Evaluation of the serum status of E-selectin and its correlation with disease severity among steady state patients with sickle cell anaemia

Abstract

Introduction: Sickle cell anemia (SCA) is a disease in which hemoglobin S exists in a homozygous state, with prominent clinical features and a wide range of phenotypes. This study aimed to determine the levels of E-selectin and how it correlated with the severity of disease in sickle cell anemia patients.

Methodology: This was a comparative cross-sectional study. Socio-demographic characteristics, full blood count, body mass index and serum E-selectin levels of patients with Sickle cell anemia (HbSS) in steady state as well as age and sex-matched controls of HbAA of HbAS phenotype. were obtained.

Results: Ninety individuals participated in the study and were made up of 30 HbSS, 30 HbAS and 30 HbAA subjects with age range of 10 to 50 years and mean age of 27.3 ±6 years, 27.1 ±6years and 26 ± 6 years respectively. Mean E-selectin level in patients with HbSS was 434.3 ±306ng/l which was significantly higher than those of HbAS and HbAA with mean value of 203.4 ±120ng/l and 238.9 ±171ng/l respectively ($p<0.05$). There was a positive correlation between mean level of E-selectin with disease severity among patients with HbSS, although it was not significant statistically

Conclusion: E-selectin level was significantly higher in patients with HbSS when compared to the control group (HbAA and HbAS). However, the positive correlation of E-selectin with disease severity in patients with HbSS, did not reach statistical significance. Further studies should be carried out on a wider scale to determine the actual effect of E-Selectin as a biomarker for early detection of disease severity in patients with HbSS.

Keywords: E-selectin, Steady state, Phenotypes, Haemoglobin SS, Haemoglobin AS, Haemoglobin AA.

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Introduction:

Sickle cell anemia (SCA) is a disease in which hemoglobin S exists in a homozygous state, with prominent clinical features and a wide range of phenotypes [1]. In sub-Saharan Africa, it is very prevalent, affecting 1-4% of newborns [2]. It is also very prevalent in India, the Middle East and the Mediterranean. However, Nigeria, partly because of its large population, bears a great burden of the disease [3].

It is inherited as an autosomal recessive disease caused by a point mutation in the 6th position of the beta chain of hemoglobin, which brings about the substitution of glutamic acid for valine and results in the formation of hemoglobin S (HbS). When oxygen is lacking, Hb S polymerizes and sets of a cascade of molecular events that activates the coagulation system and results in the release of proinflammatory cytokines, leading to an elevated leucocytes count, and a background chronic inflammation. These culminates in the initiation and propagation of Vaso-occlusion and hence prominent clinical features [4,5]. E-selectin has been found to play a crucial role in this process and may therefore be a potential therapeutic target in the management of SCA [6].

The selectin family is an important class of adhesion molecules involved in the pathogenesis of SCA. These adhesion molecules are lectins situated on leukocytes, platelets, and endothelial cells referred to as the L-, P-and E-selectins [7]. They are calcium-dependent adhesion molecules and are upregulated by diverse inflammatory stimuli [8]. The P- and E-selectins referred to as the vascular selectins [7] are found on platelets and endothelial cells respectively and have been demonstrated to be elevated during vaso-occlusive crises (VOCs) compared to the steady state [9]. The expression of E-selectin is induced by proinflammatory cytokines such as interleukin -1 (IL-1) and tumor necrosis factor α (TNFα) produced by activated leukocytes. [7,10]. The increased expression of E-selectin on the endothelial cell surface leads to the recruitment and

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adherence of leukocytes to these surfaces. This plays a crucial role in Vaso-occlusion and the prominent clinical features found in SCA [11].

Body mass index (BMI) is used to assess if an individual's weight is healthy compared to height and it's usually expressed in kg/m^2. It may be a pointer to present or future morbidity depending on its value. Some lines of evidence have shown that it may be positively correlated with some full blood count parameters in individuals of hemoglobin phenotype AS and AA [12].

