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RESEARCH ARTICLE

# Coagulation profile in pathophysiology of sickle cell anemia

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## ABSTRACT

**Background:** Sickle cell anemia (SCA) is an emerging public health challenge in India as well as globally. The WHO recognized it as a global problem since long time. In Western Odisha, its prevalence varies from 5% to 30%. **Aim and Objective:** The objective of this study was to determine the alteration of the coagulation profile in SCA in comparison to healthy control. **Materials and Methods:** This study was completed with 60 subjects that included 30 cases and 30 controls. This study was approved by the Institutional Ethical Committee. The hematological parameters such as bleeding time, clotting time, total platelet count, prothrombin time (PT), and activated partial thromboplastin time (aPTT) were tested. The data were analyzed through SPSS 20. **Results:** In this study, it was found that patients with SCA have increased clotting time ($P = 0.007$), PT ($P = 0.000$), aPTT ($P = 0.003$), and total platelet count ($P = 0.183$), but decreased bleeding time ($P = 0.000$) in comparison to healthy control. **Conclusion:** We found a significant increase in clotting time, PT, and aPTT, but decrease in bleeding time in SCA patients in comparison to healthy adults.

**KEY WORDS:** Coagulation profile; Sickle cell anemia; Hypercoagulability

## INTRODUCTION

Sickle cell disease (SCD) or sickle cell anemia (SCA) is an emerging public health challenge not only in India but also across the whole world. Due to a better survival rate and movement of people, the burden of SCA is increasing globally.[1] It has been estimated that by the year 2050, nearly 14.2 million SCA babies will be born.[2] The World Health Organization declared that SCD is a public health problem throughout the globe.[3] In Western Odisha, SCD is highly prevalent and the range varies from 5 to 30%.[4-7] It is an autosomal recessive pattern of hereditary hemoglobinopathy syndrome, in which the 6$^{th}$ codon of β globin gene undergo mutation resulting in the replacement valine (Val) in place of glutamic acid (Glu).[8] This inherited hemoglobinopathy in the homozygous state is called SCA. This is a disease of hypercoagulable state that leads to comorbidities in the form of vaso-occlusive disorder and cerebrovascular accidents.[9] The previous studies have shown that SCA patients are at higher risk of venous thrombosis. Due to more chances of thrombotic complications and hemostatic alterations, SCA is considered to be a truly hypercoagulable state.[10]

Therefore, our study was conceived to determine the coagulable profile of SCA patients and the result was compared with the healthy subjects.

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## MATERIALS AND METHODS

This study was a hospital-based case–control analytical study conducted in Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Odisha, India. The Institutional Ethical Committee approved this study to

conduct. Patients hospitalized to the medicine indoor were selected as subjects of case. It was conducted between November 2017 and October 2019. Subjects were described about the study protocol and its output. All subjects gave their consent to participate in the study and written consent was taken from all subjects. To avoid age-related confounding, we selected subjects between the age group of 18–33 years both in case and control group. Diagnosed SCA patients were selected as case and healthy subjects were selected from the same study setting as control. Sickling slide test, alkaline agarose gel hemoglobin electrophoresis (pH 8.6), and cation-exchange high-performance liquid chromatography were used to diagnose SCA.[11] Patients with sepsis, G6PD deficiency, pregnancy, and patients on anticoagulants and oral contraceptives were excluded from the study.

### Bleeding Time

It was done by Duke's method. The tip of the left middle finger was pricked with aseptic precautions. The time of puncture was noted. The blood was gently blotted every 30 s with a filter paper. This procedure was repeated until no blot appeared on the filter paper. The time was noted again. The number of blots on the paper was counted. Number of blots ×30 s was taken as the bleeding time. Normal reference range of bleeding time is 1–5 min.[12]

