Influence of β -Globin Haplotypes on Oxidative Stress, Antioxidant Capacity and Inflammation in Sick Cell Patients of Chhattisgarh

Sanjana Bhagat¹ · Amar Singh Thakur²

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Abstract The present study was conducted to investigate the frequency of β -globin gene haplotype and their influence on oxidative stress, antioxidant level and inflammation of sickle cell patient of Chhattisgarh. The serum levels of total antioxidant capacity, oxidative stress and inflammation were measured in hundred SCD patients. The mean serum CRP and MDA levels were significantly ($p < 0.0001$) lower in Arab-Indian haplotype when compared with Bantu and other haplotype. However, the antioxidant capacity and HbF levels were significantly higher in Arab-Indian haplotype. These result indicate that β -globin gene haplotype have a role in modulating disease severity in SCD patients of Chhattisgarh.

Keywords Sickle cell disease · Haplotype · Oxidative stress · Inflammation · HbF level

Introduction

Sickle cell disease (SCD) is caused by point mutation in the β -globin gene resulting in the expression of hemoglobin S (HbS). Deoxygenated HbS polymerizes, leading to erythrocyte rigidity, distortion, membrane damage and hemolysis [1]. Its polymers deform erythrocyte and lead to a diverse and complex pathophysiology. The β -globin gene

haplotypes are important in the comprehension of the clinical diversity of sickle cell anemia (SCA) patients [2].

Many attempts have been made to establish haplotype-phenotype relationship in SCD but a robust correlation has not emerged. HbS polymerization is a primary pathophysiological event in sickle cell anemia [3]. Oxidation is mediated by oxidants and free radicals, generically called reactive oxygen species (ROS), are formed as a by-product of the oxygen metabolism [4]. Antioxidant enzymatic and non-enzymatic molecules play a crucial role in maintaining the balance of ROS, an imbalance may lead to attack on all the components of the cell including protein, lipid and DNA. Collectively, oxidative stress is associated with imbalance between oxidant/free radicals and antioxidants [4]. Sickle cell disease promotes a harmful pathological effect that includes sickling of erythrocytes, vaso-occlusive episode, oxidative stress, endothelial cell dysfunction and inflammatory response, which manifests as increased levels of inflammatory cytokines, decreased bioavailability of nitric oxides and oxidative stress [5]. Increasing evidence points towards an oxidative stress response responsible for increased pathophysiology of secondary dysfunction in sickle cell patients [3, 4, 6, 7]. Recently, several studies have shown that oxidative stress is a complex mechanism in SCD patients as compare to simple imbalance between the production and elimination of ROS [8–11]. Oxidative stress is one of the factors that modulate the phenotypic expression of SCA. This stress influences the vaso-occlusive process by increasing the adhesive properties of erythrocytes, leukocytes and platelets to the endothelium. Furthermore, an activated endothelium and leukocytosis, the inflammatory phenotype of SCA is characterized by high levels of acute-phase proteins and cytokines [12]. In addition, some other reports also indicate that SCD patients

✉ Sanjana Bhagat
sanjana_bhagat@rediffmail.com

¹ Department of Biotechnology, Govt. N.P.G. College of Science Raipur, GE Road, Raipur, Chhattisgarh 492010, India

² Department of Biochemistry, Govt. Medical College, Jagdalpur, Chhattisgarh, India

have lower levels of total antioxidant capacity as compare to normal healthy individuals [11].

The β -globin gene cluster haplotype is a *cis*-acting determinant, which serves as a marker for the genetic background of HbSS disease patients and for predicting disease severity [13]. Five main haplotypes Benin, Bantu, Cameroon, Senegal and Arab-Indian are associated with their putative geographical origin. The Senegal and Arab-Indian haplotype usually have the highest HbF levels and associated with less clinical course. The Benin and Cameroon haplotypes, have intermediate clinical features. The Bantu haplotype have the lowest HbF level and associated with greater clinical severity [14, 15]. Several studies have suggested that the clinical severity of SCA is associated with β -globin gene haplotypes [16–18]. The data obtained from our earlier studies indicate that the Arab-Indian haplotype is associated with higher level of HbF [19]. No information to the best of our knowledge is available on the influence of β -globin gene haplotypes on oxidative stress, antioxidant capacity and inflammation in sickle cell patients of Chhattisgarh.

In this context, the objective of this study was to assess the frequency of β -globin gene haplotype in sickle cell patients of Chhattisgarh and their influence on oxidative stress, antioxidant capacity and inflammation.

