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Investigation of JAK2 V617F mutation prevalence in a sample of Iraqi patients with Beta thalassemia major

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Abstract

Introduction: β-thalassemia major is a common inherited hemoglobin disorder worldwide, characterized by shortened red cell survival, defective hemoglobin synthesis, resulting in hemolytic anemia, ineffective erythropoiesis, severe anaemia, hypoxia, and hepatosplenomegaly. This study aimed to evaluate the occurrence of the JAK2 V617F mutation and JAK2 polymorphism in β-thalassemia patients compared to non-β-thalassemia controls. Additionally, the relationship between JAK2 mutations/polymorphisms and specific clinical and hematological parameters was investigated.

Methods: This was a case-control study involving 71 participants, comprising 51 individuals with β-thalassemia major (patient group) and 20 healthy individuals matched for age and gender (control group). Hematological parameters were measured using an automated analyzer. Venous blood samples were collected with EDTA anticoagulant and stored at 4 °C for subsequent detection of JAK2 V617F mutation using DNA sequencing methods.

Results: Analysis of β-thalassemia patients receiving regular blood transfusions revealed significantly lower levels of red blood cells (RBCs), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and higher levels of white blood cells (WBC) and ferritin compared to the control group. However, the JAK2 V617F mutation was not detected in any patients or controls. The most common genotype observed in β-thalassemia patients was TC (52.94%), followed by the TT genotype, but this did not show significant associations with clinical and hematological parameters. However, there were significant differences in the frequencies of genotypes of the rs12343867 polymorphism between patients and controls.

Conclusion: The study did not find evidence supporting the role of JAK2 V617F mutation in the development or clinical course of β-thalassemia. Furthermore, the mutation was not detected in the normal subjects either. The frequency of JAK2 gene polymorphism did not significantly differ between thalassemia patients and normal subjects, and different genotypes of the gene polymorphism did not show associations with demographic, clinical, or hematological parameters.

Keywords: Investigation, JAK2 V617F mutation, prevalence, Iraqi patients, Beta thalassemia major.

Introduction

Thalassemia is a genetic disorder that affects the haemoglobin in the red blood cells. The condition is caused by a partial or complete deficiency of one of the globin chains that make up hemoglobin, with the most common types being α- and β-thalassemia [1]. While heterozygotes are usually asymptomatic, severely affected patients are homozygotes for α- or β-thalassemia or compound heterozygotes for different molecular forms of α- or β-thalassemia. The disorder causes a shortening of red cell survival and leads to a hemolytic picture, as well as death in erythroid precursor cells in the bone marrow. This ineffective erythropoiesis leads to severe anemia, hypoxia, and bone marrow expansion with hepatosplenomegaly, and requires continuous blood transfusions for β-thalassemia major patients [2]. The JAK2 gene is an important gene that plays a role in the JAK2-STAT signal transduction pathway [3, 4]. The JAK2-V617F mutation is an acquired somatic mutation that results in the constitutive activation of the JAK2-STAT pathway, leading to the regulated expansion of affected hematopoietic precursor cells. This mutation is highly prevalent in myeloproliferative neoplasms, including polycythemia Vera, essential thrombocytosis, and primary myelofibrosis [5, 6]. Hypoxia and anemia both cause an increase in erythropoietin levels, leading to increased JAK2 cytoplasmic pathway activity and an increase in erythroid precursor cells in the bone marrow.

The relationship between JAK2 and β-thalassemia suggests that small-molecule JAK2 inhibitors could be used to reduce ineffective erythropoiesis and splenomegaly [7-9]. The study aims to evaluate the prevalence of JAK2 V617F mutation and JAK2 polymorphism in β-thalassemia patients and controls, as well as to correlate these mutations with clinical and hematological parameters. This study could provide valuable insight into the relationship between JAK2 and β-thalassemia, and lead to the development of new therapies for this genetic disorder.