### Clotting Time

It was done using the capillary glass tube method. The tip of the left middle finger was pricked with aseptic precautions, and the time of puncture was noted. One end of the capillary tube was dipped into blood drop and by capillary action, it was filled with blood. After 1 min, a small bit of tube was broken every 30 s until a fine thread of fibrin appeared between the broken ends. The time was noted again. The interval between the prick and appearance of fibrin thread was taken as the clotting time. Normal reference range of clotting time is 3–8 min.[12]

### Platelet Count

It was done using Rees-Ecker method. The diluting fluid was sucked up to 0.5 mark in the RBC pipette. Then, the pipette was filled with blood so that the diluents reached up to the mark 1.0. The pipette was then filled with diluents up to mark 101 once again. Then, the pipette was rolled between the palms gently for 3 to 4 min. Then, the Neubauer's chamber was charged by discarding the first two drops of blood. Platelets were seen as bluish, tiny bodies fairly regular in outline, highly refractile on racking the microscope. Total platelet count was done by counting the platelet number in five groups of 16 squares each, and then, their number in 1 $mm^3$ of undiluted blood was calculated. Normal reference range of platelet count is 150 to 450 × $10^9$/L.[13]

### PT

0.1 ml of plasma was taken in a glass tube. The tube was placed in a water bath adding 0.1 ml of thromboplastin. The mixture was warmed for 1–3 min. 0.1 ml of warmed calcium chloride was added and stopwatch was started. The contents of the glass tube were mixed and the endpoint was recorded. Normal reference range of PT is 11 to 16 s.[14]

### Activated Partial Thromboplastin Time (aPTT)

0.5 ml of phospholipid reagent was mixed with 0.5 ml of kaolin suspension in a test tube and kept in a water bath. In another test tube, 0.2 ml kaolin phospholipid solution was mixed with 0.1 ml of plasma. Then, 0.1 ml pre-warmed calcium chloride solution was added and the time taken by mixture to clot was recorded. Normal reference range of aPTT is 26–40 s.[14]

The data were analyzed using statistical software SPSS (Statistical Package for the Social Sciences, IBM Corporation, Armonk, New york) version 20. Data were presented in mean ± standard deviation form. Statistical analysis was done by unpaired *t*-test. The corresponding values in different groups were compared statistically by determining "*P*" value. $P < 0.05$ was considered to be significant statistically.

## RESULTS

We recruited 60 subjects for the study. Thirty subjects who were diagnosed as SCA constituted the case group and 30 subjects who were normal healthy individuals with similar age and sex constituted the control group.

Table 1 depicts the sex distribution of case and control. The percentage of male in case and control groups was 56.6% and 63.3%. The percentage of females in case and control groups was 43.4% and 36.7%.

Table 2 compares mean values of clotting time, bleeding time, platelet count, PT, and aPTT between the case and control groups. The cases had a range of bleeding time from 2.42 min to 3.44 min with a mean of 2.77 min. Taking the reference value of bleeding time as 1–5 min, all the cases had normal bleeding time. A significant difference was shown between cases and controls in the bleeding time ($P = 0.000$). The bleeding time was higher in the control group than cases. The range of clotting time was 3.63–9.64 min with

**Table 1:** Sex distribution of case and control

| Variable | Case | Control |
|---|---|---|
| | Number (%) | Number (%) |
| Male | 17 (56.6) | 19 (63.3) |
| Female | 13 (43.4) | 11 (36.7) |

This table depicts the sex distribution of subjects in number and percent form

**Table 2:** Comparison of coagulation profile parameters

| Variable | Case (*n*=30) (Mean±SD) | Control (*n*=30) (Mean±SD) | *P* |
|---|---|---|---|
| BT (min) | 2.77±0.25 | 3.88±0.98 | 0.000 |
| CT (min) | 6.07±1.43 | 5.13±1.17 | 0.007 |
| PT (s) | 14.48±1.88 | 12.16±0.67 | 0.000 |
| aPTT (s) | 34.0±7.0 | 29.6±3.1 | 0.003 |
| PC ($10^9$/μl) | 375±138 | 334±93 | 0.183 |