Materials and Methods

Subject

The study was performed on hundred non-consanguineous sickle cell patients from different (Raipur, Bilaspur, Durg, and Dhamtari) region of Chhattisgarh. The mean age of sickle cell patients was 23.84 years. The 5 ml blood samples were collected in EDTA vacutainers after obtaining proper consent from all individuals. Genomic DNA was then extracted using HiPuraA kit (Himedia). The presence of β -globin (β^S) sickle mutation was confirmed for all DNA samples using DdeI restriction enzyme.

Haplotype Analysis

The β -globin haplotypes were determined by PCR based restriction fragment length polymorphism (RFLP) using following restriction enzymes—HindII- ϵ , XmnI- γ^G , Hind III- γ^G , Hind III- γ^A , HincII-5' $\psi\beta$, HincII-3' $\psi\beta$, AvaII- β , HinfI- β followed by agarose gel analysis.

Measurement of HbF Level

EDTA blood was used for the determination of the fetal haemoglobin (HbF) % by high-performance liquid

chromatography. Serum or plasma were prepared from of whole blood and stored at -80°C until further biochemical analysis.

Determination of Lipid Peroxidation

The oxidative stress or lipid peroxidation was evaluated by measuring the concentrations of MDA. The concentrations of MDA were evaluated from the reaction resulting in the formation of thiobarbituric acid reactive substances according to satoh method [20]. Samples were precipitated using 20% trichloroacetic acid and after centrifugation, wash the precipitate with 0.05 M H_2SO_4 . Then add 3 ml TBA (0.22%) and H_2SO_4 in the precipitate and incubate in boiling water bath for 30 min and cool under tap water. Add 4 ml n-butanol by vigorous shaking. After centrifugation, upper butanol layer were taken for absorbance at 532 nm using spectrophotometer.

Determination of C-Reactive Protein, Ferric Reducing Ability of Plasma and Total Thiol

Inflammatory biomarker C-reactive protein was determined by nephelometric method [21]. The total antioxidant capacity of plasma was determined by ferric reducing ability of plasma (FRAP) and total thiol levels were estimated using the spectrophotometric method [22, 23].

Statistical Analysis

Statistical analysis was performed by using the SPSS (Statistical Package for Social Sciences) statistical 16.0 version software. Data were tested for normality and homogeneity of variance by using Shapiro–Wilk test and Levene's test. Student's *t* was used to compare normally distributed variables between groups. Karl Pearson correlation test was used to identify associations between parameter. Results were presented as mean $\pm$ standard deviation and $p < 0.05$ was considered statistically significant.

Result

RFLP analysis was performed on genomic DNA samples isolated from 100 sickle cell patients of Chhattisgarh for the presence (+) or absence (–) of the restriction site in the β -globin locus. The sample were marked by the presence (+) or absence (–) of each of the eight restriction sites analysed in the beta globin gene cluster.

We identified sixty-five (65%) sickle cell patients with Arab-Indian haplotype, 11 (11%) Atypical Arab-Indian, 3 (3%) Atypical Benin, 3 (3%) Atypical Bantu, 5 (5%)

Atypical Cameroon, 3 (3%) Benin, 4 (4%) Atypical Cameroon/Benin, 1 (1%) Cameroon, 3 (3%) Rare 1, 2 (2%) Rare 2 atypical haplotype (Table 1).

Influence of Haplotypes on Inflammation, Oxidative Stress, Total Thiol and FARP Among Sickle Cell Disease

Table 2 illustrates a comparison of the oxidative stress, antioxidant capacity, inflammation, total thiol and HbF levels between β -globin gene haplotypes of SCA patients. The mean values of FARP, THIOL and HbF levels were significantly higher in Arab-Indian ($p < 0.001$) and atypical Arab-Indian haplotype while the MDA and CRP levels was significantly lower than other haplotypes. The Bantu haplotype was observed with higher levels of lipid peroxidation and inflammation. This statistically significant ($p < 0.001$) difference in all biochemical parameters was also observed when comparison was made between β -globin gene haplotypes of sickle cell patients excluding between Arab Indian/atypical Arab-Indian, Arab Indian/Rare 2, atypical Arab-Indian/Rare 2 haplotypes (Table 3).

