Original

Molecular Determination of Factor V Leiden Gene Mutations and Natural Coagulation Inhibitors among Sickle Cell Patients in Ekiti State

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ABSTRACT

Background: The sickle red cell mutation is the result of a single base change in the sixth codon of exon 1 of the β-globin gene responsible for the synthesis of the β-globin polypeptide of the Hb molecule.
Aim: Molecular determination of factor V Leiden gene mutations and natural coagulation inhibitors among sickle cell disease in Ekiti State
Methodology: One hundred and fifty consented sickle cell disease subjects were grouped into 50 SCD in crisis, 50 SCD in steady state and 50 SCD that had never fallen into crisis. 50 control subjects were recruited from apparently healthy individuals, whose haemoglobin phenotype is AA. K_2EDTA samples were used for confirmation of haemoglobin genotype using alkaline cellulose acetate electrophoresis method and for analysis of factor V Leiden gene mutation using real-time polymerase chain reaction. Serum samples were used for the analysis of protein C and S using sandwich enzyme linked immunosorbent assay method
Results: Prevalence of Factor V Leiden gene mutation among the study population revealed that four subjects were heterozygous genotype for FVL mutation. Protein C and S were significantly lower in SCD in crisis compared to other study groups
Conclusion: Prevailing normal factor V Leiden genotype among study subjects may be a possible reflection of a low incidence of this genetic risk factor for thromboembolic phenomena in our environment. Mechanism of low levels of proteins C and S among SCD as observed in this study could be associated with either haemolysis or continued vaso-occlusion

Keywords: Sickle cell disease, factor V Leiden gene mutation, natural coagulation inhibitors

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INTRODUCTION

Sickle cell disease is a hereditary and autosomal recessive genetic disorder of haemoglobin (Hb) in the red blood cell where the haemoglobin S gene is inherited homozygously from both parents.[1] The sickle red cell mutation is the result of a single base change in the sixth codon of exon 1 of the beta-globin (β-globin) gene responsible for the synthesis of the β-globin polypeptide of the Hb molecule ($\alpha_2\beta_2$). This change, in turn, results in replacement of the normal glutamic acid with valine at position six of the β-globin chain and the formation of an altered form of haemoglobin is called haemoglobin S[2]. A well-established genetic predisposition to venous thrombosis that occurs is a single-point mutation in the gene encoding coagulation factor V (G1691A) or Factor V Leiden (FVL). Thrombosis is a common complication in patients with sickle cell disease, where coagulation and fibrinolytic abnormalities lead to the development of a hypercoagulability state in such patients.[3,4] Factor V-Leiden (FVL) mutation is an autosomal dominant trait; the mutant gene is located on chromosome locus F5, 1q 23. The mutation causes a substitution of glutamine for arginine at position 506 (Arg 506 GLn); this is the major site of factor V (FVa) cleavage by activated protein C (APC). Mutant factor V- Leiden FVL (Arg 506GLn) is therefore resistant to APC leading to hypercoagutable state.[5] The latter results from the inability of APC to limit activated factor V (FVa) enhancement of factor X in the conversion of prothrombin to thrombin, consequently the conversion of fibrinogen to fibrin clot continues uninhibited. A low prevalence of factor V Leiden (FVL) was reported in sickle cell disease patients from Saudi Arabia with African descent[6]. Proteins C and S are vitamin K-dependent proteins with an essential anticoagulant function. Protein C exists in an inactive form and is activated by a thrombin-thrombomodulin complex. Protein S combines with protein C and forms a stoichiometric complex which regulates coagulation in the presence of calcium.[7] Protein C and its cofactor, protein S, are the main anticoagulant proteins that inhibit the coagulation by inhibiting the active V and VIII factors. The probability of protein C deficiency has been reported from 0.2% in general population to 20% in sickle cell patients and considering the protein S these numbers are from 0.7% in general population to 15% among patients with sickle cell disease[8]. Protein S and C levels are lower in sickle cell disease patients and it decrease further significantly during crisis, reduced activity of naturally occurring anticoagulants protein C and protein S may contribute to vaso-occlusion in sickle cell disease. The aim of this study is, molecular determination of factor V Leiden gene mutations and natural coagulation inhibitors among sickle cell disease in Ekiti State.

