International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 14, No. 1, January 2025 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_14.1.2025.37

ORIGINAL RESEARCH

Association Between *HBG2* rs7482144 Polymorphism and HbF Levels in Patients with Sickle Cell Anemia in Odisha

Manaswini Panda[1], Binata Nayak[2], Pratima Kumari Sahu[3], Alok Kumar Mohapatra[4], Dr. Niranjan Mohapatra[5]

[1]Assistant Professor, Department of Biochemistry, Shri Jagannath Medical College and Hospital, Puri, Odisha, India
[2]Professor, School of Life Science, Sambalpur University, Burla, Odisha, India
[3]Professor, Department of Biochemistry, Srirama Chandra Bhanja Medical College and Hospital, Cuttack, Odisha, India
[4]Senior Consultant, Department of Nephrology, Ashwini Hospital, Cuttack, Odisha, India
[5]Assistant Professor, Department of Medicine, Srirama Chandra Bhanja Medical College and Hospital, Cuttack, Odisha, India

Corresponding author
Binata Nayak
Professor, School of Life Science, Sambalpur University, Burla, Odisha, India
Email: binata.bga@gmail.com

Received: 16 November, 2024 Accepted: 21 December, 2024 Published: 17 January, 2025

ABSTRACT

Background: Sickle cell anemia (SCA), the most common type of sickle cell disease (SCD), is associated with reduced morbidity and mortality in individuals with high fetal hemoglobin (HbF) levels. Variation in HbF levels is linked to genetic polymorphisms that influence its production. This study evaluated the frequency of the *HBG2* rs7482144 polymorphism and its association with HbF levels and disease severity in patients with SCA in Odisha. **Methods:** This observational, cross-sectional study was conducted at the Department of Medicine, Srirama Chandra Bhanja Medical College and Hospital, Cuttack, from August 2023 to August 2024. Patients with homozygous SCD, confirmed through Hb electrophoresis and in steady state, were included. Disease severity was classified according to van den Tweel et al. Deoxyribonucleic acid (DNA) was extracted using theZybio Nucleic Acid Extraction Kit. Genotyping of the *HBG2* rs7482144 polymorphism was performed by polymerase chain reaction and restriction fragment length polymorphism. **Results:** A total of 94 patients were included. The mean (SD) age of the patients was 29.79 (9.90) years. The average number of hospitalizations was 12.79 (9.15), and the mean number of blood transfusions was 8.67 (8.86). The mean (SD) HbF level was significantly higher in patients with the *HBG2* rs7482144 TT genotype [20.82 (3.16) %] compared to those with the CT [18.99 (4.82) %] and CC genotypes [16.18 (4.57) %] (P<0.001).The TT genotype (71.1%) was significantly higher in patients with mild SCA (P=0.018). **Conclusion:** The T allele of the *HBG2* rs7482144 polymorphism was associated with higher HbF levels, and the TT genotype did not correlate with increased disease severity.

Keywords: CC genotype, *HBG2*rs7482144polymorphism, HbF, HbSS, TT genotype.

INTRODUCTION

Sickle cell disease (SCD) is a hereditary disorder of red blood cells resulting from either the homozygous inheritance of the hemoglobin S mutation (HbSS) or the compound heterozygous inheritance of the hemoglobin S mutation along with another β-globin chain abnormality. India ranks among the countries with the highest frequencies of the hemoglobin S allele globally and has the third-highest birth rate of infants born with HbSS [1].

The most prevalent form of SCD is sickle cell anemia (SCA), characterized by the homozygous expression of the defective gene responsible for producing hemoglobin S [2].It is a severe single-gene disorder affecting hemoglobin (Hb), caused by the substitution of nucleotide thymine (T) for adenine (A) at codon 6 of the Hb subunit beta(HBB) gene. This mutation leads to the replacement of glutamic acid with valine in the amino acid sequence, impairing β-globin chain synthesis [3, 4]. The severity and presentation of SCA exhibit significant variability [5].

Fetal hemoglobin (HbF) is a normal Hb variant and the primary form of Hb during fetal development [6]. Fetal Hb plays a crucial role in modulating the

DOI: 10.69605/ijlbpr_14.1.2025.37

morbidity and mortality associated with SCA. Research has shown that elevated HbF levels can alleviate symptoms in patients with SCA and lead to fewer hospitalizations [7,8]. Elevated HbF levels, resulting from the reactivation or overexpression of the Hb subunit gamma (HBG) gene and the subsequent production of γ-globin, help reduce the severity of anemia, particularly in hemoglobinopathies such as SCD and thalassemia [7,9].

