.R.	C.I.
	Control N=45 (%)	Patients N=60 (%)	Control N=45 (%)	Patients N=60 (%)			
AA	34 (75.5)	38(63.33)	31 (68.89)	31.1 (51.84)	0.182	0.55	0.236 - 1.319
AT	7 (15.5)	10 (16.6)	12.7 (28.2)	24.19 (40.32)	0.878	1.08	0.378 - 3.115
TT	4 (8.8)	12 (20)	1.30 (2.89)	4.70 (7.84)	0.117	2.56	0.767 - 8.558

NS: Non-Significant

Table 3 shows that the frequency of allele A was higher in the control group (83.33%) than in the patient group (71.66%). Conversely, the T allele showed a higher frequency in the patient group (28.33%) than in the control group (16.66%). The analysis revealed significant differences in allele frequencies between the two groups

Table 3: Distribution of CAT SNP-21 A/T (rs7943316) allele frequency in thalassemia patients and healthy control.

Allele Frequency	Observed		χ^2	P-value	O.R.	C.I.
	Control N=45 (%)	Patients N=60 (%)				
A	75(83.33)	86(71.66)	3.91*	0.041	0.05	0.225 - 1.000
T	15(16.66)	34(28.33)	3.91*	0.041	1.97	0.999 - 3.909

* (P≤0.05),- Significant.

5. Discussion

The study evaluated the Hb levels of both groups and observed a significant difference between them, showing higher potential Hb levels in healthy individuals than in thalassemia patients. There was a significantly higher average ferritin level in the β-thalassemia major group than in the control group, and several studies have reported significantly higher average ferritin levels in β-thalassemia major patients compared to control groups, as demonstrated in a study by Susanah *et al*. β-thalassemia, Journal of General Hospital, the median serum ferritin level of newly diagnosed patients was significantly higher than controls [17]. A similar study in Pakistan showed that high serum ferritin levels (>1000 ng/ml) were observed in 86.57% of patients with β-thalassemia major, with a mean serum ferritin of 2752.33 ± 945.41 ng/ml. This is significantly higher than that observed in healthy individuals [18]. These studies consistently demonstrated that patients with β-thalassemia have significantly elevated serum ferritin levels compared to healthy controls, reflecting the iron overload commonly observed in this condition due to frequent blood transfusions and increased iron absorption.

In this study, we evaluated the levels of catalase in thalassemia. This outcome showed that the level of catalase in the serum of patients with thalassemia was considerably higher than that in healthy volunteers. With some findings aligned with the observation of significantly higher average catalase in the β-thalassemia major group, a study Shekhar *et al*. found that catalase activity was significantly increased in both β-thalassemia major and minor patients compared to healthy controls. This increase was more pronounced in patients with thalassemia major [19]. Research conducted by Bou-Fakhredin et al. reported elevated levels of antioxidant enzymes, including catalase, in patients with β-thalassemia major. This increase is interpreted to be a compensatory mechanism against oxidative stress [20]. Hanan et al. found elevated catalase levels in patients with β-thalassemia, which they attributed to a protective mechanism against oxidative damage caused by iron overload [21] These conflicting results highlight the complexity of oxidative stress mechanisms in β-thalassemia and suggest that catalase levels may vary depending on factors such as disease severity, treatment status, and individual patient characteristics.

A comprehensive analysis of CAT rs7943316 polymorphisms in thalassemia patients has been conducted across multiple populations, yielding diverse results. Skakir et al. observed three genotype patterns (TT, AA, and AT) in 50 thalassemia patients and 20 controls and found no statistically significant differences between the groups [22]. Tripathi et al. investigated 200

β-thalassemia major (β-TM) patients and 200 healthy controls in India and found no significant association between CAT rs7943316 polymorphisms and β-TM, although they noted the potential influence of rs7943316 on gene expression owing to its promoter region location [23]. Conversely, a Brazilian study by Yahouédéhou et al. reported a higher frequency of the T allele and AT genotype of rs7943316 in patients with β-thalassemia than in healthy controls, suggesting a potential association between this polymorphism and conditions related to oxidative stress. These conflicting findings underscore the complexity of genetic associations in thalassemia and highlight the need for further research to elucidate the role of CAT rs7943316 polymorphism in different populations [24].

6. Conclusion:

Serum catalase levels were significantly higher in β-thalassemia patients than in healthy controls, and there was no significant association between the rs7943316 polymorphism and an increased risk or severity of β-thalassemia.

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