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Common Thrombophilic Mutations among Sickle Cell Disease Patients in the Western Province of Saudi Arabia

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ABSTRACT Sickle cell disease (SCD), linked to vascular thrombosis, is an autosomal recessive disorder. Among the various thrombophilic mutations, Factor V Leiden (FVL) G1691A, prothrombin (PRT) G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T stand out as genetic alterations linked to thrombotic consequences. Thus, the study aims to examine the prevalence of these mutations in SCD patients, utilizing the polymerase chain reaction-based technique. The study design is an observational study. The study results revealed that there are significant levels of the heterozygous form of FVL G1691A and PRT G20210A in the SCD patient population compared to control subjects. While, MTHFR C677T showed no statistical significance. None of the homozygous forms of the three mutations was statistically significant. However, the incidence of FVL, PRT, and MTHFR mutations was higher among female participants than male participants in the study. The high prevalence of these mutations suggests that they may be significant risk factors for the vascular complications in this population. However, further studies are required to validate the current outcomes.

INTRODUCTION

Sickle cell disease (SCD) is one of the most prevalent hemoglobinopathies globally, impacting approximately 20 million people (Zarghamian et al. 2023). It is an autosomal recessive disorder caused by an A-to-T transversion (the E6V substitusion) at codon 6 of the HBB gene, which codes for the adult hemoglobin component €globin (Levesque and Bauer 2023). The mutation in the HBB causes alteration in the structure of normal hemoglobin of the red blood cells (RBCs), resulting in the production of Hemoglobin S (HbS). The severe form (homozygous - HbSS) is inherited by an offspring in case both parents carry defective HBB. The mild heterozygous form (Hb AS) is produced when one defective sickle HBB is inherited from one of the parents, which is called sickle cell trait. Other forms of SCD result from the combination of HbS with other abnormal HBB variants like HbC or β-thalassemia (Eaton and Bunn 2017; Zarghamian et al. 2023). HbS is polymerized under low oxygen condition and forms fibers that make RBCs rigid, dense and less flexible. The RBCs injury caused by HbS leads to intra- and extravascular hemolysis. Further, sickling RBCs get stuck in the blood vessels and block blood flow to different areas of the human body including chest, abdomen, joints, and brain. This leads to chronic hemolytic anemia (Hatzlhofer et al. 2012) and acute vaso-occlusive crisis (van Hamel Parsons et al. 2016; Kato et al. 2017).

There is no specific cure for SCD. Hematopoietic stem cell (HSC) transplantation remains the best curative treatment for such genetic disorder. However, it requires compatible related or unrelated donors in terms of human leukocyte antigen (HLA) types besides other criteria and the risk of graft versus host disease may restrict this procedure for SCD patients (Bolaños-Meade et al. 2019; Eapen et al. 2019). To overcome this limitation, one potential solution involves utilizing autologous HSCs that are corrected through gene therapy techniques to restore the normal expression of hemoglobin. In SCD, there are common genetic polymorphisms that contribute to the occurrence of vascular thrombosis, including factor V Leiden (FVL) G1691A, prothrombin (PRT) G20210A and methylenetetrahydrofolate reductase (MTHFR) C677T which are considered as significant genetic risk factors for thrombotic complications (Belhaj et al. 2018). Furthermore, inheritance of these poly-

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morphisms has been identified as a major cause of vascular thrombosis and found to be linked to genetic modifiers in SCD patients. Their prevalence increased by 4-8 fold among SCD patients in the study by Geard et al. (2016) resulting in a hypercoagulable state. The hypercoagulable state is associated with the activation of coagulation cascade and increased blood predisposition for clotting, which may contribute to vaso-occlusive crisis and subsequently considered as a prominent feature of SCD. It is caused by increased biomarkers level of thrombin generation and enhanced platelet function as well as an increase in the expression of tissue factor, abnormal activation of the fibrinolytic system with the consumption of the natural anticoagulants (Faes et al. 2018). Moreover, FVL G1691A, PRT G20210A and MTHFR C677T mutations demonstrate distinctive worldwide distribution evidenced by previous studies that analyzed the association between these mutations and the development of venous thromboembolism among SCD in their populations (Abdullah et al. 2010; Yousif et al. 2017; Bhaskar 2019).

Though previous studies have been done on these mutations in various populations, their prevalence and clinical significance among SCD patients have not been extensively investigated. Understanding the interplay between these genetic factors and SCD could offer valuable insights into its heterogeneity and associated complications, such as vaso-occlusive crises and other thrombotic events. Due to the significant role of vascular manifestations in the pathophysiology of SCD, this study aims to determine the frequency of factor V Leiden G1691A, prothrombin G20210A and (MTHFR) C677T polymorphisms among SCD patients from Western Saudi Arabia. By elucidating the role of these genetic variants in the context of SCD, we may identify additional risk factors for thrombotic complications and pave the way for personalized therapeutic strategies to improve the management and outcomes of SCD.

