Article

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Association of Hypoxia-Inducible Factor-1 Alpha (C1772T) Polymorphism With Hematological Indices in Sickle Cell Anemia Patients From Taif City

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Background: Sickle cell anemia (SCA) is an inherited disorder characterized by chronic hemolysis and persistent hematological abnormalities. This study investigated the association of the hypoxia-inducible factor-1 alpha (*HIF-1*α) C1772T genetic variant, a key regulator of the hypoxic response, with hematological indices in SCA patients.
Methods: This case-control study included 100 adult SCA patients and 100 age- and gender-matched healthy controls in Taif. Hematological parameters were measured, and *HIF-1*α C1772T genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Statistical analyses were performed using Student's *t*-test or the Mann-Whitney *U* test, Chi-square (χ^2) test, and odds ratio (OR) calculations with confidence intervals (CI).
Results: SCA patients exhibited markedly reduced hemoglobin ($t = 27.59$, $p < 0.0001$) and hematocrit levels ($t = 41.54$, $p < 0.0001$), and elevated white blood cell ($t = 7.45$, $p < 0.0001$) and platelet counts ($t = 9.22$, $p < 0.0001$). The CT genotype occurred more frequently in SCA patients (18%) than in controls (7%) ($\chi^2 = 5.5$, $p = 0.019$; OR = 2.91, 95% CI: 1.16–7.33). Similarly, the T allele frequency was higher among SCA patients (9%) than controls (3.5%) ($\chi^2 = 5.2$, $p = 0.023$; OR = 2.72, 95% CI: 1.11–6.68). Among SCA patients, carriers of the CT genotype had significantly higher hemoglobin ($t = 2.83$, $p = 0.005$), hematocrit ($t = 3.05$, $p = 0.002$), and fetal hemoglobin levels ($p = 0.047$), but lower serum ferritin levels ($p = 0.026$) than CC carriers.
Conclusions: The *HIF-1*α C1772T polymorphism is associated with SCA in this population. The presence of the T allele, particularly in the heterozygous CT genotype, may increase susceptibility to SCA. Additionally, CT carriers exhibited a more favourable hematological profile than those with the CC genotype. These findings suggest that this polymorphism may influence susceptibility to SCA, pathophysiology, and clinical severity of SCA.

Keywords: hypoxia-inducible factor-1 alpha; sickle cell anaemia; polymorphism; hematological indices

Introduction

Sickle cell anemia (SCA) is a severe autosomal recessive hemoglobin disorder with global prevalence, disproportionately affecting populations of African, Middle Eastern, Mediterranean, and South Asian ancestry [1]. It represents a major public health challenge in Saudi Arabia, with notably high incidence reported in regions such as Taif [2].

This inherited blood disorder is characterized by the presence of abnormal hemoglobin S (HbS), which leads to the formation of rigid red blood cells with a sickle-like morphology that impedes blood flow through small vessels [3]. The fundamental molecular mechanism underlying SCA arises from a single point mutation in the β-globin gene, resulting in the substitution of valine for glutamic acid at the sixth residue of the β-globin chain [4]. This single amino acid replacement profoundly alters the physicochemical properties of hemoglobin, promoting the polymerization of deoxygenated HbS. The resultant hydrophobic interactions between hemoglobin molecules produce rigid, insoluble fibers that distort red blood cells into their characteristic sickle shape [5].

These deformed erythrocytes are inflexible and prone to hemolysis, resulting in chronic anemia. Crucially, their altered shape also obstructs microvascular blood flow, causing vaso-occlusive crises that manifest as severe pain episodes and ischemic damage to multiple organs throughout the body [3]. Repeated cycles of sickling and unsickling further damage the red blood cell membrane, promot-

ing premature erythrocyte destruction and contributing to the chronic inflammatory state characteristic of SCA [6].

The hypoxia-inducible factor 1 (HIF-1) is a key transcriptional regulator that mediates cellular responses to hypoxia, facilitating adaptive changes that support cell survival and proliferation under low oxygen conditions [7]. HIF-1 is a heterodimer composed of two subunits: an oxygen-regulated alpha subunit (HIF-1α) and a constitutively expressed beta subunit (HIF-1β or ARNT) [8]. Under normoxic conditions, HIF-1α is rapidly degraded via the ubiquitin-proteasome pathway, primarily regulated by prolyl hydroxylase domain enzymes. In contrast, hypoxic conditions suppress the activity of these enzymes, stabilizing HIF-1α and enabling its nuclear translocation, dimerization with HIF-β, and subsequent activation of hypoxia-responsive target genes [7].