*n*: Number of subjects. $P<0.05$ was considered to be significant. BT: Bleeding time, CT: Clotting time, PT: Prothrombin time, aPTT: Activated partial thromboplastin time, PC: Platelet count, and SD: Standard deviation

a mean of 6.07 min. As the normal value of clotting time is 3–8 min, 86.7% of cases had normal clotting time, and 13.3% had prolonged clotting time. The mean clotting time is more in the cases than controls ($P = 0.007$), although both were within the normal range. The mean PT among the cases was 14.48, ranging from 11.5 to 19.7 s. Taking the reference value of PT as 11–16 s, 86.7% of cases had normal PT, and 13.3% showed prolongation. The mean of PT was more in the cases than controls ($P = 0.000$), although both were within the normal range. The cases had a range of aPTT from 20.4 to 58.6 s with a mean of 34.0 s. Taking the reference value of aPTT as 26–40 s, 73.3% of cases had normal aPTT, 20.0% showed prolongation, and only 6.7% were shortened. A significant difference was shown in aPTT when case and control groups were compared ($P = 0.003$), aPTT in cases was higher than that of controls, although both were within the normal range. The cases had a range of platelet counts from 158 to 689 × $10^9$/L with a mean of 375.37 × $10^9$/L. Taking the reference value of platelet count as 150–450 × $10^9$/L, 70% of cases had normal count, and 30% showed thrombocytosis. No significant difference was shown in platelet count when case and control groups were compared ($P = 0.183$). Platelet counts in cases were higher than that of controls, although both were within the normal range.

## DISCUSSION

This study showed that the mean values of bleeding time in case and control groups were 2.77 min (±0.25) and 3.88 min (±0.98), respectively. The mean values of clotting time in case and control groups were 6.07 min (±1.43) and 5.13 min (±1.17), respectively. The mean values of PT in case and control groups were 14.48 s (±1.88) and 12.16 s (±0.67), respectively. The mean values of aPTT in case and control groups were 34.0 s (±7.0) and 29.6 s (±3.1), respectively. The mean values of total platelet count in case and control groups were 375.37 × $10^9$/L (±138.49) and 334.33 × $10^9$/L (±93.00), respectively.

We found that bleeding time in healthy subjects was more than the cases. This result was contradicted other reports that showed that bleeding time was less in SCA patients than the healthy subjects.[15,16] SCA patients may be saved from blood loss quickly in a natural way of hemostasis.[16] This study reported that clotting time in cases was more than the controls. Most of the patients with SCA had normal clotting time and four patients had increased clotting time. Acquired circulating inhibitors may be the cause of prolonged clotting time.[16] This study suggested that PT was more in the case than control, this was supported by some researches.[15] Most of the patients showed normal PT and four patients showed prolonged PT. Being a hypercoagulable state, the finding of prolonged PT is SCA that is rather paradoxical. Researchers suggested that abnormal liver function and deficiency of clotting factors V and VII may contribute to more PT in cases.[17-19] This study showed that aPTT is more in cases than control which was supported by other studies.[15,20] It was normal in 22 patients, shortened in two patients, and prolonged in six patients. Prolongation of aPTT may be due to acquired circulating inhibitors and reduced level of factor Xa.[18] In our study, we found that platelet count in case is more than control but not significant. In a study, researchers reported that platelet count is normal in SCA.[21]

### Strength and Limitations

This study helps to understand the role of coagulation in the development of clinical complication of SCA. A routine test of coagulation profile in sickle cell patients will be helpful to avoid complications. This study could have been better by recruiting a larger number of subjects. It could have been better if the study of fibrinogen and other clotting factors was done to establish a better correlation of coagulation profile in SCA.

## CONCLUSION

This study showed an abnormal coagulation profile characterized by decreased bleeding time but increased clotting time, PT, aPTT, and platelet count in sickle cell patients comparing with healthy adults.