Study Design and Population: Consented one hundred and fifty sickle cell disease patients were grouped based on their health status as at the time of this study into fifty sickle cell disease subjects in crisis, fifty sickle cell disease subjects in steady state and fifty sickle cell disease subjects that had never fallen into crisis attending clinic at Federal Teaching Hospital, Ido Ekiti. The fifty control subjects were recruited from apparently healthy individuals, whose haemoglobin phenotype was AA. Subjects were both gender (male and female), pediatrics subjects were between age 1 and 16 while adults subjects were between age 17 and 40. Ethical approval was obtained for this study from Federal Teaching Hospital, Ido Ekiti.

Sampling Methodology: Simple random sampling method was employed in this study; blood samples were collected under aseptic conditions. Four milliliters (4ml) of blood samples were collected, two milliliters (2ml) of blood samples were dispensed into dipotassium ethylene-diamine tetra-acetic acid (K_2EDTA) bottle for determination and confirmation of haemoglobin electrophoresis genotype for all research subjects using alkaline cellulose acetate electrophoresis method at pH 8.6 using tris-buffer. Blood sample collected into K_2EDTA was also used for the analysis of factor V Leiden gene mutation using real-time polymerase chain reaction. Peripheral blood was processed to guanidium isothiocyanate (GITC) and trizol lysate. Extraction of total RNA was done using RNeasy Mini Kit (Qiagen). cDNA and Primers were Synthesized by Inverted Deoxy Thymidine Europe. Molecular analysis was done using FVL-180F (10 μM) 5'-GTAAGAGCAGATCCCTGGACAGTC-3' (Forward Primer) and FVL-180R (10 μM) 5'-TGTTATCACACTGGTGCTAA-3' (Reverse primer). The procedure was described in the laboratory manual. Remaining two milliliters (2ml) of blood sample was dispensed into plain bottles, blood was allowed to clot and centrifuged at 5000 rpm for 5 minutes. Serum was thereafter extracted from the clotted sample into another clean plain tubes and stored at -80^{0}C for protein C and S analysis using sandwich enzyme linked immunosorbent assay (ELISA) method following manufacturer's instruction.

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Study Procedures

Analysis of Factor V Leiden Gene Expression: K2EDTA blood sample was used to analysis factor V Leiden gene expression using real time polymerase chain reaction (PCR). Peripheral blood was processed to guanidium isothiocyanate (GITC) and trizol lysate.

Principle of Processing Peripheral Blood to Guanidium Isothiocyanate (GITC)

Several molecular assays require nucleic acids from white cells. Red cell lysis using ammonium chloride solution provides a faster means of harvesting white cells. The white cell pellet is then lysed in guanidium isothiocyanate solution. The GITC lysate is stable for long term storage and amenable to various techniques of nucleic acid extraction. GITC requires activation by β-mercaptoethanol. White cell pellets were lysed in trizol reagent for nucleic acid extraction[9].

Procedure of Processing Peripheral Blood to Guanidium Isothiocyanate

Whole blood sample was transfer into a 15ml tube and label accordingly, 10ml of cold RCLB buffer was added to each sample, tube was closed properly and mixed by inversion, 15ml tube (sample tube) was placed on ice for 10minutes, the tubes were wiped carefully and centrifuged at 4000 rpm for 7minutes. The supernatant was carefully decanted into the waste bucket, 10ml of cold RCLB buffer was added to the cell pellet, mixed by vortexing, 10ml of sterile PBS was added to the cell pellet, mixed by vortexing and centrifuged at 4000 rpm for 7minutes. The supernatant was decanted; 5ml of sterile PBS was added into each tube, mixed by vortexing and centrifuged at 4000rpm for 5minutes. The supernatant was decanted into the waste bucket, the 15ml tubes was drained with a clean towel. Activated GITC buffer was prepared which contained 10µl BME and 1ml of GITC, 1ml of activated GITC buffer was added to the white cell pellets in one tube. For the tube of patient, 1ml of trizol reagent was added and incubate for 5 minutes. Using blunt end 18G needles and 2ml syringe, homogenise the GITC lysate 18 times, trizol was not homogenize. With the aid of sterile pasteur pipettes, GITC lysate was transferred into 2ml cryovial and label accordingly for storage at minus 20^{o}C. The lysate can also be used immediately for nucleic acid extraction, 1ml trizol lysate was transferred into another 2ml tube and label accordingly. For quality control (QC) purpose, 1ml of the GITC buffer containing BME was transferred into a cryovial, label as QC control and treat it as a sample during nucleic acid extractions. All samples were store at the minus 20^{o}C freezer[9].