These target genes participate in diverse biological processes essential for hypoxic adaptation including, erythropoiesis (e.g., *EPO*), angiogenesis (e.g., *VEGF*), glucose metabolism (e.g., *GLUT1*, glycolytic enzymes), and cellular proliferation, survival, and invasion [9]. Genetic variations, specifically single-nucleotide polymorphisms (SNPs), within the *HIF-1*α gene can significantly influence its expression, stability, and transcriptional activity, thereby impacting individual responses to hypoxia and susceptibility to various diseases. Located on chromosome 14q21–24, the *HIF-1*α gene contains several functional SNPs, notably C1772T (P582S) and G1790A (A588A) within exon 12's oxygen-dependent degradation domain (ODD) [10]. These polymorphisms may alter the susceptibility of proteins to prolyl hydroxylation or ubiquitination, modifying their degradation rate and overall abundance, and consequently affecting their transcriptional efficiency [11].

In oncological studies, these *HIF-1*α polymorphisms are associated with variations in tumor angiogenesis, metastasis, drug resistance, and clinical prognosis, highlighting their significance as genetic modifiers of disease progression and therapeutic response [12]. Beyond cancer, *HIF-1*α SNPs are also implicated in non-malignant disorders such as hypertension, coronary artery disease, chronic kidney disease, and adaptation to high altitude, influencing erythropoietic and ventilatory responses [12,13]. This broad impact spectrum of effects underscores the pivotal role of *HIF-1*α genetic variation in regulating oxygen homeostasis and related pathophysiological processes.

Although SCA is widely recognized as a hypoxic-driven disease, limited research has investigated the influence of *HIF-1*α genetic polymorphisms on hematological indices among SCA patients. Therefore, this study aimed to examine the association of *HIF-1*α polymorphisms with hematological parameters in SCA patients residing in Taif. The findings of this study are expected to contribute to an improved understanding of SCA prognosis and the development of targeted therapeutic approaches for improved disease management.

Materials and Methods

Sample Size Calculation

The sample size was determined using G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), based on a Chi-square (χ^2) test for two-group allele frequency comparisons. The calculation assumed a statistical power (1-β) of 80%, a two-sided α error of 0.05, and a medium effect size (w = 0.3) [14]. The analysis indicated that approximately 72 participants per group were required. To ensure sufficient statistical power, 100 participants were recruited for each group. Participants were enrolled from the outpatient clinics of King Faisal Medical Complex, Taif, Saudi Arabia, between April 2024 and March 2025.

This case-control study enrolled a total of 200 participants, equally divided into a patient group and a healthy control group (n = 100 each). Inclusion criteria: Adult patients aged 20–40 years with a confirmed diagnosis of SCA who had not received blood transfusions within the preceding three months. Eligible patients included those with mild SCA who had not yet developed complications, as well as those with a documented history of SCA-related complications such as chronic anemia, acute chest syndrome, or vaso-occlusive crises, provided that they had not experienced an acute sickle cell crisis within 14–30 days prior to enrollment. Exclusion criteria: Patients with severe disease, defined as recurrent vaso-occlusive crises and evidence of end-organ damage, or those with severe comorbidities, including advanced renal failure, decompensated liver disease, uncontrolled cardiovascular disease, malignancy, or any other life-threatening illness, were excluded.

The control group consisted of healthy subjects selected through frequency matching to ensure a comparable distribution of age, sex, and sociodemographic background with the SCA patient group. Controls had no history of SCA or other hematological disorders. All participants (cases and controls) were recruited from the same geographical region (Taif, Saudi Arabia) and self-identified as ethnically Saudi Arabian with no known recent foreign ancestry. Participants reporting mixed ethnic backgrounds or recent immigration were excluded.

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. All participants provided informed consent before enrollment. Ethical approval was obtained from the Ethics Review Committee of King Faisal Medical Complex, Taif, Saudi Arabia (Approval No. 2023-B-48).