Objectives of the Study

The study aims to:

- Assess the hematological parameters of SCD patients
- Assess the prevalence of FVL (G1691A), PRT (G20210A), and MTHFR (C677T) mutations among SCD patients.
- Compare the incidence of FVL (G1691A), PRT (G20210A), and MTHFR (C677T) mutations in female and male SCD patients and identify gender-specific risk factors for thrombotic events.

MATERIAL AND METHODS

Sample Collection and Laboratory Investigation

Blood samples of SCD patients and healthy individuals were obtained after signing consent forms from the main tertiary hospitals. 90 samples from each category were utilized for downstream analyses. Clinical and diagnostic data such as Complete Blood Count, sickle cell screening and Hb electrophoresis were obtained from the electronic patient records. The inclusion criteria included confirmed cases of SCD who gave consent to participate. For the control group, the inclusion criteria included healthy volunteers who agreed to participate in the study. This study was approved by the ethics and research committee at FAMS with a reference number FAMS-2019-002.

DNA Extraction and Preservation

Genomic DNA was extracted from peripheral samples using The Wizard® Genomic DNA purification kit (Promega, USA) according to the manufacturer's instruction. The purity and concentration of extracted DNA were assessed using the Nanodrop 2000 spectrophotometer (ThermoFisher SCIENTIFIC, USA). The extracted DNA was stored at -20 C° for the next experimental phase.

Detection of Factor V Leiden G1691A and Prothrombin G20210A Mutations

FVL (G1691A) and PRT (G20210A) mutations were detected using multiplex polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay with MnlI digestion as previously described (Koksal et al. 2007). Briefly, the reaction contained the primer pairs: forward, 52 -ACA TCG CCT CTG GGC TAA TA-32 ; and reverse, 52 -TTG AAG GAA ATG CCC CAT TA-32 for factor V Leiden amplification and a forward primer sequence, 52 -ATG GGG TGA AGG CTG TGA CC-32 ; with reverse sequence, 52 -AGC ACT GGG AGC ATT GAG CCT-32 for the PRT (G20210A).

The PCR products were digested with MnlI restriction enzyme as per the manufacture's protocol ((ThermoFisher SCIENTIFIC, USA) and subsequently analyzed on a 2% agarose gel. The normal G allele of factor V Leiden (G1691A) was confirmed by the presence of 115 bp fragment while the mutant A heterozygous allele was confirmed by the presence of two fragments (115 and 152 bp). For the PRT (G20210A), the normal G allele was confirmed by 192 bp fragment while heterozygous A allele was identified by the presence of additional 221 bp fragment.

Detection of MTHFR C677T Mutation

The MTHFR C677T mutation was detected based on single plex PCR-RFLP with HinfI digestion as previously described (Nishank et al. 2013). A pair of primers including forward sequence 52 - TGA AGG AGA AGG TGT CTG CGG GA-32 and reverse sequence 52 -AGG ACG GTG CGG TGA GAG TG-32 . PCR products were then treated with HinfI restriction enzyme to generate one band of 198 bp long in case of the wild type while 175 bp and 23 bp fragments in the homozygous state. The presence of heterozygous MTHFR allele generated three bands of 198 bp, 175bp and 23 bp.

Statistical Analysis

Data entry and statistical analysis were done using Microsoft excel to find out statistical significance among the participants. Illustrative charts and graphs for diagnostic workup and molecular testing were described. Categorical variables were reported and explained as frequency and percentage, whereas quantitative continuous variables were described in the form of mean and standard deviation (SD). Chi-square test was applied to investigate the association between categorical variables. Fischer Exact test was applied in case of small frequencies (<5 in one or more of the cells in 2 by 2 table). Statistical significance was determined at P-values of less than 0.05.

RESULTS

Clinical Characteristics

The study included 90 sickle cell patients and an equal number of controls. The general and hematological characteristics of sickle cell cases were summarized. The male gender comprised 41 cases compared to 49 females with a mean age of 21.96 ± 10.67 years. Table 1 provides valuable insights into the characteristics of SCD patients. The white blood cell count (WBCs) was 13.05±4.57 x103 /µL, which exceeded the normal range of 4.5-11 x103 / µL. The red blood cell count (RBCs) was significantly reduced at 2.84±0.74 x106 /µL, compared to the normal range of 4.3-5.7 x106 /µL. The hemoglobin level (Hb) was also lower than the reference values, with an average of 8.23±1.78 g/dL. Hematocrit (Hct) was 24.13±5.03 percent, Mean Corpuscular Volume (MCV) was 85.74±10.76 fL, Mean Cell Hemoglobin (MCH) was 30.26±7.55 pg, and Mean Cell Hemoglobin Concentration was 34.16±1.59 g/dL. The RBC Distribution Width (RDW) was 19.44±3.62 percent, higher than the normal range of 11.6-14.6 percent. Platelet count (Plt) was 386.51±147.88 x106 /µL, within the normal range of 150-440 x106 /µL. Hb electrophoresis results indicated a decrease in HbA (normal range: 95-98%), an increase in HbF (normal range: 0.8-2%), a significant elevation in HbS (normal range: 1.8-3.5%), and a normal level of HbA2 (normal range: 1.8-3.5%). These findings offer valuable insights into the hematological characteristics of SCD patients and provide essential information for diagnosing and managing the disease. The comparison with the reference ranges helps to identify any abnormalities and deviations from the standard values in these patients.