Correlation of β -Globin Gene Haplotypes with HbF, Inflammation, Oxidative Stress and Antioxidant Capacity Marker Among SS Patients

Table 4 indicates the correlation of inflammatory biomarker CRP, oxidative stress marker (malondialdehyde), total antioxidant power (ferric reducing ability of plasma) and total thiol (T-Sh) with HbF levels in different haplotypes among SCA patients. The CRP and MDA levels were significant negatively ($p < 0.0001$) correlated with HbF level and FARP and total thiol concentration were significant ($p < 0.0001$) positively correlated with HbF levels among different haplotypes in SCA patients.

Significant correlation was found between the HbF levels and CRP, MDA, FARP, total thiol (T-Sh) levels with Arab-Indian haplotype, Atypical Arab-Indian, Atypical Benin, Rare 1 and Rare 2 haplotypes. No significant correlations were found between CRP, MDA, FARP, total

thiol (T-Sh) levels with fetal haemoglobin level in Cameroon haplotype.

Discussion

Fetal haemoglobin (HbF) is related to the haplotype and protects against many clinical complications of sickle cell anemia [9, 24]. Oxidative stress appears to contribute to the pathophysiology of SCD including hemolysis, infection, inflammation, painful episode and vaso-occlusive and reperfusion injury [3]. Many previous studies have described that the different ethnic groups of SCA patients with different haematological and clinical parameter suggested that β -globin gene cluster haplotype may be useful as a predictor of disease severity [14, 25]. This study highlights that significantly ($p < 0.001$) higher level of HbF were observed in SCA patients with Arab-Indian haplotype.

It is believed that other genetic markers in the β -locus also exerted a significant effect on fetal haemoglobin synthesis [26]. In carriers of Senegal and Arab-Indian haplotype, the presence of XmnI C-T polymorphism at—158 positions upstream of γ gene strongly associated with high HbF [24]. In present study we found that patients with Arab-Indian haplotype have higher levels of HbF ($p < 0.001$) than other haplotypes. This finding is consistent with the other reported influence of the Arab-Indian haplotype on HbF level [27]. Furthermore, the different types of haplotypes contain a range of HbF concentration that act as inhibitors of HbS polymer formation.

In light of evidence suggesting that an excess of reactive oxygen species has implication in SCD pathophysiology including hemolysis, inflammation and vaso-occlusive. In sickle cell disease, the chronic inflammatory state promotes the production of ROS and predicts the disease severity. CRP is a acute phase protein that is produced by hepatocytes upon stimulation by the cytokines. The results of this study showed that the levels of oxidative stress for the Bantu haplotype were significantly higher than those for the Arab-Indian haplotype. Therefore, our findings

Table 1 β -globin haplotypes frequency among sickle cell patients

	Arab-Indian	Atypical Arab-Indian	Atypical Benin	Atypical Bantu	Atypical Cameroon	Benin	Atypical Cameroon/Benin	Cameroon	Rare 1	Rare 2
Genotype frequency n (%)	65 (65%)	11 (11%)	3 (3%)	3 (3%)	5 (5%)	3 (3%)	4 (4%)	1 (1%)	3 (3%)	2 (2%)

Genotype Frequency = No. of subjects with espective haplotype

n Number of subjects

Table 2 Influence of β -globin gene haplotypes on malondialdehyde (MDA), C-reactive protein, total antioxidant power (FARP) and total thiol levels among SCA patients

Haplotypes with genotype frequency	CRP	MDA	FARP	THIOL	HbF
Arab-Indian haplotype (n = 65)	4.35 $\pm$ 4.30	3.46 $\pm$ 2.43	435.22 $\pm$ 92.11	229.15 $\pm$ 41.44	22.71 $\pm$ 5.23
Atypical Arab-Indian (n = 11)	4.47 $\pm$ 4.08	3.80 $\pm$ 1.14	392.55 $\pm$ 68.11	225.0 $\pm$ 30.50	20.85 $\pm$ 3.02
Atypical Benin (n = 3)	15.76 $\pm$ 2.55	5.56 $\pm$ 0.57	254.0 $\pm$ 3.46	156.67 $\pm$ 11.37	9.73 $\pm$ 0.59
Atypical Bantu (n = 3)	21.93 $\pm$ 1.74	7.13 $\pm$ 0.32	245.33 $\pm$ 13.61	101.0 $\pm$ 3.60	3.83 $\pm$ 0.46
Atypical Cameroon (n = 5)	13.32 $\pm$ 3.79	4.92 $\pm$ 0.45	305.60 $\pm$ 15.70	148.80 $\pm$ 19.67	12.84 $\pm$ 0.67
Benin haplotype (n = 3)	19.0 $\pm$ 0.14	6.76 $\pm$ 0.15	245.67 $\pm$ 14.0	135.0 $\pm$ 9.53	7.0 $\pm$ 0.21
Atypical Cameroon/Benin (n = 4)	18.20 $\pm$ 0.85	6.37 $\pm$ 0.55	273.50 $\pm$ 11.12	148.50 $\pm$ 12.87	9.17 $\pm$ 0.38
Cameroon haplotype (n = 1)	17.91	4.60	266.0	169.0	10.1
Rare 1 (n = 3)	6.64 $\pm$ 6.38	4.70 $\pm$ 0.26	303.67 $\pm$ 33.17	148.0 $\pm$ 13.85	13.7 $\pm$ 1.48
Rare 2 (n = 2)	10.08 $\pm$ 9.88	4.35 $\pm$ 0.35	365.0 $\pm$ 49.49	195.0 $\pm$ 60.81	14.7 $\pm$ 0.1