Blood Sampling

Five milliliters of peripheral venous blood were collected from each participant at King Faisal Medical Complex into K_3-EDTA vacutainer tubes (0.1 mL of 7.5% K_3-EDTA) for complete blood count (CBC) analysis and DNA extraction. Hematological indices were measured using a

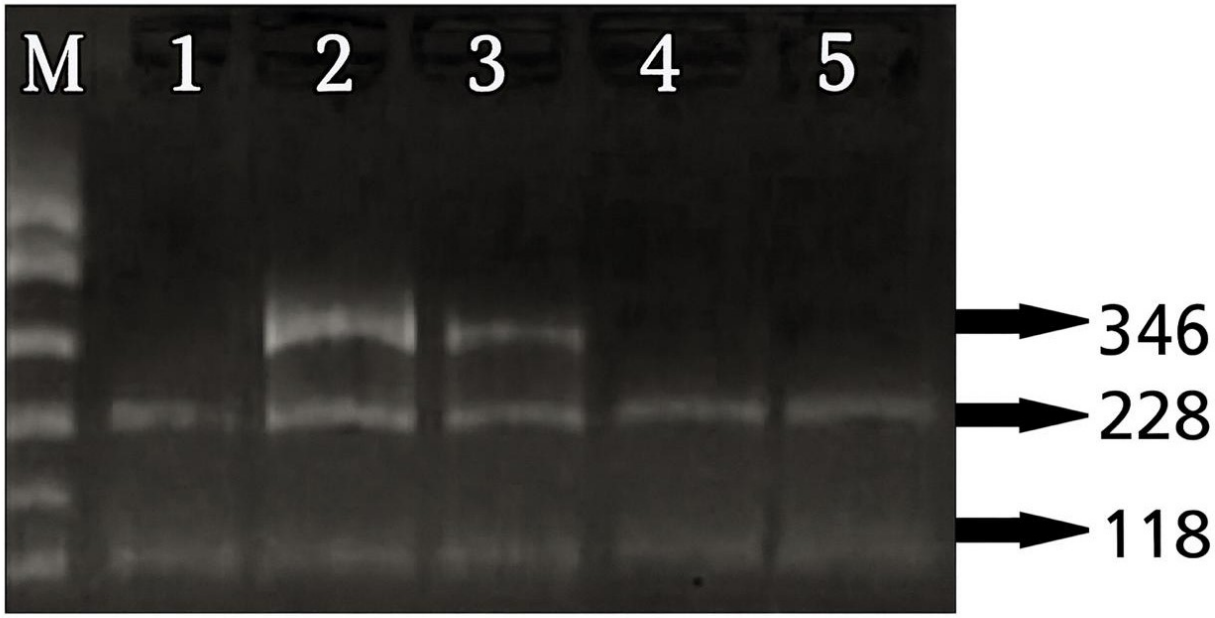

Fig. 1. Agarose gel electrophoresis of the RFLP analysis for the *HIF-1α* C1772T (rs11549465) polymorphism. The heterozygous CT genotype produced three DNA fragments of 346, 228, and 118 base pairs (lanes 2 and 3), while the homozygous CC genotype produced two fragments of 228 and 118 base pairs (lanes 1, 4 and 5). A 50 bp DNA ladder was used as a molecular weight marker and is shown on the left. RFLP, restriction fragment length polymorphism; HIF-1α, hypoxia-inducible factor-1 alpha.

Sysmex XP-300 automated hematology analyzer (Sysmex Corporation, Kobe, Japan).

DNA Extraction

Genomic DNA was extracted from whole blood using the spin column method, following the manufacturer's guidelines (Thermo Scientific GeneJET Genomic DNA Purification Kit, Ref. K0721) (Thermo Fisher Scientific Inc., Waltham, MA, USA). DNA concentration and purity were assessed using a Thermo Scientific NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) by measuring absorbance at 260/280 nm. DNA samples with absorbance ratios between 1.8 and 2.0 were considered of acceptable quality.