Procedure for Extraction of Total RNA using RNeasy Mini Kit (Qiagen): Guanidium isothiocyanate (GITC) lysates was allowed to thaw and was arranged orderly. Working sheet was created and vortex the GITC lysate gently. 350µl of 70 % ethanol was added to 350µl of GITC lysate, mixed well by pipetting. Seven hundred microliter (700µl) of the sample was mixed including any precipitate that may have formed and transfer to a RNeasy spin column placed in a 2ml collection tube. Centrifuged for 15seconds at 11,000 rpm. The flow was discarded through liquid. Spined columns were placed into new collection tubes, 700µl of RW1 was added to the spin columns, centrifuged for 15 seconds at 11,000 rpm and discard the flow through liquid. Five hundred microliter (500µl) of buffer RPE was added to the spin columns, centrifuged for 15 seconds at 10,000 rpm, discard the flow through liquid. Five hundred microliter (500µl) of buffer RPE was added to the spin columns, centrifuged for 2 minutes at 11,000 rpm, collection tube was discarded. Spin columns were placed into new collection tubes and centrifuge at 12,000 rpm for 1 minute to dry the membrane. Spin columns were placed in sterile 1.5ml tube. Label the tubes and add 30µl of RNase-free water directly into the membrane. Centrifuged at 10,000 rpm for 1 minute to elute the RNA. Eluted was kept on ice for immediate cDNA synthesis or store at minus 80^{o}C.

Procedure for cDNA Synthesis: About 25µl of RNA elute was added to 15µl of the cDNA synthesis, mixed by pipetting up and down for 4 times (Discard pipette tip and use a new one for another sample). To each tube, 0.3µl of RT was added and mixed with RNAse inhibitor by pipetting up and down 100 for 4 times and incubated at 42^{o}C for 60 minutes in dry thermal block. The tubes were transferred to another dry thermal block at 85^{o}C for 10 minutes to inactivate the reverse transcriptase. Stored at -80^{o}C until required for testing or used immediately for testing if needed.

Real-time - Quantitative PCR protocol for Factor V Leiden gene: The primer concentrations were normalized and gene-specific forward and reverse primer pair were mixed for each gene primer. Each primer (forward or reverse) for each gene concentration in the mixture was 5pmol/µl, samples were assayed using the BIO-RAD CFX96TM Real –Time System. PCR cycles (used for later dissociation curve analysis) is as follows; 50^{o}C for 2 minutes, 1 cycle; 95^{o}C for 10

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minutes, 1 cycle; 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds, 40 cycles; 72°C for 10 minutes 1 cycle. The real-time PCR reaction mixture for 25l was prepared in each optical tube as follows; 12.5l SYBR Green Mix (2x); 0.2l liver cDNA 1l primer pair mix (5 pmol/l each primer); 11.3l H_2O. The primer for each gene was indicated below

Primers: (Synthesize by Inverted Deoxy Thymidine (IDT) Europe)

FVL-180F (10 µM) 5'-GTAAGAGCAGATCCCTGGACAGTC-3' (Forward Primer) FVL-180R (10 µM) 5'-TGTTATCACACTGGTGCTAA-3' (Reverse primer)