The *HBG2* rs7482144 polymorphism is caused by a nucleotide substitution at position -158 of the γ-globin gene, where cytosine (C) is replaced by T [10]. The "T" allele of the *HBG2* rs7482144 polymorphism reduces the binding affinity of transcriptional inhibitors to the β-globin locus control region(LCR), thereby allowing the *HBG2* gene to remain actively expressed in adulthood [11]. Clinical studies have demonstrated that patients with SCA carrying the "T" allele of the *HBG2* rs7482144 polymorphism exhibit significantly higher mean HbF levels compared to those with the "C" allele;this polymorphism is linked to elevated HbF in certain SCA cases [12]. However, the prevalence of the *HBG2* rs7482144 polymorphism in the *HBG2* gene and its association with HbF levels in patients with SCA has not yet been studied in Odisha [13].

Therefore, the present study aimed to estimate the frequencies of *HBG2* rs7482144 polymorphism and investigate its association with HbF levels and the severity of disease in patients with SCA in Odisha.

MATERIALS AND METHODS

Study design

Thisobservational, cross-sectional study was conductedat theDepartment of Medicine, Srirama Chandra Bhanja Medical College and Hospital, Cuttack,from August2023 toAugust2024.The study protocol was approved by the 52nd Institutional Ethics Committee (Approval number: 1491/16.08.2023).Written informed consent was obtained from adult study participants, and the legal guardians provided a written consent on behalf of minors prior to the commencement of the study.

Inclusion criteria

The study included patients with homozygous SCD, confirmed by Hb electrophoresis. All patients were known to be in a steady state at the time of inclusion.

Exclusion criteria

Exclusion criteria included a lowplatelet count (<100,000/mm^3), neutropenia (polymorphonuclear neutrophils<1,200/mm^3), pregnancy, patients receiving interferonorhydroxyureatreatment, other sickle cell syndromes (such as HbS-β thalassemia, HbSE, HbSC,HbSD),recent blood transfusion (within the last 3 months), or a VOC attack in the past month.Additionally, patients who were part of a special program or trial that couldaffect their clinical or hematological status were excluded from the study.

Data collection

Baseline values of complete blood count (CBC) and Hb high-performance liquid chromatography(HPLC) were obtained from patient records. Allpatients were evaluated for clinical phenotypes,including pain, anemia, jaundice, pneumonia, stroke, andosteonecrosis. Severity scores were determined by usinga combination of anemia, complications, total leukocytecount (TLC), and transfusion scores. The criteria for assessing the severity score were based on those described by van den Tweel et al [14].

Deoxyribonucleic acid extraction

About 5ml of blood was collectedfrom each patient. Deoxyribonucleic acid (DNA) was extracted from whole anticoagulated blood using theDNA extraction kit (Zybio Nucleic Acid Extraction Kit, Magnetic Bead Method).

Genotyping of HBG2 rs7482144 polymorphism

Genotypingofthe *HBG2*rs7482144polymorphismwasperformed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).A 310-base pair (bp) fragment in the upstream region of *HBG2*wasamplified with the following primers: 5′-AAC TGT TGC TTT ATA GGATTT T-3′ and 5′-AGG AGC TTA TTG ATA ACC TCA GAC-3′. The PCRwascarried outinatotalreactionvolumeof20µL,containing approximately80ng of genomic DNA, 10µL ofAmaROne PCR master mix, and 2µL of forward and reverse primers. Cycling conditionsincluded aninitialdenaturation at 95°C for 5 minutes, followed by 35cycles ofdenaturationat 95°Cfor40seconds, annealing at 58°C for 40seconds, and extension at 72°C for 40seconds, with a final7-minute extension step at 72°C. The PCR amplicons were then incubated with the XmnI restriction enzyme as recommended by the manufacturer (FermentasThermo Fisher Scientific, United States). The digested products were resolved by electrophoresis on 2% agarose gel. Upon digestion, the650bp PCR product was cleaved into two fragments of 450 and 200bp for the T allele. The presence of uncleaved 650bp product indicated the presence of the C allele.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 22. Descriptive statistics were used to describe categorical variables (frequency and percentages) and continuous variables (mean and standard deviation [SD]). The chi-squared test was performed to investigate the association between the*HBG2* rs7482144 polymorphism and SCA severity. The association between the*HBG2* rs7482144 genotypes and HbF levels was analyzed

DOI: 10.69605/ijlbpr_14.1.2025.37

using ANOVA.The risk associated with genotypes was calculated usingthe odds ratio and 95% confidence interval (CI),withMedCalc statistical software (https://www.medcalc.org/calc/odds_ratio.php).A P value <0.05 was considered statistically significant.