Values are expressed in mean with standard deviation (Mean $\pm$ SD)

n Number of subjects

Table 3 Significant (*p* value) difference of biochemical parameter between haplotypes among sickle cell patients (SCA) in present study

Significant between haplotypes	CRP (<i>p</i> value)	MDA (<i>p</i> value)	FARP (<i>p</i> value)	THIOL (<i>p</i> value)
Arab-Indian/AtpArab-Indian	0.370	0.466	0.087	0.698
Arab-Indian/AtpBenin	0.053	< 0.003*	< 0.001*	< 0.001*
Arab-Indian/AtpBantu	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Arab-Indian/AtpCameroon	0.167	< 0.001*	< 0.001*	< 0.001*
Arab-Indian/Benin	< 0.003*	< 0.001*	< 0.001*	< 0.001*
Arab-Indian/Atp Cameroon-Benin	< 0.007*	< 0.001*	< 0.001*	< 0.001*
Arab-Indian/Cameroon	< 0.003*	< 0.001*	< 0.001*	< 0.001*
Arab-Indian/Rare 1	0.809	< 0.001*	< 0.005*	< 0.001*
Arab-Indian/Rare 2	0.816	0.067	0.270	0.572
Atp Arab-Indian/Atp Benin	< 0.002*	< 0.008*	< 0.001*	< 0.001*
Atp Arab-Indian/Atp Bantu	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Atp Arab-Indian/Atp Cameroon	< 0.003*	< 0.015*	< 0.002*	< 0.001*
Atp Arab-Indian/Benin	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Atp Arab-Indian/Atp Cameroon-Benin	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Atp Arab-Indian/Cameroon	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Atp Arab-Indian/Rare 1	0.622	< 0.036*	< 0.015*	< 0.001*
Atp Arab-Indian/Rare 2	0.567	0.151	0.574	0.611

t significant (2-tailed), Comparisons were made by independent sample *t*' test with Levene's test for equality of variance, Atp atypical

demonstrate that HbF and β -globin haplotype were found as modulator for biochemical parameters (MDA, CRP, FARP and total thiol) predicting for disease severity among SCA patients of Chhattisgarh. Several studies have reported in favour of our results [4, 9, 11]. Thus many researchers have extensively studied that significant increase in oxidative stress marker in SCA are correlated with haplotypes as predictors of disease severity [28–30]. According to the Akinsheye et al. 2011 reported that HbF was identified as one of the first determinants of disease

severity, with high HbF associated with mild disease. Additionally, attempts were also made that oxidative stress responsible for increased pathophysiology in SCA patients cause secondary dysfunctions. Recently, many studies suggested that oxidative stress (ROS and RNS) useful for as potential markers for SCA variable clinical severity [3, 4].

The present study demonstrated that oxidative stress was higher in sickle cell patients. One of the main finding of the current study is that the fetal haemoglobin level was

Table 4 Association β -globin gene haplotypes with HbF level and CRP, MDA, FARP, T-Sh parameters in sickle cell patients in present study