Method

This cross-sectional case-control study included 51 patients with transfusion-dependent β-thalassemia and a control group of 20 healthy individuals. The patients were recruited from the Thalassemia Center of AL-Karama Teaching Hospital in Baghdad over a period of three and a half months. Verbal permission was obtained from the participants or their caregivers, and all information was kept confidential. The patient group consisted of individuals diagnosed with β-thalassemia major, with ages ranging from 5 to 31 years. The diagnosis was based on a complete blood count, blood film examination, and HPLC or hemoglobin electrophoresis. The control group comprised healthy individuals between the ages of 6 and 25 years, with no family history of thalassemia or other hemoglobinopathies. A questionnaire was used to collect information from the participants, including age, sex, type of thalassemia, blood transfusion details, family history, paternal consanguinity, medical and drug history, as well as investigation results such as complete blood count, blood film, virology screen, serum ferritin, and reports of abdominal ultrasound and echocardiogram. Venous blood samples were collected from both groups using EDTA anticoagulant and stored at 4°C for subsequent analysis. DNA extraction was performed following the ABIO pure Extraction protocol. The concentration of extracted DNA was quantitated using a Quantus Fluorometer. PCR amplification was carried out using specific primers, and the annealing temperature of the primers was optimized. Agarose gel electrophoresis was performed to confirm the presence of PCR amplification. Statistical analysis was conducted using SPSS software version 25.0. Descriptive statistics, Student's t-test, ANOVA, chi-square test, and logistic regression analysis were used to analyze the data. A P-Value less than 0.05 was considered statistically significant. The study aimed to determine the prevalence of JAK2 V617F mutation and JAK2 polymorphism in β-thalassemia patients compared to healthy controls. It also aimed to explore the association of these genetic variations with clinical and hematological parameters in β-thalassemia. This study collected data from patients with transfusion-dependent β-thalassemia and healthy controls, followed by DNA extraction, PCR amplification, and agarose gel electrophoresis. Statistical analysis was performed to assess the prevalence of JAK2 mutations and their relationship with clinical parameters.

Results

Patients and controls were comparable in terms of age and gender with no significant difference. However, consanguinity marriage was far more common among patients than controls (66.67% vs. 15%) with a highly significant difference. The mean blood transfusion in patients was 1.82±0.48 times (range: 1-3 times). All patients originally had splenomegaly, 8 of whom (15.69%) underwent splenectomy. About half of the patients had some kind of comorbidity, the most common of which was osteoporosis and growth delay accounting for 21.57% of the patients for each (Table 1).

Table 1: Demographic and clinical baseline of the study population

Characteristics	Patients (N=51)	Controls (N=20)	P-Value
Age, years			
Mean ± SD	15.1±6.3	17.8±5.5	0.208
Range	5.0-30.0	6.0-28.0	
Gender			
Male	28(54.9%)	8(40%)	0.259
Female	23(45.1%)	12(60%)	
Consanguinity marriage			
No	17(33.33%)	17(85%)	< 0.001
Yes	34(66.67%)	3(15%)	
Blood transfusion/month			
Mean ± SD	1.82±0.48	---------	-----
Range	1.0-3.0		
Splenomegaly	43(84.13%)	---------	-----
Splenectomy	8(15.69%)	---------	-----
Comorbidities			
None	26(50.98%)	----------	-------
Osteoporosis	11(21.57%)		
Growth delay	11(21.57%)		
Others*	8(15.69%)		

SD: Standard Deviation. *Other comorbidities include hepatomegaly (2 cases), glucose 6 phosphate deficiency (2 cases), and one case of each of diabetes, cholecystectomy, nasopharyngeal cancer, gall stone.

Most hematological parameters were significantly variated between patients and controls. The mean total RBC, PCV and Hb in patients was 3.3±0.34×10^9/L, 25.12±3.8%, and 8.67±1.39 g/dl, respectively compared with 4.89±0.31×10^9/L, 42.02±2.8% and 13.85±1.15 g/dl, respectively in controls with highly significant differences. Similarly, patients demonstrated a lower mean of MCV and MCH (77.6±6.82 ft and 25.97±3.26 pg, respectively) than controls (85.07±3.26 ft and 28.12±1.19pg, respectively) with highly significant differences. In contrast, patients had a higher mean of total WBC and ferritin (10.41±4.2 ×10^9/L and 3623±2674 ng/ml, respectively) than controls (7.13±1.84 ×10^9/L and 65.8±39.36 ng/ml, respectively) with highly significant difference (table 2).

Table 2: Hematologic parameters in patients and controls.