Preparation of Primer Mix

For 50 reactions, 40µl of FVL-180 Forward (10µM), 40µl of FVL-1 80 Reverse (10µM) and 220µl of Nuclease free water was pipetted into 1.5ml tube. The mixture was vortex gently and spin at low speed for 30 seconds and labeled as FVL Mix- high resolution meting (HRM)

Setting up the Reaction

Worksheet was created which indicate samples and quality control (QC). Appropriate number of micro-PCR tubes was selected and placed unto micro-PCR rack. All materials including primer mix and evagreen master mix were placed inside BSL-Class 2. Ten microliters (10µl) of 2x evagreen master mix, 6µl of the FVL mix were pipetted into each micro-PCR tube and then 4µl DNA Template of sample or QC was added into appropriate tube. The tubes were caped and placed on the machine carousel. The template was already set up with a thermal profile as follows: a. 95°C for 2 minutes, b. 95°C for 5 seconds, c. 60°C for 30 seconds, d. b-c for 40 minutes

***Resulting*:** After the run, results and temperature were checked in the high-resolution curve and also in the melt curve. The interpretation of run is as follows:

Wild type is G/G
Heterozygous (Trait) A/G
Homozygous mutant: A/A

Human Protein C and Protein S Assay using quantitative Sandwich Enzyme linked immunosorbent method

Protein C and protein S are vitamin K-dependent protein that is synthesized in the liver. Protein C through complex interactions with thrombin from blood clots, endothelial cells and other factors of the coagulation cascade, contribute to the maintenance of normal hemostatic mechanisms by down-regulating clot formation and by promoting fibrinolysis. Protein S functions as a cofactor for activated protein C on the vascular membrane to facilitate the degradation of clotting factors Va and VIIIa. Protein C anticoagulant system is activated by the binding of thrombin to thrombomodulin, a transmembrane protein receptor on endothelial cells. Thrombin thrombomodulin binding on endothelial cell membranes activates circulating protein C. Activated protein C binds to protein S on the membrane of endothelial cells or platelets. In this protein C and protein S complex, activated protein C is now capable of inactivating the coagulation cascade factors Va and VIIIa, down regulating clot formation

***Principle*:** This is a quantitative sandwich ELISA technique, competitive enzyme immunoassay technique utilizing a polyclonal anti-PROC antibody. Specific biotin-conjugate with the assay sample is incubated for one hour. After the incubation period, the wells were washed five times. The wells are then incubated with a substrate protein C and S horseradish peroxidase conjugate enzyme in a separate well. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the protein C and S concentration since protein C and S from samples and HRP conjugate compete for the anti-protein C and S antibody binding site. Hence, the number of sites is limited, as more sites are occupied by protein C and S from the sample, fewer sites are left to bind protein C and S HRP conjugate. A standard curve is plotted relating the intensity of the color optical density to the concentration of standards. The protein C and S concentration in each sample is interpolated from standard

Procedure: All reagents and samples were allowed to reach room temperature before analysis. Aliquot of 100µl of biotin was added into all the well except blank well, 100µl of standard solutions, sample and control were added into the wells according to protocol table except blank well, plate well was seal with a cover and incubated at 37 °C for 90 mins. The well plate was washed with wash buffer solution using ELISA auto washer, after the last wash, removal of any remaining wash buffer was done by decanting or invert the plate and blot it against clean paper tissue, 100µL of horseradish peroxidase (HRP) solution was added into

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each well except blank, the well plate was cover and incubate at 37°C for 30 mins. After incubation, the process of washing was repeated, 90µL of tetramethylbenzidine (TMB) substrate was added into each well, the well plate was cover and incubate at 37°C in dark for 15 mins, 50µL of stop solution was added into each well. The color changes into yellow immediately. Optical density of each well was determined within 30 minutes, using an ELISA microplate reader set to 450 nm with wavelength set to 540 nm or 570nm

RESULTS

A total of 200 subjects were recruited for this study, study participants were grouped into four namely; 50 SCD patients that were in crisis, 50 SCD patients that were in steady state, 50 SCD patients that had never fallen into crisis and 50 control subjects that were haemoglobin genotype AA. Out of 200 study participants, 84 were females and 116 were males. However, sex distribution among SCD subjects on crisis were 40 male and 10 female; in SCD subjects that are in steady state 38 were male and 12 were female; SCD subjects that never fall into crisis were 15 male and 35 female; control group had 23 male and 27 female as shown in figure 1.