RESULTS

A total of 94 patients with HbSS were included in the study. The demographic and baseline characteristics of the patients are presented in Table 1.The mean (SD) age of the patients was 29.79 (9.90) years. The study population consisted of 52.1% females and 47.9% males. The mean (SD) Hb level was 7.71 (1.79) g/dL,andTLC was 11,958.41 (3,090.84) cells/mm³.The mean (SD) number of hospitalizations was 12.79 (9.15), while the mean (SD) number of blood transfusions was 8.67 (8.86).

Table 2 and Figure 1 showsthe correlation between the *HBG2* rs7482144 genotypes (CC, CT, TT) and HbF levels in patients with SCA. Of the study population, 38 had the *HBG2* rs7482144 TT genotype, while 31 and 25 had the CT and CC genotypes, respectively. The mean (SD) HbF level was significantly higher in patients with the *HBG2* rs7482144 TT genotype [20.82 (3.16)%] compared to those with the CT genotype [18.99 (4.82)%] andthe CC genotype [16.18 (4.57)%] (P<0.001).

Table3depicts the distribution of *HBG2* rs7482144 genotypes(CC, CT, TT)in patients with SCA.Among the study population, the severity of SCA was mild, moderate, and severe in 53, 18, and 23 patients, respectively. The*HBG2* rs7482144 CC genotype was significantly higher in patients with severe SCA(44%), whereas the CT (58.1%) and TT (71.1%) genotypesweresignificantly higher in those with mild SCA (P=0.018 for all).

Table 1: Demographic and baseline characteristicsof the patients

Parameters	Number of patients (N=94)
Age (years)	29.79 (9.90)
Sex, n (%)	
Female	49 (52.1)
Male	45 (47.9)
Hemoglobin(g/dL)	7.71 (1.79)
TLC (cells/mm^3)	11958.41 (3090.84)
Hematocrit (%)	24.46 (4.29)
MCV(fL)	79.16 (10.75)
MCH(pg)	25.51 (3.30)
Number of hospitalizations	12.79 (9.15)
Number of blood transfusion	8.67 (8.86)
Data shown as mean (SD), unless otherwise specified. MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume;SD, standard deviation; TLC, total leukocyte count.	

Table 2: Correlation between the*HBG2*rs7482144 genotypes and fetal hemoglobin

Parameters	CC (N=25)	CT (N=31)	TT (N=38)	Total (N=94)	P value
HbF(%)	16.18 (4.57)	18.99 (4.82)	20.82 (3.16)	18.98 (4.51)	<0.001
Data presented as mean (SD). CC, cytosine-cytosine genotype; CT, cytosine-thymine genotype;HbF, fetal hemoglobin; TT, thymine-thymine genotype.					

Table 3: Genotype frequencies of *HBG2* rs7482144 polymorphism according to the severity of patients with sickle cell anemia

Parameters	Mild (N=53)	Moderate (N=18)	Severe (N=23)	P value
***HBG2*rs7482144**				
CC	8 (32.0)	6 (24.0)	11 (44.0)	0.018
CT	18 (58.1)	8 (25.8)	5 (16.1)	
TT	27 (71.1)	4 (10.5)	7 (18.4)	
Data presented as n (%). CC, cytosine-cytosine genotype; CT, cytosine-thymine genotype; TT, thymine-thymine genotype.				