Prevalence of Thrombophilic Mutations in SCD Patients

Table 2 presents the distribution of three genetic mutations (FVL G1691A, PRT G20210A, and MTHFR C677T) among SCD patients and control subjects. Among the 90 sickle cell patients, 29 (32.2%) had the G/A heterozygous genotype, and none had the A/A homozygous genotype for FVL G1691A mutation. In contrast, in the control group, 97.8 percent had the G/G normal genotype, and 2.2 percent had the G/A heterozygous genotype. The difference in distribution between the SCD patients and controls was statistically significant ($p<0.001$). Similarly, for the PRT G20210A mutation, 77.8 percent of the SCD patients had the G/G normal genotype, 20.0 percent had the G/A heterozygous genotype, and 2.2 percent had the A/A homozygous genotype. In contrast, in the control group, 98.9

Table 1: Diagnostic characteristics of sickle cell patients

Variables	*SCD (n=90)*	
Gender, Male - Female	41- 49	
Age, years	21.96± 10.67	
Complete Blood Count	*SCD (n=90)*	*Reference Range*
WBCs (X10^3 /µL)	13.05± 4.57	4.5-11 x10^3 /µL
RBCs (X10^6 /µL)	2.84± 0.74	4.3-5.7 x10^6 /µL
Hb (g/dl)	8.23± 1.78	Male 13.2- 17.3 g/dl, Female 11.7- 15.5 g/dl
Hct (%)	24.13± 5.03	Male 39- 49 %, Female 35- 45%
MCV (fL)	85.74± 10.76	80-100 fL
MCH (pg)	30.26± 7.55	26-34 pg
MCHC (g/dl)	34.16± 1.59	32-36 g/dL
RDW	19.44± 3.62	11.6-14.6%
Plt (X106 /µL)	386.51±147.88	150-440 x106 /µL
Hb Electrophoresis	*SCD (n=90)*	*Reference Range*
HbA (%)	28.80± 21.37	95-98%
HbF (%)	11.91± 8.28	0.8-2%
HbS (%)	68.11± 18.87	1.8-3.5%
HbA2 (%)	3.13± 0.54	1.8-3.5%

Abbreviations: WBC=White Blood Cell, RBC=Red Blood Cell, Hb=Hemoglobin, Hct=Hematocrit, MCV=Mean Corpuscular Volume, MCH=Mean Cell Hemoglobin, MCHC=Mean Cell Hemoglobin Concentration, RDW=Red Cell Distribution, Plt=Platelets, SCA=Sickle Cell Anemia

Table 2: Frequency of Factor V Leiden G1691A, Prothrombin G20210A and MTHFR C677T mutations in the study population

Mutations	*Participants*	*G/GNormal*	*G/A Heterozygous*	*A/A Homozygous*	*P value*
Factor V Leiden G1691A	SCD Patients (n=90)	61 (67.8%)	29 (32.2%)	0 (0.0%)	P-value of Fischer Exact test<0.001
	Controls (n=90)	88 (97.8%)	2 (2.2%)	0 (0.0%)	
Prothrombin G20210A	SCD Patients (n=90)	70 (77.8%)	18 (20.0%)	2 (2.2%)	χ^2=19.48, p<0.001
	Controls (n=90)	89 (98.9%)	1 (1.1%)	0 (0.0%)	
MTHFR C677T	*Participants*	*C/CNormal*	*C/THeterozygous*	*T/T Homozygous*	*P value*
	SCD Patients (n=90)	73 (81.1%)	16 (17.8%)	1 (1.1%)	χ^2=3.38, p=0.185
	Controls (n=90)	81 (90.0%)	9 (10.0%)	0 (0.0%)	

percent had the G/G normal genotype, and 1.1 percent had the G/A heterozygous genotype. The difference in distribution between the SCD patients and controls was also statistically significant (p<0.001). However, for MTHFR C677T, there was no significant difference in distribution between the SCD patients and controls: 81.1 percent of SCD patients and 90.0 percent of controls had the C/C normal genotype, 17.8 percent of SCD patients and 10.0 percent of controls had the C/T heterozygous genotype, and 1.1 percent of SCD patients had the T/T homozygous genotype. The difference in distribution between the two groups was not statistically significant (p=0.185).