In this study, pediatrics subjects were between age 1 and 16 while adults subjects were between age 17 and 40. Sickle cell disease (SCD) in crisis had 36 pediatrics (29 male, 7 female) and 14 adults (11 male, 3 female). SCD that are in steady state had 34 pediatrics (26 male, 8 female) and 16 adults (12 male 4 female). SCD that never fall into crisis had 48 pediatrics (15 male, 33 female) and 2 adults (male nil, 2 female). Control subjects had 34 pediatrics (14 male, 20 female) and 16 adults (9 male, 7 female). The majority of participants in this study were male and pediatrics. Study subjects were between age 1 and 40 with over all mean age 13.44±8.90. The Socio-demographic characteristics shows that age group 33-40 and Tertiary had lowest frequency among the age group and educational level respectively while, male and Christianity had highest prevalence in gender and religion respectively among the study group as shown in table 1

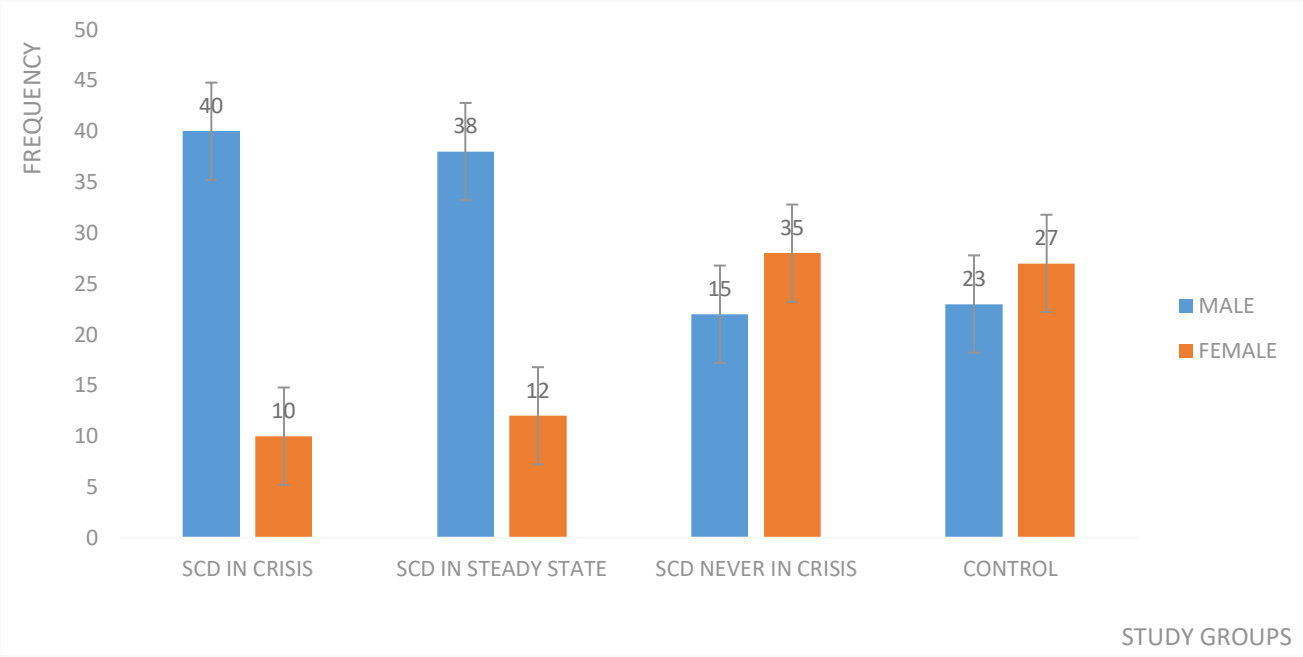

Figure 1: Sex Distribution among Study Participants

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Table 1: Socio-demographic characteristics of Study Participants