Genotyping of HIF-1α C1772T Polymorphism

Genotyping for the *HIF-1α* C1772T (rs11549465) polymorphism was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. PCR amplification was conducted using the forward primer 5′-AAG GTG TGG CCA TTG TAA AAACTC-3′ and the reverse primer 5′-GCA CTA GTA GTT TCT TTA TGT ATG-3′ (Macrogen, Integrated Gulf Biosystems, Riyadh, Saudi Arabia). PCR conditions included an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes.

PCR products were digested with the restriction enzyme RseI (Thermo Scientific, USA) at 37 °C for 2–4 hours to detect the C1772T polymorphism. RseI recognizes the palindromic sequence 5′-GT↓AC-3′ and cleaves between T and A to generate blunt-ended fragments. The C→T substitution at rs11549465 abolishes this restriction site; hence, only the wild-type CC genotype is digested, yielding two fragments of 228 bp and 118 bp, while the mutant TT remains uncut (346 bp). Heterozygous (CT) samples yield three fragment sizes (346 bp, 228 bp, and 118 bp).

Digested and undigested PCR products were separated on a 3% agarose gel, stained with ethidium bromide, and visualized using a Thermo Fisher iBright Imaging System. This system includes a UV transilluminator to make the gel bands visible, a digital camera to capture images, and analysis software for band interpretation. A 50 bp molecular weight marker was used as a reference (Fig. 1).

Genotyping Quality Control:

Duplicate testing: 20 randomly selected samples (10%) were re-genotyped, demonstrating 100% concordance.

Table 1. Demographic and hematological characteristics of sickle cell anemia (SCA) patients and the control group.

Parameter	SCA Group (n = 100)	Control Group (n = 100)	Significance
Age (years)	29.6 ± 7.2	30.0 ± 6.8	$t = 0.40$, $p = 0.686$
Sex – Male	56 (56.0%)	55 (55.0%)	$\chi^2 = 0.02$, $p = 0.886$
Sex – Female	44 (44.0%)	45 (45.0%)	
Hemoglobin (g/dL)	8.8 ± 1.3	13.5 ± 1.1	$t = 27.59$, $p < 0.0001$*
WBCs ($\times 10^9$/L)	10.4 ± 2.7	7.5 ± 2.8	$t = 7.45$, $p < 0.0001$*
MCV (fL)	85.3 ± 6.2	88.1 ± 4.6	$t = 3.62$, $p = 0.0004$*
Hematocrit (%)	26.8 ± 3.7	44.9 ± 2.3	$t = 41.54$, $p < 0.0001$*
Platelets ($\times 10^3$/µL)	368 ± 81	268 ± 72	$t = 9.22$, $p < 0.0001$*
Ferritin (ng/mL) (median, IQR)	30.4 (21.5–43)	16 (9.7–26.5)	$Z = 6.00$, $p < 0.0001$*
HbF (%) (median, IQR)	8.0 (2–26)	—	—

Note: Significance calculated using unpaired sample *t*-test and Chi-square test where applicable. *Statistically significant at $p < 0.05$. WBCs, white blood cells; MCV, mean corpuscular volume; IQR, interquartile range; HbF, hemoglobin.

Call rate: The overall genotyping success rate was 97.5% (96.0% for cases and 99.0% for controls).

Hardy-Weinberg equilibrium: Control genotype frequencies conformed to the Hardy-Weinberg equilibrium ($\chi^2 = 0.132$, $p = 0.71$).

Statistical Analysis

Statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The distribution of variables was evaluated using the Kolmogorov–Smirnov test with Lilliefors significance correction to determine data normality. Quantitative variables were expressed as mean ± standard deviation (SD). Differences in continuous variables between two groups were analyzed using the Student's *t*-test or the Mann-Whitney U test, depending on data distribution. Qualitative variables were summarized as frequencies and percentages and compared using the Chi-square (χ^2) test.

The genotypic distribution of the *HIF-1α* C1772T polymorphism in the control group was examined for Hardy–Weinberg equilibrium (HWE) using the Chi-square goodness-of-fit test. Allele frequencies were calculated, and expected genotype frequencies were determined based on HWE assumptions. A p-value < 0.05 was considered indicative of significant deviation from equilibrium. All statistical tests were considered statistically significant at a $p < 0.05$.

Results

Demographic and Hematological Characteristics

Details of the demographic and hematological characteristics of SCA patients and controls are presented in Table 1.