Haplotypes	Correlation (r^2) CRP and HbF	Correlation (r^2) MDA and HbF	Correlation (r^2) FARP and HbF	Correlation (r^2) Total thiol and HbF
Arab-Indian haplotype (n = 65)	– 0.946*	– 0.998*	0.498*	0.603*
Atypical Arab-Indian (n = 11)	– 0.447*	– 0.692	0.497*	0.700*
Atypical Benin (n = 3)	– 0.679*	– 0.191	0.994*	0.891*
Atypical Bantu (n = 3)	– 0.016	– 0.111	0.996*	0.769*
Atypical Cameroon (n = 5)	– 0.579*	– 0.215	0.256*	0.103
Benin haplotype (n = 3)	– 0.209	0.137	0.080	0.076
Atypical Cameroon/Benin (n = 4)	– 0.896*	– 0.461*	0.169	0.178
Cameroon haplotype (n = 1)	0.0	0.0	0.0	0.0
Rare 1 (n = 3)	0.081	– 0.455*	0.586*	0.998*
Rare 2 (n = 2)	– 1.0*	– 1.0*	1.0*	1.0*

* $p < 0.0001$, r^2 Pearson correlation coefficient

correlate with CRP, MDA, FARP and total thiol among β -globin gene haplotypes. A positive correlation of FARP and total thiol with HbF level and negative correlation for CRP and MDA with HbF level were found among all sickle β -globin gene haplotypes. No significant difference was observed for Benin haplotype (only for FARP + HbF parameter) and Cameroon haplotypes (CRP, MDA, FARP, THIOL) because low frequency in the study.

In this study the highest mean value for oxidative stress (MDA) were observed for Atypical Bantu > Benin haplotype > Atypical Cameroon/Benin > Atypical Benin > Atypical Cameroon > Rare 1 > Cameroon > Rare 2 > Atypical Arab-Indian > Arab-Indian haplotypes. Additionally, for inflammatory marker (CRP) highest mean value was observed for Atypical Bantu > Benin > Cameroon/Benin > Cameroon > Atypical Benin > Atypical Cameroon > Rare 2 > Arab-Indian haplotype > Rare 1 > Atypical Arab-Indian. Therefore with these finding indicate that Arab-Indian haplotype was observed with highest fetal haemoglobin (HbF) level and lowest oxidative stress (MDA) and associated with mildest disease severity in Chhattisgarh state. The results of this study showed that inflammation profile was higher for Bantu haplotype. Other studies have also described that the influence of β globin gene haplotype on oxidative stress in SCA patients, where the Bantu haplotype is associated with poor prognosis too. Recent studies also found association between inflammations with β globin haplotypes suggest that the levels of the proinflammatory cytokine for Bantu haplotypes were significantly greater than those for Benin haplotype [5].

In conclusion, the result of this study provides information about the Arab-Indian haplotype and HbF level influence the clinical presentation in SCA patients of Chhattisgarh. Our data suggest that the inflammation and

oxidative stress were higher in Bantu haplotype. The β -globin gene haplotype may possibly act as a form of protection oxidative stress by increasing level of antioxidant.

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References

1. Jison ML, Munson PJ, Barb JJ, Suffredini AF, Talwar S, Logun C, et al. Blood mononuclear cell gene expression profiles characterize the oxidant, hemolytic, and inflammatory stress of sickle cell disease. *Blood*. 2004;104:270–80.
2. Okumura JV, Lobo CL, Bonini-Domingos CR. Beta-S globin haplotypes in patients with sickle cell anemia: one approach to understand the diversity in Brazil. *Rev Bras Hematol Hemoter*. 2013;35:71–2.
3. Nur E, Biemond BJ, Otten HM, Brandjes DP, Schnog JJ. Oxidative stress in sickle cell disease; pathophysiology and potential implication for disease management. *Am J Hematol*. 2011;86:484–9.
4. Queiroz RF, Lima ES. Oxidative stress in sickle cell disease. *Rev Bras Hematol Hemoter*. 2013;35:16–7.
5. Bandeira IC, Rocha LB, Barbosa MC, Elias DB, Querioz JA, Freitas MV, et al. Chronic inflammatory state in sickle cell anemia patients is associated with HBB* S haplotype. *Cytokine*. 2014;65:217–21.
6. Stuart MJ, Nagel RL. Sickle cell disease. *Lancet*. 2004;364:1343–60.
7. Belcher JD, Beckman JD, Balla G, Balla J, Vercellotti G. Heme degradation and vascular injury. *Antioxid Redox Signal*. 2010;12:233–48.
8. Chirico EN, Pialoux V. Role of oxidative stress in the pathogenesis of sickle cell disease. *IUBMB Life*. 2012;64:72–80.
9. Loggetto SR. Sickle cell anemia: clinical diversity and beta S-globin haplotypes. *Rev Bras Hematol Hemoter*. 2013;35:155–7.
10. Belini Junior E, da Silva DG, Torres Lde S, de Almeida EA, Cancado RD, Chiatone C, et al. Oxidative stress and antioxidant capacity in sickle cell anaemia patients receiving different