Variation of Thrombophilic Mutations According to Gender

Table 3 presents the distribution of mutations (FVL, PRT G20210A, and MTHFR C677T) in the

Table 3: Variation of Factor V Leiden mutations, Prothrombin G20210A and MYHFR C677T in males and females with sickle cell disease

Mutations	*Genotype*	*Male*	*Female*	*P value*
Factor V Leiden (SCD n=90)	G/G Normal	29 (70.7%)	32 (65.3%)	χ^2=0.30, p=0.583
	G/A Heterozygous	12 (29.3%)	17 (34.7%)	
	A/A Homozygous	0 (0.0%)	0 (0.0%)	
Prothrombin G20210A (SCD n=90)	G/G Normal	33 (80.5%)	37 (75.5%)	χ^2=3.55, p=0.170
	G/A Heterozygous	6 (14.6%)	12 (24.5%)	
	A/A Homozygous	2 (4.9%)	0 (0.0%)	
MTHFR C677T (SCD n=90)	G/G Normal	31 (75.6%)	42 (85.7%)	χ^2=2.21, p=0.331
	G/A Heterozygous	9 (22.0%)	7 (14.3%)	
	A/A Homozygous	1 (2.4%)	0 (0.0%)	

SCD patients, categorized by genotype and gender. For FVL, among the 90 SCD patients, 70.7 percent of male patients and 65.3 percent of female patients had the G/G normal genotype, while 29.3 percent of male patients and 34.7 percent of female patients had the G/A heterozygous genotype. No individuals had the A/A homozygous genotype. For PRT G20210A, 80.5 percent of male patients and 75.5 percent of female patients had the G/G normal genotype, 14.6 percent of male patients and 24.5 percent of female patients had the G/A heterozygous genotype, and 4.9 percent of male patients had the A/A homozygous genotype, with no occurrence in female patients . Regarding MTHFR C677T, 75.6 percent of male patients and 85.7 percent of female patients had the G/G normal genotype, 22.0 percent of male patients and 14.3 percent of female patients had the G/A heterozygous genotype, and 2.4 percent of male patients had the A/A homozygous genotype, with no occurrence in female patients . The statistical analysis using the Chi-square test showed no significant gender differences ($p > 0.05$) for all three mutations.

DISCUSSION

SCD is an inherited hemoglobinopathy disorder that leads to multiple organ dysfunction (Hassell 2010). Thrombosis is a significant aspect of clinical complication. Vaso-occlusive crises and acute chest syndrome have been evidenced in sickle cell patients as major causes of mortality (Manci et al. 2003; Naramreddy et al. 2023). Apart from the sickle cell gene, many genetic variables influence the variation in SCD phenotypes. The pathophysiology of thrombosis is significantly influenced by inherited thrombophilia polymorphisms, which are also considered as genetic modifiers of SCD. The presence of FVL G1691A, PRT G20210A, and MTHFR C677T mutations increases the risk of thrombotic complications in SCD patients (Pandey et al. 2012). In this study, the frequencies of factor V Leiden G1691A, MTHFR C677T and PRT G20210A mutations were assessed among SCD using traditional PCR and a multiplex polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques. The findings provide valuable insights into the hematological profile of SCD patients and offer essential information for diagnosing and managing the disease.

Regarding the hematological parameters, the findings of this study showed that the levels of WBC and Plt counts were higher in SCD patients than in healthy individuals, which are in consistent with other previous studies (Curtis et al. 2015; Antwi-Boasiako et al. 2018; Belhaj et al. 2018). The elevated WBC and Plt counts in the SCD patients can be attributed to the chronic inflammatory state and vascular damage associated with the disease (Damanhouri et al. 2015; Yousif 2022). SCD causes the deformation and destruction of RBCs, releasing cellular debris and heme molecules, which trigger inflammation and activate the immune system (Sesti-Costa et al. 2023). This ongoing inflammation stimulates the bone marrow to produce more WBCs as a defense mechanism. Additionally, the damaged blood vessels in SCD can activate platelets, leading to increased Plt production and aggregation to maintain hemostasis (Periayah et al. 2017). These factors collectively contribute to the higher WBC and Plt counts observed in SCD patients compared to healthy individuals.