Variable	Crisis (%) N = 50	Steady (%) N = 50	Never in Crisis (%) N = 50	Control (%) N=50	Statistical test	
					t-value	p-value
Age group					21.35	**0.00**
1–8 (N=98)	25 (50%)	19 (38%)	38 (76%)	16 (32%)		
9–16 (N=54)	11 (22%)	15 (30%)	10 (20%)	18 (36%)		
17–24 (N=34)	12 (24%)	10 (20%)	02 (04%)	10 (20%)		
25–32 (N=11)	02 (04%)	05 (10%)	0 (0%)	04 (8%)		
33-40 (N=03)	0 (0%)	01 (02%)	0 (0%)	02 (4%)		
Overall Mean Age *13.44±8.90*						
Age at diagnosis of SCD						
1-8	38 (76%)	32 (64%)	40 (80%)			
9-16	12 (24%)	16 (32%)	08 (16%)	---		
17-above	0 (0%)	02 (4%)	02 (04%)	---		
Gender						
Male (N=116)	40 (80%)	38 (76%)	15 (30%)	23 (46%)		
Female (N=84)	10 (20%)	12 (24%)	35 (70%)	27 (54%)		
Educational level						
Primary	25 (50%)	19 (38%)	38 (76%)	16 (32%)		
Secondary	18 (36%)	22 (44%)	10 (20%)	24 (48%)		
Tertiary	07 (14%)	09 (18%)	02(04%)	10 (20%)		
Religion						
Christianity	46 (92%)	47(94%)	48(96%)	45(90%)		
Islamic	04 (08%)	03(06%)	02(04%)	05 (10%)		

Socio-demographic characteristics shows that age group, educational level and religion among the study group

The results of real time polymerase chain reaction in this study shows amplification curves and cycle threshold values. Sigmoidal curve indicates successful amplification of targeted DNA with a clear exponential phase. The fluorescence signal increases exponentially which is proportional to the amount of DNA in the sample. The cycle threshold shows the cycle number at which the fluorescence signal exceeds the threshold. Figure 2 shows multiple peaks or shifts in the melting temperature which indicate sequence variations or heterozygosity. Table 2 shows the prevalence of Factor V Leiden (FVL) gene mutation among the study population; detection was carried out by high resolution melting curve analysis using real time polymerase chain reaction system (RT-PCR). The frequency of FVL mutation in the study revealed that 03 of 50 SCD in crisis and 01 of 50 SCD in steady state was heterozygous genotype, while none of the SCD that had never fallen into crisis and control subjects were neither heterozygous genotype nor homozygous genotype for FVL mutation. High resolution melting curve for factor V Leiden gene mutation was shown in figure 2. Table 3 shows the mean value of protein C and protein S among study participants. Protein C and protein S were significantly lower (p=0.00 and p=0.00) in SCD in crisis compared to other study groups

Table 2: Prevalence of Factor V Leiden gene Mutation among the Study Participants

Participants	FVL Leiden Genotype		
	G/G	G/A	A/A
SCD in Crisis (N=50)	47	03	-
SCD in Steady State (N=50)	49	01	-
SCD Never in Crisis (N=50)	50	-	-
Control Subjects (N=50)	50	-	-

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Key: G/G- Normal Genotype for FVL Gene Mutation, G/A- Heterozygous Genotype for FVL Gene Mutation, AA- Homozygous Genotype for FVL Gene Mutation, FVL- Factor V Leiden