A total of 200 individuals participated in this study, comprising 100 adult patients with SCA and 100 healthy controls. The two groups were comparable in age (29.6 ± 7.2 vs. 30.0 ± 6.8 years; $p = 0.686$) and gender distribution ($p = 0.886$). Significant differences were observed across all measured hematological parameters. SCA patients exhibited significantly lower hemoglobin (8.8 ± 1.3 g/dL) and hematocrit (26.8 ± 3.7%) levels compared with controls ($p < 0.0001$). In contrast, white blood cell and platelet counts were markedly elevated in the SCA group ($p < 0.0001$). Mean corpuscular volume (MCV) was significantly lower among SCA patients than controls ($p = 0.0004$). Serum ferritin levels were markedly higher in SCA patients ($p < 0.0001$). Fetal hemoglobin (HbF) levels in patients had a median value of 8.0% (interquartile range [IQR]: 2–26%).

Genotypic and Allelic Distribution of HIF-1α C1772T Polymorphism

The distribution of *HIF-1α* C1772T genotypes and alleles among study participants is shown in Table 2.

The genotype distribution within the control group was consistent with Hardy–Weinberg equilibrium ($\chi^2 = 0.132$, $p = 0.71$). The frequencies of *HIF-1α* C1772T genotypes and alleles showed significant differences between the two groups. The heterozygous CT genotype occurred more frequently among SCA patients (18%) than in controls (7%), representing a statistically significant difference ($p = 0.019$). No individual carried the TT genotype in either group. The T allele frequency was 9% among SCA patients compared with 3.5% in controls ($p = 0.023$). Odds ratio analysis revealed that individuals carrying the CT genotype had 2.91-fold higher odds of being in the SCA group, while carriers of the T allele exhibited 2.72-fold higher odds. These findings suggest that the *HIF-1α* C1772T polymorphism may contribute to genetic susceptibility to SCA.

Hematological Indices According to HIF-1α C1772T Genotypes

Hematological indices of SCA patients stratified by *HIF-1α* C1772T genotypes are shown in Table 3.

Comparison of hematological parameters between CC and CT genotypes in SCA patients demonstrated signifi-

Table 2. Distribution of genotype and alleles of the HIF-1α C1772T polymorphism in SCA patients and controls.

Genotype/Allele	SCA Group (n = 100)	Control Group (n = 100)	χ^2	*p*-value*	OR (95% CI)
Genotype					
CC	82 (82.0%)	93 (93.0%)			Reference
CT	18 (18.0%)	7 (7.0%)	5.5	0.019*	2.91 (1.16–7.33)
TT	0 (0.0%)	0 (0.0%)			
Allele					
C	182 (91.0%)	193 (96.5%)	5.2	0.023*	Reference
T	18 (9.0%)	7 (3.5%)			2.72 (1.11–6.68)

Note: **p*-values calculated using Fisher's exact or Chi-square test. OR, odds ratio; CI, confidence interval.

Table 3. Hematological indices in SCA patients according to HIF-1α C1772T genotypes.

Parameter	CC Genotype (n = 82)	CT Genotype (n = 18)	Test Statistic	*p*-value
Hemoglobin (g/dL)	8.7 ± 1.1	9.5 ± 1.0	$t = 2.83$	0.005*
WBCs ($\times 10^9$/L)	10.6 ± 2.5	9.5 ± 2.3	$t = 1.71$	0.089
MCV (fL)	85.0 ± 6.4	89.0 ± 5.1	$t = 2.48$	0.014*
Hematocrit (%)	26.3 ± 3.5	29.1 ± 3.6	$t = 3.05$	0.002*
Platelets ($\times 10^3$/µL)	372 ± 83	340 ± 68	$t = 1.52$	0.130
Ferritin (ng/mL) (median, IQR)	35.2 (22–54)	23.1 (11–37)	$Z = 2.21$	0.026*
HbF (%) (median, IQR)	7.3 (2–19)	11.1 (5–26)	$Z = 1.99$	0.047*

Note: *p*-values were calculated using independent *t*-test or Mann–Whitney U test, as appropriate. *Statistically significant at $p < 0.05$.