Several studies have shown that inherited thrombophilia contributes to SCD complications. Such complications were investigated in relation to the prevalence of FVL G1691A, PRT G20210A,

and MTHFR C677T polymorphisms and their link to the occurrence of thrombosis in SCD where wide geographic variation was detected (Fawaz et al. 2004; Rahimi et al. 2008; Al-Saqladi et al. 2010; Hatzlhofer et al. 2012; Nishank et al. 2013). A lower frequency of FVL was reported in Western India, Brazil, Africa, and Eastern Saudi Arabia. In the present study, the frequency of FVL gene was significantly higher in SCD patients than in the control group ($p< 0.05$) where the majority of SCD patients (32.2%) were heterozygous (G>A) compared to the control group (2.2%). Additionally, the prevalence of heterozygous form of FVL was higher among females than males. This is similar to a previous study performed on Egyptian SCD children where a significant high prevalence of the heterozygous form of FVL (30%) were detected compared to 16 percent of normal controls ($p< 0.05$). Furthermore, the odds ratio was calculated in another study and it was revealed that FVL G1691A increased the risk of thrombosis by two folds in patients with SCD (OR=1.7, 95% CI= 1.01-3.43) (Hamdy et al. 2013). Samarah and Srour (2018) reported similar findings in Palestinian SCD patients in their study. The study showed a significantly higher prevalence of FVL mutation among SCD patients (18.64%) than normal individuals. It was related to a higher occurrence of disease complications, including pain in the chest, joints and abdomen (OR= 5.6, 95% CI= 1.9-39.4, p-value = 0.039) in SCD (Samarah and Srour 2018). Another study also demonstrated a significant increase ($p< 0.05$) in the prevalence of FVL among Iranian SCD (14.3%) than normal individuals (1.6%); there was a significant correlation between FVL mutation and SCD among Southern Iranian patients (Rahimi et al. 2008). India's population is diverse, and the distribution of thrombophilic mutations varies in different regions. Pandey et al. (2012) demonstrated that the heterozygous form of FVL (G>A) was significantly higher in Asian Indian patients (8%) than in the control ($p= 0.02$). In central India, Nishank et al. (2013) also observed the heterozygous FVL in 14.6 percent of SCD patients compared to normal individuals. The high frequency of FVL mutation in SCD patients can be associated with a hypercoagulable state due to chronic inflammation, endothelial dysfunction, and increased platelet activation. This prothrombotic environment may create favorable conditions for the FVL mutation to confer a selective advantage, leading to its higher prevalence in SCD patients. In contrast, lower frequency of FVL was previously reported in SCD patients from Eastern province of Saudi Arabia in Dammam city, which contradicts with this study's finding (Fawaz et al. 2004). This is probably due to genetic diversity caused by geographical distance. Also, FVL mutation is more prevalent in certain populations, and SCD commonly affects individuals from specific ethnic backgrounds where FVL mutation is more frequent.

Regarding the prevalence of PRT G20210A, the result of this study showed a significant difference between the heterozygous type of the SCD patients compared to the heterozygous state of control group ($p<0.001$) only; while other types remained non-significant. The findings of this study align with a study that revealed that the PRT G20210A mutation was strongly related with an elevated risk ($p=0.0150$) (Shafia et al. 2018). However, previous studies showed no statistical significance seen in Central India, Asian Indian, southern Iran, and Africa. Hamdy et al. (2013) found that the distribution of PRT G20210A mutation among Egyptian children was 6 percent in SCD patients compared to 2 percent in the normal individuals. This shows there was no statistical significance (Hamdy et al. 2013). Samarah and Srour (2018) reported similar finding in Palestine. None of the Palestinians SCD patients were homozygous for PRT G20210A; however, 8 of the 117 enrolled patients were heterozygous. The overall prevalence of PRT G20210A mutation was 6.83 percent compared to 5.08 percent healthy controls, which was not significantly different. This gives an indication that the PRT G20210A mutation is not a risk factor for vascular complications in SCD (Samarah and Srour 2018). In addition, according to a meta-analysis, patients with SCD are at a high risk of developing vaso-occlusion due to the FVL mutation, but not the PRT G20210A mutation (Bhaskar 2019).

Regarding MTHFR 677T, this study's results showed no statistical significance difference between the genotypes among SCD. This is in agreement with other previous studies (Al-Saqladi et al. 2010; Kangne et al. 2015). Al-Saqladi et al. (2010) examined the prevalence and clinical impact of MTHFR C677T mutation among SCD children in Yemen. The homozygous T/T genotype for MTHFR C677T was found in only 2 out of 102 SCD patients (2%) and heterozygous C/T in 11 out of 102 sickle cell patients (10.8%). The overall allele