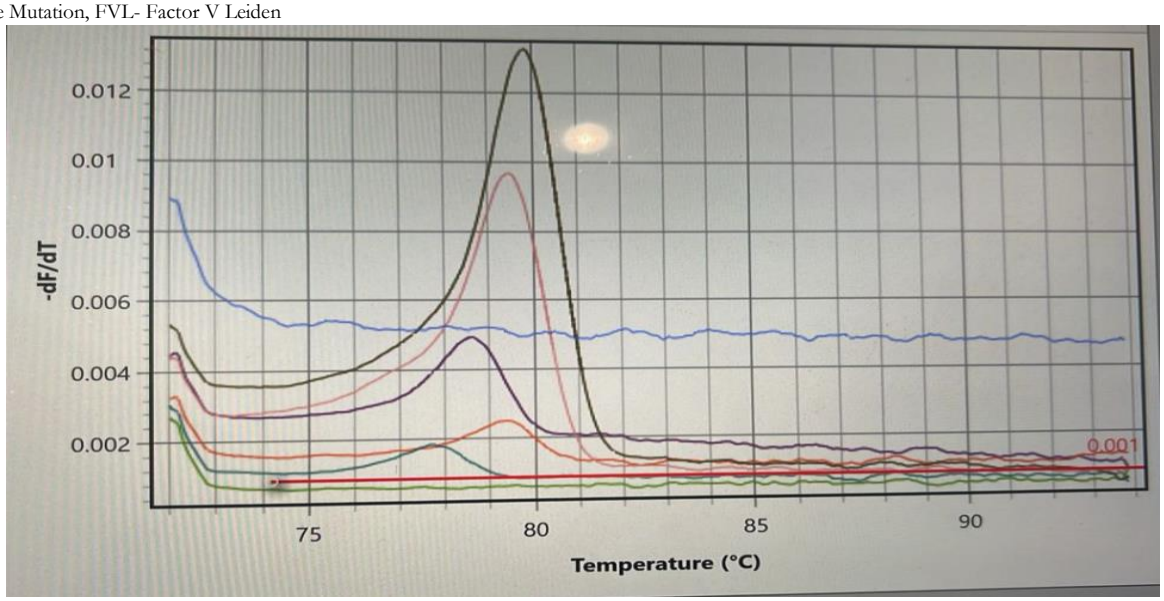

Figure 2: High Resolution Meting Curve for factor V Leiden gene mutation

Table 3: Mean ± SD of Protein C and Protein S among Study Participants

Parameters	SCD in Crisis N=50	SCD in Steady N=50	Never in Crisis N=50	Control N=50	f (p-value)
Protein C(UI/dL)	46.68± 3.19	59.79± 5.00	69.05 ±5.44	93.24 ±8.91	535.01 **(0.00)**
Protein S (UI/dL)	40.15± 2.29	48.09± 4.32	57.62 ±3.80	83.70 ±5.69	101.33 **(0.00)**

Mean value of Protein C and S were lower in SCD in crisis compared to other study groups. p≤0.05 was considered significant, parameters were compared using ANOVA

DISCUSSION

This is a cross-sectional study on molecular determination of factor V leiden gene mutations and natural coagulation inhibitors among sickle cell disease in Ekiti State. Pediatric and adult mean ages in this study were in keeping with previous studies across the geo-political regions in Nigeria.[10] Gradual improvement in care of people living with SCD in the study region and early detection has contributed to the increase in the number of adults with SCD. This fact supported the findings in this study as the peak age prevalence in this study was forty years. This may be an indication of reduction in mortality rate of SCD as the disease advances from childhood to adulthood. The results in this study showed that majority of the participants were males and pediatrics, this may be a true reflection of sickle cell disease (SCD) among the study population also the males might have more preponderance to having a crisis, and may also be attributed to perhaps a reflection of the proportion of males in the community.[3] Although the incidence of sickle cell disease SCD is not strictly gender-related as it is transmitted as an autosomal recessive disorder.[11] Ceglie reported that, SCD male patients presented with the very severe cases, which take more time to resolve, this explains their frequent hospitalization for persisting Pain[17] as observed in this study.