- treatments and medication for different periods of time. *Ann Hematol.* 2012;91:479–89.
11. El-Ghamrawy MK, Hanna WM, Abdel-Salam A, El-Sonbaty MM, Youness ER, Adel A. Oxidant-antioxidant status in Egyptian children with sickle cell anemia: a single center based study. *J Pediatr (Rio J).* 2014;90:286–92.
 12. Torres Lde S. Comment on “Influence of β S-globin haplotype and hydroxyurea on tumor necrosis factor- α levels in sickle cell anemia”. *Rev Bras Hematol Hemoter.* 2014;36:102–3.
 13. Ogedegbe HO. β -Globin gene cluster haplotype analysis as a predictor of sickle cell disease severity. *Lab Med.* 2007;38:563–8.
 14. Steinberg MH. Predicting clinical severity in sickle cell anemia. *Br J Haematol.* 2005;129:465–81.
 15. Cabral CH, Serafim ES, de Medeiros WR, de Medeiros Fernandes TA, Kimura EM, Costa FF, et al. Determination of β^S haplotypes in patients with sickle cell anemia in the state of Rio Grande do Norte, Brazil. *Genet Mol Biol.* 2011;34:421–4.
 16. Silva MAL, Friedrichs JR, Bittar CM, Urnau M, Merzoni J, Valim VS, et al. β -globin gene cluster haplotypes and clinical severity in sickle cell anemia patients in Southern Brazil. *Open J Blood Dis.* 2014;4:16–23.
 17. Steinberg MH. Genetic etiologies for phenotypic diversity in sickle cell anemia. *Sci World J.* 2009;9:46–67.
 18. Steinberg MH, Sebastiani P. Genetic modifiers of sickle cell disease. *Am J Hematol.* 2012;87:795–03.
 19. Bhagat S, Patra PK, Thakur AS. Fetal haemoglobin and β -globin gene cluster haplotypes among sickle cell patients in Chhattisgarh. *J Clin Diagn Res.* 2013;7:269–72.
 20. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta.* 1978;90:37–43.
 21. Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effect of laboratory variables on levels of protein in blood. *Clin Diagn Lab Immunol.* 2003;10:652–7.
 22. Benzie FFI, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “Antioxidant Power”: the FRAP Assay. *Anal Biochem.* 1996;239:70–6.
 23. Costa CM, dos Santos RCC, Lima ES. A simple automated procedure for thiol measurement in human serum samples. *J Bras Patol Med Lab.* 2006;42:345–50.
 24. Ngo D, Bae H, Steinberg MH, Sebastiani P, Solovieff N, Baldwin CT, et al. Fetal hemoglobin in sickle cell anemia: genetic studies of the Arab-Indian haplotype. *Blood Cells Mol Dis.* 2013;51:22–6.
 25. Shimauti ELT, Silva DGH, Souza EM, Almeida EA, Leal FP, Bonini-Domingos CR. Prevalence of β^S -globin gene haplotypes, α -thalassemia (3.7 kb deletion) and redox status in patients with sickle cell anemia in the state of Paraná, Brazil. *Genet Mol Biol.* 2015;38:316–23.
 26. Thein SL, Menzel S, Lathrop M, Garner C. Control of fetal hemoglobin: new insight emerging from genomics and clinical implications. *Hum Mol Genet.* 2009;18:R216–23.
 27. Kulozik AE, Kar BC, Satapathy RK, Serjeant BE, Serjeant GR, Weatherall DJ. Fetal hemoglobin levels and β S globin haplotype in an Indian population with sickle cell disease. *Blood.* 1987;69:1742–6.
 28. Camilo-Araujo RF, Amancio OM, Figueiredo MS, Cabanas-Pedro AC, Braga JA. Molecular analysis and association with clinical and laboratory manifestation in children with sickle cell anemia. *Rev Bras Hematol Hemoter.* 2014;36:334–9.
 29. Schnog JJ, Lard LR, Rojer RA, Van der Dijs FP, Muskiet FA, Duits AJ. New concepts in assessing sickle cell disease severity. *Am J Hematol.* 1998;58:61–6.
 30. Sebastiani P, Solovieff N, Hartley SW, Milton JN, Riva A, Dworkis DA, et al. Genetic modifiers of the severity of sickle cell anemia identified through a genome-wide association study. *Am J Hematol.* 2010;85:29–35.