frequency was 7.35 percent, which was not higher than in the common Yemeni population (2.0 vs 2.2%). Thus, the MTHFR C677T mutation was not significantly associated with SCD severity (Al-Saqladi et al. 2010). Kangne et al. (2015) also reported that the frequency of MTHFR C677T mutation in the Western India was not higher among SCD patients (5.6%) compared to the normal control. This suggests that MTHFR C677T mutation is not linked to the SCD pathogenesis and its vascular complications (Kangne et al. 2015). Regarding the Indian populations, the prevalence of MTHFR 677 C>T genotype increased among SCD patients (20%) as well as healthy individuals, but this was not statistically significant. The P value was 0.13 (Pandey et al. 2012). This is also in agreement with the study done in Eastern Saudi Arabia, where MTHFR 677 T/T genotype was seen in 9.20 percent of SCD compared to 3.81 percent in healthy individuals (p=0.232). While the prevalence of C/T genotype was 32.18 percent in SCD and 20.95 percent in normal subjects (p=0.109). The frequency of MTHFR C677T mutation increased more among SCD patients than healthy individuals, though it was not statistically significant (Fawaz et al. 2004). In contrast, MTHFR C677T was detected in 40.2 percent of cases in a heterozygous state, 43.4 percent of SS cases, and 34.5 percent of S/â thalassemia cases. There was a significantly higher prevalence of this genetic variant in cases (SCD patients) compared to normal individuals (P = 0.001) (Sokkar et al. 2022). Similarly, Nishank et al. (2013) compared the frequencies of clinical symptoms between SCD with or without mutations for FVL and MTHFR C677T. The results revealed that SCD patients having FVL or MTHFR C677T mutations had higher incidence of joints pain, chest pain, and abdomen pain along with frequent requirement of blood transfusion than the SCD patients without FVL or MTHFR C677T mutations. This indicates that FVL G1691A and MTHFR C677T mutations are considered as risk factors for the development of vascular thrombosis in Central Indian patients with sickle cell anemia. Furthermore, higher frequency of MTHFR C677T polymorphism was detected among SCD in Africa. Homozygous form was detected in 1 patient (1.8%), while 18 patients (34%) were found to have the heterozygous form. On the other hand, association between the 677T allele and vascular complications in SCD patients was identified in Africa. The findings showed that the presence of the MTHFR C677T allele was highly related with the incidence of vascular thrombosis in SCD patients, and the Odds ratios (OR) was 3.8 (95% CI: 1.3-17.3, P= 0.020). Higher prevalence of MTHFR C677T polymorphism among SCD may be a risk factor for vascular thrombosis in SCD (Moreira Neto et al. 2006). Additionally, an increased prevalence of MTHFR C677T mutation was found among Pernambuco Brazilian patients with SCD. The study authors selected two groups of patients: the first group was suffering from vascular complications and the other group had no vascular complications. The genotypes 677 C/T and T/T revealed a significant risk for ischemic stroke and deep vein thrombosis in the SCD patients. This is likely to be an indication for the development of vascular complications in patients with sickle cell disease (Hatzlhofer et al. 2012).

CONCLUSION

SCD patients exhibited abnormal blood cell counts, with elevated WBCs, lowered RBCs, and reduced Hb levels compared to standard reference values. Hct, MCV, MCH, MCHC, and RDW also demonstrated deviations from the normal ranges. The investigation into thrombophilic mutations among SCD patients and controls revealed significant differences in the distribution of FVL G1691A and PRT G20210A mutations. However, there was no notable distinction in the prevalence of MTHFR C677T between the SCD patients and controls. Furthermore, the study explored the influence of gender on the occurrence of these mutations but found no significant gender-related differences for FVL, PRT G20210A, and MTHFR C677T mutations. These findings provide valuable insights into the hematological characteristics and thrombophilic mutations associated with SCD, contributing better ways that can be used for the diagnosis and management of the condition.

RECOMMENDATIONS

Further studies on different regions are suggested to be done to validate the current results. Other rare risk factors contributing to vascular complications in SCD may need to be addressed by future analysis, such as mutations in the factor VII gene, platelet glycoprotein IIIa (GPIIIa) gene, fi-

brinogen gene and others. There is also a need to explore the interplay between SCD and thrombophilic mutations. Longitudinal studies can help to understand the impact of these mutations on the disease course and the effectiveness of various therapeutic interventions. Additionally, investigating the role of these mutations in influencing the severity of SCD-related complications could provide valuable insights for improved patient care. Thus, it is possible to improve therapy interventions to reduce the symptoms that these patients experience.

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DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest to be reported with this work.

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ETHICAL APPROVAL STATEMENT

This study was approved by the ethics and research committee at FAMS with a reference number FAMS-2019-002.

REFERENCES

Abdullah WZ, Kumaraguru S, Ghazali S, Yusoff NM 2010. Factor V Leiden and Prothrombin G20210A mutations among healthy Indians in Malaysia. *Laboratory Medicine,* 41(5): 284-287. doi: 10.1309/LM9W9L 8GQPCZVAYO %J Laboratory Medicine.

Al-Saqladi AW, Harper G, Delpisheh A, Fijnvandraat K, Bin-Gadeem HA, Brabin BJ 2010. Frequency of the MTHFR C677T polymorphism in Yemeni children with sickle cell disease. *Hemoglobin,* 34(1): 67-77. doi: 10.3109/09687630903554111.