DOI: 10.69605/ijlbpr_14.1.2025.37

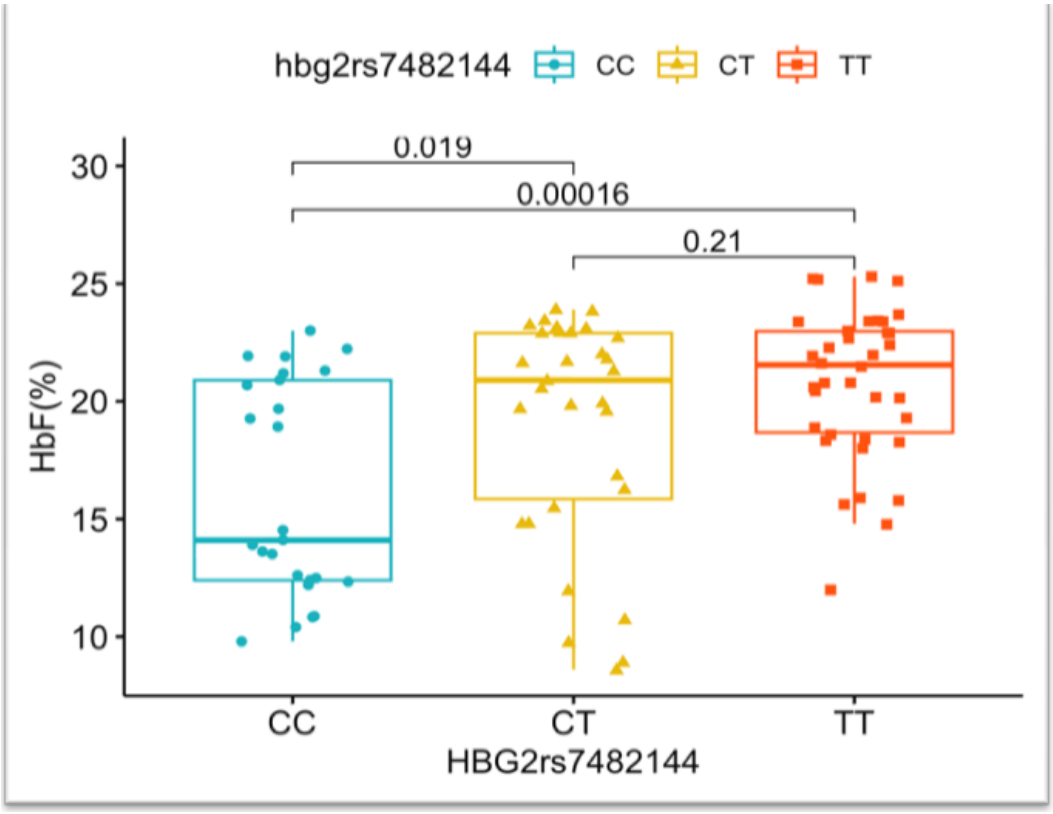

Figure1: Genes correlation between *HBG2* rs7482144 with HbF (%)

DISCUSSION

Sickle cell anemia is a severe monogenic disorder marked by anemia, acute complications, and reduced life expectancy. Fetal hemoglobin levels are key modulators of SCA morbidity and mortality [16], with higher HbF reducing disease severity. While HbF levels vary significantly, this variation is heritable and influenced by genetic polymorphisms at three quantitative trait loci (QTL), namely Xmn1-HBG2, HMIP-2, and BCL11A [17]. This study evaluated the frequency of the *HBG2* rs7482144 polymorphism and its association with HbF levels and SCA severity in Odisha.

In the present study, the mean age of the patients was 29.79 years. In contrast, the mean age reported in a study by Lakkakula et al. was 16.52 years [16]. Moreover, the mean hemoglobin (8.5 g/dL), hematocrit (25.13%), mean corpuscular volume (86.23 fL), and mean corpuscular hemoglobin (29.03 pg) levels were higher in the previous study [16]. However, the average number of hospitalizations (8.24) and blood transfusions (6.98) was lower compared to the present study.

The present study showed higher HbF levels in patients with the *HBG2* rs7482144 TT genotype, followed by those with the CT and CC genotypes. A similar trend was observed in the study by Lakkakula BVKS et al., where the mean HbF level was 20.09% for the TT genotype, 18.41% for the CT genotype, and 7.50% for the CC genotype. These findings support the notion that the presence of the T allele is associated with increased HbF levels.

In this study, the distribution of the *HBG2* rs7482144 polymorphism varied significantly across the severity groups of SCA. The *HBG2* rs7482144 CC genotype was more prevalent in severe cases, while the CT and TT genotypes were more common in patients with mild SCA. The results suggest a significant association between the TT genotype and a milder form of the disease. In contrast, Lakkakula BVKS et al. reported that the TT genotype was linked to severe SCA, with 50.3% of patients with the TT genotype experiencing severe disease.