The results in this study showed that majority of the participants expressed normal genotype (G/G) for factor V Leiden (FVL), four were heterozygous (A/G) genotype and none of the study participants were Homozygotes (A/A) genotype. This study shows that

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FVL had no effect on sickle cell complications which shows the need for performing more studies in this field. Findings in this study was in consonance with previous reports that factor V Leiden gene mutation incidence is low among the black African population. The different findings concerning prevalence of factor V Leiden among sickle cell disease (SCD) patients may be partially due to the different genetic background of the different ethnicities of study subjects as well as the limited sample size in some studies.[12] Similar to the findings in this current study, Fekri and Mahmoud reported that analysis of the sickle cell disease patients for the factor V Leiden gene mutation in his study revealed that, eleven sickle cell disease patients were heterozygotes, while none of the sickle cell disease patients were homozygote for this mutation, it was reported that there is no single clinical or laboratory test that can predict which patients are at high risk for developing thrombotic complications of sickle cell disease.[13] Confirming the findings in this study, it was reported that the mutant allele factor V Leiden gene incidence is low in African with prevalence varies between 0% - 15%. Heterozygotes (A/G) was reported to have 3-5 times increased risk of thrombotic events. Homozygotes (A/A) are much less common but associated with higher thrombotic risk which was about 80 times increased.[12] The apparently prevailing normal factor V Leiden genotype among our study subjects in Ekiti State may be a possible reflection of a low incidence of this genetic risk factor for thromboembolic phenomena in our environment.[3]

The value of protein C and protein S recorded in this study were significantly lower in sickle cell disease in crisis compared to other SCD study groups. Results obtained in this study was consistent with previous studies. Mohamed reported low level of protein C and protein S in sickle cell disease patients compared to controls. He explained that protein C and S are natural inhibitors proteins and mechanism of low levels of these natural anticoagulant proteins in SCD could be as a result of hepatic dysfunction that can decrease synthesis of protein C and S, in addition to consumptive coagulopathy.[14] Similar to the findings in this study, Piccin reported that protein C and S levels were lower in sickle cell disease patients and it decrease further significantly during crisis, suggesting that protein C and S may be consumed acutely and chronically. He stated that reduced activity of naturally occurring anticoagulants protein C and protein S may contribute to vaso-occlusion in sickle cell disease compared to controls.[15] It was reported that low protein C and S level may be due to sustained tissue destruction associated with either haemolysis or continued vaso-occlusion or the liver being frequently affected in SCD which cause impaired liver function secondary to chronic intra-hepatic sickling in the hepatic sinusoids.[16] Therefore, impaired hepatic function due to chronic hepatic sickling may affect its production since protein C and S is vitamin K-dependent protein. Low level of protein C and S in SCD patients especially in crisis state might cause hypercoagulation. However, there is also a possibility of an inherent protein C abnormality due to genetic defect that can further accentuate the prothrombotic state of SCD, this fact is subject for further investigation.

Strengths and limitations of the study

High resolution melting in real time polymerase chain reaction is a quantitative measure that offers a high-resolution sensitive analysis of DNA melting as observed in this study. However, its interpretation in relation to gel electrophoresis qualitative measure would have helps visualized amplified real-time polymerase chain reaction products to confirm the identity and presence of sequence variation or mutation in this present study.

Implications of the findings of the study

Socio-demographic characteristics and quality of life play a major role in SCD. Factors like age, levels of education, income, gender and social relations (marital status) should be considered seriously in the management of SCD to facilitate healing and restoration of quality of life. Apparently, prevailing normal factor V Leiden genotype among study subjects may be a possible reflection of a low incidence of this genetic risk factor for thromboembolic phenomena in our environment Mechanism of low levels of proteins C and S among SCD as observed in this study could be associated with either haemolysis or continued vaso-occlusion.

CONCLUSION

Socio-demographic profile of people living with SCD is a strong predictor of their clinical course, outcome of the disease can predict quality of life. Apparently, prevailing normal factor V Leiden genotype among study subjects may be a possible reflection of a low incidence of this genetic risk factor for thromboembolic phenomena in our environment. Mechanism of low levels of proteins C and S among SCD as observed in this study could be as a result of consumptive

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coagulopathy, hepatic dysfunction, tissue destruction associated with either hemolysis or continued vaso-occlusion which might increase the risk of thrombosis among the SCD subjects

Declarations

Authors' contribution: Author 1 and 2 Design the manuscript and write-up, Author 3, 4 and 5 assisted in sample collection. Author 6,7,8 assisted in sample processing and storage. Author 9 and 10 assisted in proof reading.

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