Antwi-Boasiako C, Ekem I, Abdul-Rahman M, Sey F, Doku A, Dzudzor B et al. 2018. Hematological parameters in Ghanaian sickle cell disease patients. *Journal of Blood Medicine,* 9: 203-209. doi: 10.2147/jbm.S169872.

Belhaj NR, Doggui R, Ouali F, Messaoud T, Gritli N 2018. Factor V Leiden G1691A, Prothrombin G20210A, and MTHFR C677T and A1298C mutations in patients with sickle cell disease in Tunisia. *Hemoglobin,* 42(2): 96-102. doi: 10.1080/03630269.2018.1451340.

Bhaskar LV 2019. Factor V Leiden and prothrombin G20210A mutations and risk of vaso-occlusive complications in sickle cell disease: A meta-analysis through the lens of nephrology. *Journal of Nephropharmacology,* 8(2): e16-e16. doi: 10.15171/npj.2019.16.

Bolaños-Meade J, Cooke KR, Gamper CJ, Ali SA, Ambinder RF, Borrello IM et al. 2019. Effect of increased dose of total body irradiation on graft failure associated with HLA-haploidentical transplantation in patients with severe haemoglobinopathies: A prospective clinical trial. *The Lancet Haematology,* 6(4): e183-e193. doi: 10.1016/s2352-3026(19)30031-6.

Curtis SA, Danda N, Etzion Z, Cohen HW, Billett HH 2015. Elevated steady state WBC and platelet counts are associated with frequent emergency room use in adults with sickle cell anemia. *PLoS One,* 10(8): e0133116. doi: 10.1371/journal.pone.0133116.

Damanhouri GA, Jarullah J, Marouf S, Hindawi SI, Mushtaq G, Kamal MA 2015. Clinical biomarkers in sickle cell disease. *Saudi Journal of Biological Sciences,* 22(1): 24-31. doi: https://doi.org/10.1016/j.sjbs.2014.09.005.

Eapen M, Brazauskas R, Walters MC, Bernaudin F, Bo-Subait K, Fitzhugh CD et al. 2019. Effect of donor type and conditioning regimen intensity on allogeneic transplantation outcomes in patients with sickle cell disease: A retrospective multicentre, cohort study. *The Lancet Haematology* 6(11): e585-e596. doi: 10.1016/s2352-3026(19)30154-1.

Eaton WA, Bunn HF 2017. Treating sickle cell disease by targeting HbS polymerization. *Blood* 129(20): 2719-2726. doi: 10.1182/blood-2017-02-765891.

Faes C, Sparkenbaugh EM, Pawlinski R 2018. Hypercoagulable state in sickle cell disease. *Clinical Hemorheology and Microcirculation,* 68(2-3): 301-318. doi: 10.3233/ch-189013.

Fawaz NA, Bashawery L, Al-Sheikh I, Qatari A, Al-Othman SS, Almawi WY 2004. Factor V-Leiden, prothrombin G20210A, and MTHFR C677T mutations among patients with sickle cell disease in Eastern Saudi Arabia. *American Journal of Hematology,* 76(3): 307-309. doi: 10.1002/ajh.20087.

Geard A, Pule GD, Chelo D, Bitoungui VJ, Wonkam A 2016. Genetics of sickle cell-associated cardiovascular disease: An expert review with lessons learned in Africa. *Omics: A Journal of Integrative Biology,* 20(10): 581-592. doi: 10.1089/omi.2016.0125.

Hamdy M, Heba MG, Iman AS, Mervat MK, Rania HT 2013. Prevalence of Factor V Leiden (G1619A) and Prothrombin Gene (G20210A) mutation in Egyptian children with sickle cell disease. *Comparative Clinical Pathology,* 22(4): 697-702. doi: https://doi.org/10.1007/s00580-012-1469-7.

Hassell KL 2010. Population estimates of sickle cell disease in the U.S. *American Journal of Preventive Medicine,* 38(4 Suppl): S512-521. doi: 10.1016/j.amepre.2009.12.022.

Hatzlhofer BL, Bezerra MA, Santos MN, Albuquerque DM, Freitas EM, Costa FF et al. 2012. MTHFR polymorphic variant C677T is associated to vascular complications in sickle-cell disease. *Genetic Testing and Molecular Biomarkers,* 16(9): 1038-1043. doi: 10.1089/gtmb. 2011 .0361.

Kangne HK, Jijina FF, Italia YM, Jain DL, Nadkarni AH, Ghosh KK et al. 2015. The prevalence of Factor V Leiden (G1691A) and Methylenetetrahydrofolate Reductase C677T mutations in sickle cell disease in Western India. *Clinical and Applied Thrombosis/Hemostasis,* 21(2): 186-189. doi: 10.1177/1076029613495308.