cantly improved hematological profiles among CT carriers. Hemoglobin levels were higher in individuals with CT genotypes (9.5 ± 1.0 g/dL vs. 8.7 ± 1.1 g/dL; $p = 0.005$), as were hematocrit (29.1 ± 3.6 % vs. 26.3 ± 3.5%; $p = 0.002$) and MCV (89.0 ± 5.1 fL vs. 85.0 ± 6.4 fL; $p = 0.014$). Ferritin levels were significantly lower in CT patients ($p = 0.026$). Moreover, CT carriers exhibited higher fetal hemoglobin levels (median 11.1% vs. 7.3%; $p = 0.047$). Although white blood cell (WBC) and platelet counts were lower in CT individuals, these differences did not reach statistical significance.

Discussion

Sickle cell anemia (SCA) is an inherited disorder characterized by chronic hemolysis, vascular occlusion, and systemic inflammation, resulting in notable hematological abnormalities. Investigating genetic factors that influence disease severity is essential for optimizing patient management. This study examined the association between the *HIF-1*α C1772T genetic variants and hematological indices in SCA patients from Taif. To the best of our knowledge, this is the first study to specifically explore the impact of this polymorphism in SCA, whereas previous research has primarily focused on its role in other hypoxia-related disorders, such as cancer and diabetes [15,16].

A notable strength of this study is the rigorous matching of SCA patients and controls for age and gender, thereby minimizing potential confounding by demographic variables. All participants, including both SCA patients and controls, were recruited from the same geographical region (Taif, Saudi Arabia), known for its relatively homogeneous population. Furthermore, all participants were of Saudi Arabian ethnicity. Controls were also matched for sociodemographic background, reducing the risk of population stratification between the groups.

Our findings reaffirm several well-characterized hematological abnormalities in SCA patients, consistent with established pathophysiological mechanisms [17]. Specifically, SCA patients exhibited significantly lower hemoglobin and hematocrit levels compared with controls, reflecting the premature destruction of sickled erythrocytes due to the β-globin gene mutation. This persistent hemolysis reduces the oxygen-carrying capacity of blood, contributing to the major clinical manifestations of SCA [4]. Conversely, we observed significantly elevated WBC and platelet counts in SCA patients, indicating a compensatory response to chronic hemolysis, inflammation, and vascular dysfunction. This thrombocytosis may exacerbate the risk of vaso-occlusive crises, a major complication of SCA [18]. Furthermore, ferritin levels were significantly elevated, consistent with previous reports [19] and suggesting enhanced iron turnover and chronic inflammation, both well-recognized characteristics of hemolytic disorders [20]. Unlike microcytic anemia arising from iron deficiency, SCA involves chronic hemolysis that releases iron into the

circulation, leading to systemic iron accumulation. Regular blood transfusions, a standard therapeutic intervention for SCA, further contribute to iron overload. Thus, elevated ferritin levels in SCA primarily reflect iron accumulation rather than deficiency. The significantly reduced mean corpuscular volume (MCV) observed in SCA patients aligns with the frequent presentation of microcytic anemia, resulting from chronic hemolysis and ineffective erythropoiesis [21].

SCA patients in our study demonstrated a median fetal hemoglobin (HbF) level of 8.0%, with an interquartile range of 2–26%, indicating substantial inter-individual variability. HbF mitigates the deleterious effects of deoxygenated HbS by inhibiting its polymerization. This wide variation in HbF levels underscores its role as a significant modulator of disease severity and prognosis in SCA [22].

In terms of the *HIF-1α* C1772T polymorphism, our study revealed a significant association between this genetic variation and SCA, suggesting a potential genetic susceptibility factor. The CT genotype was more prevalent among SCA patients (18%) than in controls (7%), a statistically significant difference ($p = 0.019$). Similarly, the T allele occurred more frequently among SCA patients (9%) compared with controls (3.5%; $p = 0.023$). The TT genotype was not observed in either group. Odds ratio (OR) analysis further supported this association: individuals carrying the CT genotype had a 2.91-fold higher likelihood of being in the SCA group, while the T allele was associated with a 2.72-fold increase in risk. These findings suggest that the presence of the T allele, particularly in the heterozygous state, may confer increased susceptibility to SCA.