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## REFERENCES

1. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010;115:4331-6.
2. Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global burden of sickle cell anemia in children under five, 2010-2050: Modelling based on demographics, excess mortality, and interventions. PLoS Med 2013;10:e1001484.
3. World Health Organization. Fifty-ninth World Health Assembly:

Resolutions and Decisions, Annexes. WHA59/2006/REC/1. Geneva: World Health Organization; 2006.
4. Purohit P, Mashon RS, Patel S, Dehury S, Pattanayak C, Das K, *et al*. Clinical and molecular characterization of Hb Hofu in Eastern India. Int J Lab Hematol 2014;36:71-6.
5. Meher S, Patel DK, Patel S. Epidemiology of beta thalassemia trait in Eastern India. Odisha Physician J 2012;9:24-9.
6. Patel DK, Mashon RS, Patel S, Dash PM, Das BS. β-globin gene haplotypes linked with the Hb D-Punjab [β121 (GH4) Glu→Gln, GAA> CAA] mutation in Eastern India. Hemoglobin 2010;34:530-7.
7. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, *et al*. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: A multicentre study. J Community Genet 2013;4:33-42.
8. Purohit P, Mantri S, Nayak J, Mahapatra B. Factors responsible for mortality in patients with sickle cell disease: A hospital-based study. Natl J Physiol Pharm Pharmacol 2020;10:32-6.
9. Nduka N, Owhochuku SM, Odike P. Current observations on sickle cell genotype in Nigeria. East Afr Med J 1993;70:646-9.
10. Noubouossie D, Key NS, Ataga KI. Coagulation abnormalities of sickle cell disease: Relationship with clinical outcomes and the effect of disease modifying therapies. Blood Rev 2016;30:245-56.
11. Mohanty PK, Meher S. Mortality risk factors in hospitalised late adolescent and adult sickle cell disease patients. J Evid Based Med Healthc 2018;5:135-39.
12. Guyton AC, Hall JE. Textbook of Medical Physiology. 14th ed. Philadelphia, PA: Elsevier Saunders; 2020. p. 487.
13. Bain BJ, Bates I, Laffan MA, Mitchell ES. Dacie and Lewis Practical Haematology. 12th ed. London: Churchill Livingstone; 2017. p. 12.
14. Bain BJ, Bates I, Laffan MA, Mitchell ES. Dacie and Lewis Practical Haematology. 11th ed. London: Churchill Livingstone; 2009. p. 410-1.
15. Al-Jiffri OH. Coagulation profile, platelets and endothelial activation markers among patients with steady state sickle cell anemia. Austin Hematol 2017;2:1009.
16. Ataga KI, Orringer EP. Hypercoagulability in sickle cell disease: A curious paradox. Am J Med 2003;115:721-8.
17. Hankins JS, Wang WC. Sickle cell anemia and other sickling syndromes. In: Greer JP, Arber DA, Glader B, List AF, Means RT Jr., Paraskevas F, *et al*., editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia, PA: Wolter Kluwer Lippincott Williams and Wilkins Health; 2014. p. 823-926.
18. Raffini LJ, Niebanck AE, Hrusovsky J, Stevens A, Blackwood-Chirchir A, Ohene-Frempong K, *et al*. Prolongation of the prothrombin time and activated partial thromboplastin time in children with sickle cell disease. Pediatr Blood Cancer 2006;47:589-93.
19. Nsiri B, Gritli N, Bayoudh F, Messaoud F, Fattoum S, Machghoul S. Abnormalities of coagulation and fibrinolysis in homozygous sickle cell disease. Hematol Cell Ther 1996;38:279-84.
20. Buseri FI, Jeremiah ZA, Shokunbi WA. Plasma levels of some blood coagulation parameters in Nigerian homozygous sickle cell patients (HbSS) in steady state. Hematology 2006;11:375-9.
21. Mohamed EA, Elgari MM, Babker AM, Waggiallah HA. Comparative study of hypercoagulability change in steady state and during vaso-occlusive crisis among Sudanese patients living with sickle cell disease. Afr Health Sci 2020;20:392-6.

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