Kato GJ, Steinberg MH, Gladwin MT 2017. Intravascular hemolysis and the pathophysiology of sickle cell disease. *The Journal of Clinical Investigation,* 127(3): 750-760. doi: 10.1172/jci89741.

Koksal V, Baris I, Etlik O 2007. Primer-engineered multiplex PCR-RFLP for detection of MTHFR C677T, prothrombin G20210A and factor V Leiden mutations. *Experimental and Molecular Pathology,* 83(1): 1-3. doi: 10.1016/j.yexmp.2006.12.006.

Levesque S, Bauer DE 2023. Gene correction for sickle cell disease hits its prime. *Nature Biomedical Engineering,* 7(5): 605-606. doi: 10.1038/s41551-023-01040-2.

Manci EA, Culberson DE, Yang YM, Gardner TM, Powell R, Haynes J Jr et al. 2003. Causes of death in sickle cell disease: An autopsy study. *British Journal of Haematology,* 123(2): 359-365. doi: 10.1046/j.1365-2141. 2003. 04594.x.

Moreira Neto F, Lourenço DM, Noguti MA, Morelli VM, Gil IC, Beltrão AC et al. 2006. The clinical impact of MTHFR polymorphism on the vascular complications of sickle cell disease. *Brazilian Journal of Medical and Biological Research,* 39(10): 1291-1295. doi: 10.1590/s0100-879x2006001000004.

Naramreddy S, Varma A, Taksande A, Meshram RJ, Sudheesh Reddy N, Varma Sr A 2023. The role of antiplatelet in the management of sickle cell disease patients. *Cureus,* 15(7): e42058. doi: 10.7759/cureus.42058.

Nishank SS, Singh MP, Yadav R 2013. Clinical impact of factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations among sickle cell disease patients of Central India. *European Journal of Haematology,* 91(5): 462-466. doi: 10.1111/ejh.12190.

Pandey SK, Meena A, Kishor K, Mishra RM, Pandey S, Saxena R 2012. Prevalence of factor V Leiden G1691A, MTHFR C677T, and prothrombin G20210A among Asian Indian sickle cell patients. *Clinical and Applied Thrombosis/Hemostasis,* 18(3): 320-323. doi: 10.1177/1076029611425830.

Periayah MH, Halim AS, Mat Saad AZ 2017. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. *International Journal of Hematology-Oncology and Stem Cell Research,* 11(4): 319-327.

Rahimi Z, Vaisi-Raygani A, Mozafari H, Kharrazi H, Rezaei M, Nagel RL 2008. Prevalence of factor V Leiden (G1691A) and prothrombin (G20210A) among Kurdish population from Western Iran. *Journal of Thrombosis and Thrombolysis,* 25(3): 280-283. doi: 10.1007/s11239-007-0052-6.

Samarah F, Srour MA 2018. Factor V Leiden G1691A and prothrombin G20210A mutations among Palestinian patients with sickle cell disease. *BMC Hematology,* 18: 1. doi: 10.1186/s12878-018-0097-0.

Sesti-Costa R, Costa FF, Conran N 2023. Role of macrophages in sickle cell disease erythrophagocytosis and erythropoiesis. *International Journal of Molecular Sciences,* 24(7): 6333.

Shafia S, Zargar MH, Khan N, Ahmad R, Shah ZA, Asimi R 2018. High prevalence of factor V Leiden and prothrombin G20101A mutations in Kashmiri patients with venous thromboembolism. *Gene,* 654: 1-9. doi: https://doi.org/10.1016/j.gene.2018.02.031.

Sokkar MF, Kamal L, Salama N, Hamdy M 2022. Thrombophilic mutations and risk of vascular complications in sickle cell disease. *Gene Reports,* 27: 101595. doi: https://doi.org/10.1016/j.genrep.2022.101595.

van Hamel Parsons V, Gardner K, Patel R, Thein SL 2016. Venous thromboembolism in adults with sickle cell disease: Experience of a single centre in the UK. *Annals of Hematology,* 95(2): 227-232. doi: 10.1007/s00277-015-2531-8.

Yousif AA, Abdel Rahim Mahmmoud Muddathir, Elwaleed Mohamed Elamin, Ahmed Alhadi 2017. The role of Factor V Leiden 1691G>A and Prothrombin Gene 20210G> A mutations in hypercoagulable state associated with venous thromboembolism among Sudanese patients. *Journal of Blood Disorders & Transfusion,* 8(3): 386. doi: 10.4172/2155-9864.1000386.

Yousif TYE 2022. Impact of abnormal leukocyte count in the pathophysiology of sickle cell anemia. *Journal of Blood Medicine,* 13: 673-679. doi: 10.2147/jbm.S378133.

Zarghamian P, Klermund J, Cathomen T 2023. Clinical genome editing to treat sickle cell disease-A brief update. *Frontiers in Medicine,* 9. doi: 10.3389/fmed.2022.1065377.

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