Notably, the TT genotype of the *HIF1α* C1772T polymorphism (rs11549465) was not detected in our cohort, and the mutant T allele frequency was 3.5%. This finding aligns with global allele frequency data, which consistently shows that the T allele is rare. According to Islam and Jesmin, data from the 1000 Genomes Project (Phase 3), indicate that the minor allele frequency (MAF) of the T allele of *HIF1A* rs11549465 varies across ethnic groups, with frequencies of 10% in White, 6.2% in Black, and 8.2% in Asian populations. Notably, South Asians exhibit a higher MAF (11.9%) than East Asians (4.6%) [23]. Similarly, Das *et al.* [24] reported that across 111 studies from 57 countries, the MAF for rs11549465 ranged from 0% to 25.7%, indicating substantial inter-population variability.

Regionally, a study conducted in Jordan reported a mutant T allele frequency of 17.5%, comparable to frequencies observed in Turkish, Italian, Iranian, Chinese, Korean, and American populations [25,26]. Conversely, lower frequencies were reported in Japanese, Polish, and Russian populations [27,28]. These findings reinforce that allele frequency is highly population-dependent, and the absence of the TT genotype in a moderately sized sample from Saudi Arabia is statistically plausible. Although data on rs11549465 allele frequency in Saudi Arabia remain scarce, the absence of the TT genotype in our study likely reflects both the global rarity of the T allele and potential population-specific genetic factors.

To our knowledge, no previous research has specifically examined the *HIF-1α* gene C1772T variant in SCA. Although this polymorphism has been explored in other diseases, our study provides novel insights into its potential role in SCA pathophysiology. For example, Tepebaşı *et al.* [29] reported that specific *HIF-1α* polymorphisms, including the C1772T T allele, were associated with an increased likelihood of developing erythrocytosis and polycythemia vera. Genetic variations in *HIF-1α* that modify its transcriptional activity may also contribute to lung cancer development, particularly adenocarcinomas, potentially through enhanced genomic instability [30]. Furthermore, such polymorphisms may affect treatment response and prognosis, with the C1772T CC genotype associated with improved chemotherapy responsiveness in certain studies [31]. Another study found a significantly higher frequency of the C1772T TT genotype in patients with breast cancer compared with controls, suggesting a possible correlation between the T allele and TT genotype and progesterone receptor-negative status [32].

However, emerging evidence highlights the role of *HIF-1α* in modulating hypoxic responses in SCA. *HIF-1α* stimulates the expression of pro-inflammatory cytokines and adhesion molecules, thereby aggravating inflammation and contributing to the development of vaso-occlusive crises (VOC). The interaction among hypoxia, reactive oxygen species (ROS), inflammation, and endothelial dysfunction considerably influences the frequency and severity of VOC episodes [33]. Additionally, *HIF-1α* contributes to disease modulation by enhancing fetal hemoglobin (HbF) synthesis, a protective adaptation in SCA that mitigates hemoglobin polymerization and erythrocyte sickling. This dual role of *HIF-1α*, both as a mediator of pathological inflammation and as a promoter of adaptive HbF expression, has attracted increasing attention in recent therapeutic investigations [34].

Importantly, in our study, SCA patients carrying the CT genotype exhibited improved hematological profiles compared with those with the CC genotype. This included lower ferritin concentrations, possibly indicating reduced hemolytic activity or iron burden, and elevated HbF levels, which may contribute to milder anemia and decreased sickling.

Additionally, CT carriers demonstrated higher hemoglobin and hematocrit levels, increased MCV, and non-significant reductions in WBC and platelet counts. This observation suggests a potentially protective effect of the CT genotype in SCA. Consistent with our findings, a study by Torti *et al.* [35] reported that the *HIF-1α* C1772T polymorphism was associated with improved red blood cell parameters and enhanced iron regulation following blood loss in male blood donors, suggesting its potential predictive utility for post-donation recovery, particularly in males.

Pedrosa and Lemes [36] reported elevated *HIF-1α* gene expression in SCA patients with SS hemoglobin, correlating with several hematological parameters. Specifically, higher *HIF-1α* expression was associated with increased levels of RBC, WBC, and hematocrit, but with decreased hemoglobin and MCHC levels. No significant correlations were observed with MCV, MCH, or platelet counts, highlighting the complex interplay among erythropoiesis, hypoxia, inflammation, and gene expression in SCA.