In Nigeria, there is limited report on the possible association between E-Selectin and disease severity in SCA patients. Investigating the levels of E-selectin in SCA patients may help in the evaluation and management of patients with SCA. The aim of this study was to determine the levels of E-selectin and its correlation with BMI and disease severity in patients with sickle cell anemia in steady state.

Contribution to knowledge

Sickle cell anemia (SCA) is a disease with a background chronic inflammatory process manifested by increased granulocyte counts and elevated levels of pro-inflammatory cytokines. A hypercoagulable state and chronic inflammation are important pathophysiological processes that lead to vaso-occlusion and tissue damage in sickle cell anemia. The critical role of E-selectin in inflammation makes it a potential therapeutic target in SCA.

We have shown that there is significant difference in the mean level of E-selectin in the different hemoglobin (Hb) phenotype groups. There was a significant increase in the mean level of E-selectin in subjects with homozygous sickle cell disease (HbSS) compared with the control groups (HbAS and HbAA) in our data set collected in a tertiary hospital in Nigeria, which may be partly responsible for mediating the initiation and propagation of vaso-occlusion in the subjects. We also found E-selectin levels to be positively correlated with SCD severity in the steady state, although this association did not reach statistical significance. It would be tempting to hypothesize that the drug Colchicine, which is widely available and cheap can be repurposed for prophylactic therapy in SCD patients since it can affect inflammation by modulating the distribution of E-selectin in endothelial cells and neutrophils.

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Materials and methods

Study design and study site: This was a comparative cross-sectional study carried out in a tertiary health center

Study population: This was made up of subjects with sickle cell anemia (HbSS) in steady state attending sickle cell clinic assigned Group A, normal adult haemoglobin (HbAA) – Group B and those with individuals with sickle cell trait (HbAS) – Group C who met the inclusion criteria and gave consent. Steady state was defined based on three parameters; absence of fever, crisis, and blood transfusion for at least two weeks, four weeks, and three months respectively, before recruitment [13].

Sample size: Using the method described by Charan and Biswas [14]; a sample size of 30 subjects was calculated. However, 90 participants were recruited made up of 30 participants with HbSS in steady state as subjects, 30 HbAS and 30 HbAA individuals as controls.

The sample size was calculated as follows;

$$N = \frac{(Z^2pq)}{d^2}$$

Where:

N= Desired number of sample when population of the facility is limited

Z = Z value, where Z is the standard normal variance where confidence level is 1.96 at 95%

p = prevalence rate of sickle cell disease in Nigeria is 2% (WHO, 2017)

q = 1 – p

d = 5% i.e degree of precision as desired by the researcher.

Applying the method,

$$N = \frac{Z^2 \times P \times (1-P)}{}$$

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$$N = \frac{1.96^2 \times 0.02 \times (1-0.02)}{0.05^2}$$

N = 30

Inclusion criteria: Sickle cell anemia (HbSS) subjects in steady state, heterozygous sickle cell anemia controls (HbAS) and controls with normal adult hemoglobin (HbAA) all within the age range 10-50yrs.

Exclusion criteria: Subjects outside the age range of 10-50yrs, individuals with known cardiovascular and inflammatory disease (autoimmune disease), renal disease, pregnancy, metabolic disorders such as diabetes mellitus, individuals on medication such as NSAIDs.

Sampling technique: Patients with HbSS who met the inclusion criteria were selected by simple random sampling from the Hematology outpatient clinic of a tertiary health Centre between December 2020 and May 2021. Control groups (HbAS and HbAA) were randomly selected from among students and staff of the institution.

Sample collection and analysis: Six (6 mL) of venous blood was collected aseptically by venipuncture from each participant via the antecubital vein using a plastic syringe and needle with minimal stasis; 3 mL was then dispensed into commercially prepared concentration of Ethylene Diamine tetracetic acid (EDTA) bottles for the assessment of hemoglobin phenotype using the method of Kohn [15]; [cellulose acetate paper hemoglobin electrophoresis at alkaline pH using Zip zone electrophoresis chamber and EV 243 power supply (Helena Biosciences, UK).]. Full blood count (FBC) was determined using the method described by Buttarello and Plebani [16] using Sysmex automated hematology analyzer (KX2IN model, Sysmex Corporation Kobe, Japan). The remaining 3 mL was dispensed into a plain bottle and subsequently used for the determination of E-selectin levels.