Various studies have reported an association between the *HBG2* rs7482144 polymorphism and HbF levels in patients with SCA [18-21]. In Africa, SCA patients carrying the *HBG2* rs7482144 polymorphism have higher HbF levels compared to those without this polymorphism. Additionally, SCA patients from Atlantic West Africa (Senegalese) have a lower proportion of dense red blood cells and elevated HbF levels, which are associated with the presence of the *HBG2* rs7482144 polymorphism [22]. Sickle cell anemia cases with the Arab-Indian haplotype also exhibit higher HbF levels and tend to experience a milder disease course [23]. Another study using combined linkage and association analysis of the β-globin gene cluster demonstrated a strong correlation between the *HBG2* rs7482144 polymorphism and increased HbF levels [24].

The *HBG2* rs7482144 T allele does not always correlate with high HbF levels in patients with SCA. According to the African American Cooperative Study of Sickle Cell Disease (CSSCD), the TT genotype of

DOI: 10.69605/ijlbpr_14.1.2025.37

the *HBG2* rs7482144 polymorphism accounted for only 2.2% of the variation in HbF levels. Similarly, a study by Lakkakula et al. reported that SCA patients carrying the *HBG2* rs7482144 T allele were not associated with a milder disease course. Nonetheless, those with the TT genotype exhibited higher HbF levels compared to patients with other genotypes [16].

LIMITATIONS

The study had several limitations. The small sample size may affect the generalizability of theresults.This study was conducted at a single center, which limits its generalizability to other populations.While multiple genetic loci control HbF variation between individuals, this study excluded several known trans-acting QTLs, highlighting the need for large-scale studies that consider additional clinical factors to explore these associations.

CONCLUSION

The T allele of the *HBG2* rs7482144 (C>T) polymorphism was associated with higher HbFlevels, which reduce the severity of SCA. The study also indicated thatthe TTgenotype of the *HBG2* rs7482144 polymorphism were not associatedto an increased risk of disease severity.

Acknowledgements

We would like to express our deepest gratitude to all the participants in this study, without whom this research would not have been possible. Our heartfelt thanks go to the laboratory staff of the post-graduate department of Biochemistry, SCB Medical College and Hospital, for their support and assistance during the study.

Contribution of authors

Research concept- Dr Alok Kumar Mohapatra
Research design- ProfessorDr Pratima Kumari Sahu
Supervision- Professor Dr Pratima Kumari Sahu, Dr Binata Nayak
Materials- Dr Manaswini Panda
Data collection- Dr Manaswini Panda,Dr Niranjan Mohapatra
Data analysis and interpretation- Dr Alok Kumar Mohapatra
Literature search- Dr Manaswini Panda, Dr Pratima Kumari Sahu
Writing article- Dr Manaswini Panda
Critical review- Dr Pratima Kumari Sahu, Dr Binata Nayak