Variables	Patients (N=51)	Controls (N=20)	P-Value
Total RBC×10^9/L			
Mean ± SD	3.3±0.34	4.89±0.31	< 0.001
Range	2.16-4.12	4.55-5.41	
Hematocrit (%)			
Mean ± SD	25.12±3.8	42.02±2.8	< 0.001
Range	15.6-31.5	38.6-47.7	
Hb (g/dl)			

Mean ± SD	8.67±1.39	13.85±1.15	< 0.001
Range	4.9-10.8	12.1-16.3	
MCV (ft)			
Mean ± SD	77.6±6.82	85.07±3.26	< 0.001
Range	56.5-85.9	76.9-88.2	
MCH, PG			
Mean ± SD	25.97±3.26	28.12±1.19	0.006
Range	16.4-29.9	26.0-30.1	
Total WBC×10^3/ml			
Mean ± SD	10.41±4.2	7.13±1.84	0.001
Range	4.0-24.5	4.13-9.71	
Platelets ×10^3/ml			
Mean ± SD	321.33±107.83	310.8±61.95	0.683
Range	110-589	213-465	
Ferritin, ng/ml			
Mean ± SD	3623±2674	65.8±39.36	< 0.001
Range	291-11825	28.0-148.0	

SD: Standard Deviation

The distribution of different genotypes in the included SNPs was found to be in good accordance with HWE (Hardy-Weinberg equilibrium). The heterozygous genotype TC was more frequent among patients than controls (52.94% vs. 40%); however, the difference was not significant. In contrast, the homozygous mutant genotype (CC) was more frequent in controls (20%) than patients (5.88%) with no significant difference (the wild type of gene is TT). Analysis of allele distribution revealed a higher frequency of T allele among patients than controls (67.65% versus 60%) with no significant difference (Table 3).

Table 3: The frequency of different genotypes and alleles of polymorphism rs12343867 in thalassemia patients and controls

Rs12343867	Patients (N=51)	Controls (N=20)	P-Value	OR (95%CI)
Genotypes				
TT	21(41.18%)	8(40%)		
TC	27(52.94%)	8(40%)	0.217	1.0
CC	3(5.88%)	4(20%)	0.150	0.28(0.05-1.57)
HWE	0.135	0.456	0.081	0.22(0.04-1.21)
Dominant model				
TT+TC	48(94.12%)	16(80%)	0.090	1.0
CC	3(5.88%)	4(20%)		0.25(0.05-1.24)
Recessive model				
TT	21(41.18%)	8(40%)	0.928	1.0
CC+TC	30(58.82%)	12(60%)		0.95(0.33-2.73)
Alleles				
T	69(67.65%)	24(60%)	0.389	1.0
C	33(32.35%)	16(40%)	0.389	0.72(0.34-1.53)

Generally, none of the included parameters had a significant association with a particular genotype of rs12343867 gene polymorphism. Although MCH was slightly higher in patients carrying the CC genotype (28.23±1.1 PG) than either those carrying the TT genotype (26.06±3.23 PG) or TC genotype (25.65±3.41pg), the differences were not significant. Furthermore, patients carrying CC genotype demonstrated a light elevation of PLT count (350.7±82.64) compared with those carrying the TT genotype (314.71±115.75) and TC genotype (323.2±106.72). Again, the differences were not significant (Table 4).

Table 4: Association of haematology parameters with different genotypes of rs12343867 gene polymorphism

Variables	TT genotype (N= 21)	TC genotype (N= 27)	CC genotype (N=3)	P-Value
Total RBC×10^9/L				
Mean ± SD	3.41±0.37	3.25±0.48	3.03±0.2	0.242
Range	2.83-4.12	2.16-3.95	2.85-3.24	
Hematocrit (%)				
Mean ± SD	25.53±3.74	24.93±4.06	23.9±1.78	0.748
Range	19.8-31.5	15.6-31.10	21.9-25.3	
Hb (g/dl)				
Mean ± SD	8.87±1.2	8.51±1.59	8.67±0.51	0.693
Range	6.2-10.8	4.9-10.6	8.1-9.1	
MCV (ft)				
Mean ± SD	77.8±7.35	77.28±6.66	79.1±6.4	0.900
Range	58.1-85.9	56.5-85.0	73.2-85.9	
MCH, pg				
Mean ± SD	26.06±3.23	25.65±3.41	28.23±1.1	0.432
Range	18.2-29.9	16.4-29.6	27.2-29.4	