Estimation of E-selectin

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E-selectin level was assayed by Enzyme Linked Immunosorbent Assay (ELISA) technique using ELISA machine (BIOBASE 10-E) and E-selectin test-kit. Serial dilutions of the standard were made according to manufacturer's instructions. Blank well and sample wells was set. 25 µL of E-selectin standards, controls, and samples was pipetted into appropriate wells. To each well, 50 µL HRP conjugate reagent was added except the blank well. After closing plate with closure plate membrane, the mixture was incubated for 30mins at 37℃ and washed with 300 µL of wash buffer and blotted on absorbent paper towel for color development, 50 µL chromogen solution A and 50 µL chromogen solution B was added to each well. The wells were covered and incubated for 10mins at 37℃. The reaction was stopped by adding 50 µL of stop solution into each well (the blue color changed into yellow immediately). The measurement of the optical density (OD) under 450nm wavelength was carried out within 15mins after adding the stop solutions with ELISA reader. The blank well was taken as zero.

A questionnaire was used for further acquisition of information on socio-demographic profile, medical history, and drug history for all participants.

Determination of body mass index (BMI)

This was done according to the standard method as described by Odetunde *et al.* [17].

Procedure for severity scoring in sickle cell disease

This was done by an objective SCA severity scoring system, which is contributed to by scoring four parameters, which are: degree of anemia, number of complications suffered, white blood cell count (WBC) and lifetime transfusion rate. Depending on the score, the subjects were divided into groups of mild, moderate, and severe disease [13].

Disease severity scoring was determined as follows:

Anaemia score: Haemoglobin level of ≥ 10g/dl = 0; Haemoglobin level of ≥ 8g/dl and < 10g/dl = 1; Haemoglobin level of ≥ 6 and < 8g/dl = 2; Haemoglobin level of ≥ 4 and < 6g/dl = 3; Haemoglobin level of < 4g/dl = 4.

Complications score: The complications included conditions such as retinopathy, pulmonary hypertension, stroke, liver failure, avascular necrosis (AVN) of joint, leg ulcer, nephropathy,

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priapism, anaemic heart failure and acute chest syndrome. A score of 1 was given for each complication except stroke and nephropathy which are scored 2 points each.

WBC score: Count of $< 9 \times 10^9$ cells/µl = 0; Count of ≥ 9 and $< 11 \times 10^9$ cells/µl = 1; Count of ≥ 11 and $< 15 \times 10^9$ cells/µl = 2; Count of $\geq 15 \times 10^9$ cells/µl = 3.

Transfusion score: Life Transfusion Rate = Total Number (pint) of Blood transfusion divided by Age

Convert the score to the nearest whole number.

Disease severity was scored as follows: ≤ 3 – mild, > 3 – ≤ 5 moderate, > 5– severe

Statistical Analysis

Data generated from this study was analyzed using Statistical Package for Social Sciences (SPSS) software, version 20 (IBM SPSS Inc. Chicago, IL). Descriptive statistics was used to compute means and standard deviation. Analysis of Variance was used to compare means. Correlation of the parameters with disease severity was determined using the Pearson's correlation coefficient. P-values of <0.05 was regarded as significant.

Results

Ninety individuals participated in the study and were made up of 30 HbSS in steady state, (8 had mild disease, while 20 and 2 had moderate and severe disease respectively) 30 HbAS and 30 HbAA subjects with age range of 10 to 50 years and mean age of 27.3 ±6 years, 27.1 ±6years and 26 ± 6 years respectively. Mean body Mass Index (BMI) of participants with HbSS was 21.9 ±2.8 kg/m^2 which was significantly lower than those of HbAA and HbAS participants which was 24.6 ±2.4 kg/m^2 and 23.7 ±2.5 kg/m^2 respectively (p = 0.001; p = 0.045 respectively) Table 1.