REFERENCES

1. Jain D, Gupta M, Madkaikar M, Jena R, Khargekar N, Saraf S, et al. Sickle cell disease in India: current status and progress. *Lancet Haematol.* 2024;11(5):e322-e23.
2. Wood KC, Hsu LL, Gladwin MT. Sickle cell disease vasculopathy: a state of nitric oxide resistance. *Free Radic Biol Med.* 2008;44(8):1506-28.
3. Piel FB, Steinberg MH, Rees DC. Sickle Cell Disease. *N Engl J Med.* 2017;376(16):1561-73.
4. Lakkakula BVKS, Sahoo R, Verma H, Lakkakula S. Pain Management Issues as Part of the Comprehensive Care of Patients with Sickle Cell Disease. *Pain ManagNurs.* 2018;19(6):558-72.
5. Makani J, Williams TN, Marsh K. Sickle cell disease in Africa: burden and research priorities. *Ann Trop Med Parasitol.* 2007;101(1):3-14.
6. Olaniyi JA, Arinola OG, Odetunde AB. FOETAL HAEMOGLOBIN (HbF) STATUS IN ADULT SICKLE CELL ANAEMIA PATIENTS IN IBADAN, NIGERIA. *Ann IbPostgrad Med.* 2010;8(1):30-3.
7. Thein SL, Menzel S. Discovering the genetics underlying foetalhaemoglobin production in adults. *Br J Haematol.* 2009;145(4):455-67.
8. Estepp JH, Smeltzer MP, Kang G, Chen Li, Wang WC, Christina A, et al. A clinically meaningful fetal hemoglobin threshold for children with sickle cell anemia during hydroxyurea therapy. *Am J Hematol.* 2017;92(12):1333-9.
9. Akinsheye I, Alsultan A, Solovieff N, Ngo D, Baldwin CT, Sebastiani P, et al. Fetal hemoglobin in sickle cell anemia. *Blood.* 2011;118(1):19-27.
10. Pandey S, Pandey S, Mishra RM, Saxena R. Modulating Effect of the -158 γ (C→T) Xmn1 Polymorphism in Indian Sickle Cell Patients. *Mediterr J HematolInfect Dis.* 2012;4(1):e2012001.
11. Cardoso GL, Diniz IG, Silva AN, et al. DNA polymorphisms at BCL11A, HBS1L-MYB and Xmn1-HBG2 site loci associated with fetal hemoglobin levels in sickle cell anemia patients from Northern Brazil. *Blood Cells Mol Dis.* 2014;53(4):176-9.
12. Gilman JG, Huisman TH. Two independent genetic factors in the beta-globin gene cluster are associated with high G gamma-levels in the HbF of SS patients. *Blood.* 1984;64(2):452-7.
13. Patra PK, Khodiar PK, Hambleton IR, Serjeant GR. The Chhattisgarh state screening programme for the sickle cell gene: a cost-effective approach to a public health problem. *J Community Genet.* 2015;6(4):361-8.
14. van den Tweel XW, van der Lee JH, Heijboer H, Peters M, Fijnvandraat K. Development and validation of a pediatric severity index for sickle cell patients. Am J Hematol. 2010;85(10):746-51.
15. Bhaskar L VKS, Patra P K. Sickle cell disease is autochthonous and unique in Indian populations. Indian J Phys Anthropol Hum Genet. 2015;34:201–210.
16. Lakkakula BVKS, Pattnaik S. The HBG2 rs7482144 (C > T) Polymorphism is Linked to HbF Levels but not to the Severity of Sickle Cell Anemia. *J Pediatr Genet*. 2021;12(2):129-34.
17. Friedrisch JR, Sheehan V, Flanagan JM, et al. The role of BCL11A and HMIP-2 polymorphisms on endogenous and hydroxyurea induced levels of fetal hemoglobin in sickle cell anemia patients from southern Brazil. *Blood Cells Mol Dis*. 2016;62:32-7.
18. Das S. A study to understand the relation between fetal haemoglobin, the hematological parameters and Xmni gene polymorphism. *Indian Journal of Medicine and Healthcare.* 2012; 1:206–10.
19. Alaoui-Ismaili FZ, Laghmich A, Ghailani-Nourouti N, Barakat A, Bennani-Mechita M. *Xmn*I Polymorphism in Sickle Cell Disease in North Morocco. *Hemoglobin*. 2020;44(3):190-4.
20. Mohamed GS, Lemine SM, A-S Kleib, Najjar F, Mohamed A. Modulator effect of XmnI polymorphism

DOI: 10.69605/ijlbpr_14.1.2025.37

concerning 50 Mauritanian sickle cell disease patients. *Int JSciRes*. 2018;7:1384–7.

21. Bhagat S, Patra PK, Thakur AS. Association between XmnI Polymorphism and HbF Level in Sickle Cell Disease Patients from Chhattisgarh. *Int J Biomed Sci*. 2012;8(1):36-9.
22. Nagel RL, Fabry ME, Pagnier J, et al. Hematologically and genetically distinct forms of sickle cell anemia in Africa. The Senegal type and the Benin type. *N Engl J Med*. 1985;312(14):880-4.
23. Habara AH, Shaikho EM, Steinberg MH. Fetal hemoglobin in sickle cell anemia: The Arab-Indian haplotype and new therapeutic agents. *Am J Hematol*. 2017;92(11):1233-42.
24. Garner C, Tatu T, Game L. A candidate gene study of F cell levels in sibling pairs using a joint linkage and association analysis. GeneScreen. 2000;1:9–14.