The observed association between the CT genotype of the *HIF-1α* C1772T polymorphism and improved hematological parameters in SCA patients may be partially explained by the known biological functions of *HIF-1α* in hypoxia-adaptive responses. *HIF-1α* regulates erythropoiesis by stimulating erythropoietin (EPO) production under hypoxic conditions. Elevated EPO levels enhance the proliferation and differentiation of erythroid progenitor cells in the bone marrow, thereby increasing the production of red blood cells. This mechanism could contribute to the higher hemoglobin and hematocrit levels observed in CT genotype carriers [37,38].

In addition to promoting erythropoiesis, *HIF-1α* also regulates iron metabolism by modulating the expression of genes involved in iron transport and storage, such as ferritin and transferrin. It downregulates hepcidin, a key negative regulator of iron absorption, by suppressing its transcription and stabilizing ferroportin [39–41]. These mechanisms enhance iron bioavailability for hemoglobin synthesis, potentially explaining the lower ferritin levels and improved red cell indices observed in CT individuals.

Furthermore, *HIF-1α* influences cellular metabolism through inhibition of mTORC1 via its downstream target gene *REDD1*, thereby promoting autophagy, a process essential for proper erythroid maturation, particularly under hypoxic stress. This pathway provides a plausible mechanistic explanation for the effect of *HIF-1α* on red blood cell parameters [42].

Limitations of the Study

This study has several limitations that warrant consideration. The low frequency of the T allele in our cohort may have reduced the statistical power to detect robust genotype-phenotype associations, especially for rare variants such as *HIF-1α* C1772T. Although a potential association was observed between the CT genotype and favourable hematological indices in SCA patients, the biological mechanisms underlying this relationship remain unclear and warrant further functional validation. Detailed data on hydroxyurea usage, transfusion history beyond three months, nutritional status, and disease severity were unavailable, which may have introduced residual confounding. Additionally, our study primarily focused on hematological parameters, including hemoglobin, MCV, and ferritin, providing a limited perspective on the broader physiological effects of the *HIF-1α* C1772T polymorphism. Future studies incorporating inflammatory biomarkers, oxidative stress markers, and clinical severity indices, as well as functional genomic assays, would provide a more comprehensive understanding of the impact of the variant. Finally, replication studies involving larger and more genetically diverse populations, supported by ancestry-informative markers and multi-center designs, are needed to validate our findings and elucidate the functional relevance of rare polymorphisms.

Conclusions

This study identifies distinct hematological abnormalities associated with sickle cell anemia (SCA) and highlights a potential association between *HIF-1α* C1772T genetic variants and SCA susceptibility. Individuals carrying the T allele and CT genotype exhibited a higher risk of SCA. Additionally, SCA patients with the CT genotype demonstrated a more favourable hematological profile compared with those with the CC genotype, including elevated hemoglobin, hematocrit, MCV, and fetal hemoglobin levels, alongside reduced ferritin concentrations. However, these findings are based on cross-sectional data and lack direct mechanistic evidence. Although the findings suggest that the C1772T polymorphism may influence disease pathophysiology, the proposed mechanisms remain hypothetical. Further functional and longitudinal studies are required to confirm the biological relevance of this variant and clarify its role in modulating hematological and clinical outcomes in SCA.

Availability of Data and Materials

The datasets generated during this study are available from the corresponding author upon reasonable request.

Author Contributions

AFG, EMA, WMB, TMA and HMA made substantial contributions to the conception and design of the work. RAK, TMA and AFG were responsible for data acquisition and analysis. MAlm, MAll, WMB and HMA interpreted the data. AFG, RAK, HMA, TMA, EMA, MAll, MAlm and WMB drafted or critically revised the article for important intellectual content. All authors approved the final version for publication and agreed to be accountable for the work's accuracy and integrity.

Ethics Approval and Consent to Participate

This study was approved by the Scientific Research Ethics Committee at King Faisal Medical Complex in Taif (Approval No. 2023-B-48). Informed consent was obtained from all participants, and the study adhered to the principles of the 1964 Helsinki Declaration and its subsequent amendments.

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Conflict of Interest

The authors declare no conflict of interest.

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