Total WBC×10^3/ml				
Mean ± SD	10.9±4.57	10.0±3.96	10.54±4.76	0.775
Range	5.6-24.5	4.0-19.42	7.32-16.0	
Platelets ×10^3/ml				
Mean ± SD	314.71±115.75	323.2±106.72	350.7±82.64	0.862
Range	144-589	110-580	291-445	
Ferritin (ng/ml)				
Mean ± SD	3510±2806	3762±2757	3162±724	0.909
Range	871-11825	291-9623	2744-3999	

The three genotypes were generally comparable in all included demographic and clinical characteristics with no significant differences. All patients carrying the CC genotype had comorbidity, and 66.67% of them had growth delay compared with about 32% and 52% of patients carrying TT and TC genotypes, respectively having comorbidity, and 14.27% and 22.22%, respectively having growth delay. Statically, the differences were not significant (Table 5).

Table 5: Association demographic and clinical characteristics with different genotypes of rs12343867 gene polymorphism

Characteristics	TT genotype (N= 21)	TC genotype (N= 27)	CC genotype (N=3)	P-Value
Gender				
Male	12(57.14%)	14(51.85%)	2(66.67%)	0.856
Female	9(42.86%)	13(48.15%)	1(33.33%)	
Consanguinity				
No	5(23.81%)	11(40.74%)	1(33.33%)	0.467
Yes	16(76.19%)	16(59.26%)	2(66.67%)	
Blood transfusion per month				
1 time	7(33.33%)	4(14.81%)	0(0%)	0.307
2 times	14(66.67%)	21(77.78%)	3(100%)	
3 times	0(0%)	2(7.4%)	0(0%)	
Splenomegaly	19(90.48%)	22(81.48%)	2(66.67%)	
Splenectomy	2(9.52%)	5(18.52%)	1(33.33%)	
Comorbidities				
None	13(61.9%)	13(48.15%)	0(0%)	0.122
Osteoporosis	5(23.81%)	5(18.52%)	1(33.33%)	0.796
Growth delay	3(14.27%)	6(22.22%)	2(66.67%)	0.118
Others	2(9.52%)	5(18.52%)	0(0%)	0.479

Discussion

Thalassemia (BT) is an inherited genetic disease characterized by ineffective erythropoiesis (IE), which leads to anemia and abnormal iron metabolism [10]. IE is marked by an abnormal expansion of erythroid progenitor cells and the production of defective red blood cells, resulting in anemia and hypoxia. High levels of erythropoietin (EPO) are induced by anemia and hypoxia, leading to the activation of the JAK2/STAT5 pathway in erythroid progenitors, primarily in the spleen [11]. A particular study included patients with an age range between 5 and 30 years, with a mean age of 15.1-6.3. The control group consisted of individuals aged 8 to 25 years, with a mean age of 17.8±5.5. There was no significant difference in age and sex distribution between the patients and the control group (P-value of 0.259). These findings were consistent with previous studies conducted in Iraq and other non-Iraqi populations [12, 13]. Regular blood transfusion is the primary treatment for beta-thalassemia major (TM) to improve anemia and suppress ineffective erythropoiesis. The frequency of blood transfusions typically ranges from every two to five weeks, depending on the individual's transfusion needs, to maintain pre-transfusion hemoglobin levels between 9 and 10.5 g/dL. This level promotes proper growth, enables normal physical activities, inhibits bone marrow activity, and minimizes transfusion AL iron accumulation. Patients with heart disease, clinically significant extramedullary hematopoiesis, and splenomegaly may require more frequent transfusions to maintain higher hemoglobin levels (11-12 g/dL) [14, 15]. Consanguinity marriages were found in 66.67% of the patients with beta-thalassemia, while 33.33% had non-consanguineous marriages. In the control group, 15% had consanguinity marriages, and 85% had non-consanguineous marriages. There was a significant difference between the two groups (P-Value < 0.001). Consanguinity marriages were also reported in other studies, and the higher prevalence of consanguinity in parents of patients with major hereditary hemolytic diseases, including TM, is related to the autosomal recessive mode of inheritance of the disease [16, 17]. Splenomegaly was a prominent clinical sign observed in 84.13% of the patients, while 15.69% had undergone splenectomy. Splenomegaly is commonly observed due to excessive destruction of abnormal red blood cells, extramedullary hematopoiesis, and iron overload. Splenomegaly increases the need for blood transfusions. Splenectomy is indicated when hypersplenism leads to an increased requirement for blood transfusions and interferes with effective iron control through chelation therapy [18, 19]. The study identified various complications and comorbidities in 31 patients, including osteoporosis (11), delayed growth (11), diabetes mellitus (1), hepatomegaly (2), glucose-6-phosphate dehydrogenase deficiency (2), gallstones (1), nasopharyngeal cancer (1), and cholecystectomy (1). These complications are part of the sequelae of thalassemia, resulting from anemia, hemolysis, infections, marrow expansion, and consanguinity marriages between parents. The study revealed significant differences in several hematological parameters between patients with beta-thalassemia and controls. The total red blood cell

(RBC) count, packed cell volume (PCV), hemoglobin (Hb) level, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were significantly lower in patients compared to controls, with a P-Value of < 0.001. Similar findings were reported in other studies conducted in Iraq and elsewhere (20, 21). The white blood cell (WBC) count was significantly higher in patients, with a P-Value of 0.001. This result was consistent with studies conducted in Palestine and Wasit, where an increased WBC count was attributed to the presence of nucleated red blood cells [22, 23]. However, the platelet count did not show a significant difference between patients and controls in the current study (p=0.683), which was in line with findings from a study conducted in Turkey [24]. Serum ferritin levels were significantly higher in patients compared to controls (36232.674 ng/ml vs. 65.839.36 ng/ml), with a highly significant difference (p<0.001). Similar findings were reported in other studies conducted in Iraq and Egypt [25]. The accumulation of unpaired alpha-globin chains in beta-thalassemia patients leads to cellular oxidative damage and contributes to iron overload, resulting in increased ferritin levels. Regular blood transfusions further exacerbate iron overload, as each unit of transfused blood introduces 200 to 250 mg of iron to the patient's body. The excess iron cannot be efficiently removed by the body, leading to increased serum ferritin levels. Additionally, iron is distributed to the reticuloendothelial system, leading to increased ferritin synthesis and release into the bloodstream [26, 27]. However, it's worth noting that serum ferritin levels may underestimate liver iron concentration in transfusion-independent thalassemia patients compared to regularly transfused thalassemia and sickle cell patients [28]. The JAK2 V617F mutation was not detected in any of the patients with beta-thalassemia or in the control group in the current study. This finding is consistent with a study by Vlackaki, *et al.* in 2012, where all 20 patients with beta-thalassemia tested negative for the JAK2 V617F mutation using the RG-PCR method. These results suggest that the JAK2 gene may not play a role in the pathogenesis of beta-thalassemia, ineffective erythropoiesis, or iron metabolism, as it did not influence the hematological changes observed in the complete blood count (CBC) findings or coagulopathy. However, further studies are needed to provide more conclusive evidence on the potential role of JAK2 inhibitors as a therapeutic option for beta-thalassemia [29]. A local study conducted by Ahmed et al. in 2020 on 50 patients with beta-thalassemia in Erbil also reported negative results for the JAK2 V617F mutation [30]. In contrast, a study by Asadi et al. on 75 patients found that 19% of them were positive for the JAK2 V617F mutation, while 81% were negative. This study reported a significant association between the frequency of blood transfusions and the presence of the JAK2 V617F mutation, but no significant differences were found in terms of sex, age, genotype, or the mutation itself [31]. It should be noted that the absence of the JAK2 V617F mutation in the control group is expected, considering the low prevalence of this mutation in the general population, which is around 0.1-0.2%. The clinical significance of this mutation in the general population is still unknown, and no specific changes in blood counts or laboratory tests have been demonstrated among individuals without signs of myeloproliferative neoplasms [32, 33].

Conclusion

The study aimed to investigate the relationship between JAK2 V617 F mutation and thalassemia, but the mutation was not found in any of the patients or normal subjects. The frequency of JAK2 gene polymorphism in thalassemia patients was similar to that of normal individuals, and different genotypes of the gene polymorphism were not associated with demographic, clinical, or hematological parameters. These results suggest that the JAK2 V617 F mutation and the Rs. 12343867 gene polymorphism may not be significant factors in thalassemia pathogenesis, but further research is needed to fully understand the genetic mechanisms of the disease.

Conflict of Interest
Not available

Financial Support
Not available

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