Table 1: Mean age, height, weight, and Body Mass Index (BMI) of different haemoglobin phenotype groups (HbSS, HbAA and HbAS)

Groups	Age (years)	Height (m)	Weight (Kg)	BMI (kg/m^2)
HbSS (A)	27.3 ±6.3	1.66 ±0.1	60.3 ±9.4	21.9 ±2.8
HbAA (B)	27.2 ±6.6	1.62 ±0.1	64.8 ±6.3	24.6 ±2.4
HbAS (C)	25.8 ±6.0	1.66 ±0.1	65.0 ±6.4	23.7 ±2.5
F- test	0.377	1.766	3.486	8.358
A vs B (p-value)	1.000	0.384	0.078	0.001
A vs C (p-value)	1.000	1.000	0.088	0.045

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Mean ±Standard Deviation (SD), BMI= Body mass index

Mean E-selectin level in patients with HbSS was 434.3 ±306ng/l which was significantly higher than those of HbAS and HbAA with mean value of 203.4 ±120ng/l and 238.9 ±171ng/l respectively ($p<0.05$) (Table 2 and Figure 1).

Table 2: Mean level of E-selectin in different haemoglobin phenotype groups (HbSS, HbAA and HbAS)

Groups	Mean E-selectin level (ng/l)
HbSS (A)	434.3 ±306
HbAA (B)	238.9 ±171
HbAS (C)	203.4 ±120
F-test	8.596
A vs B (p-value)	0.004
A vs C (p-value)	0.001

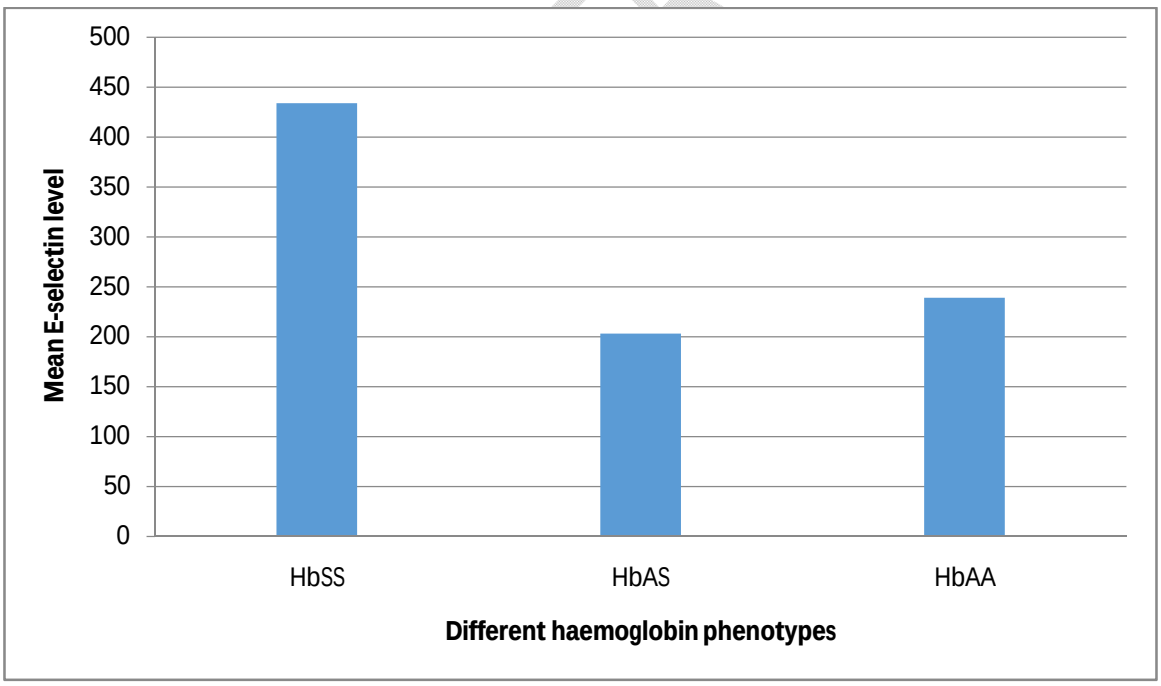

Figure 1: Mean E-selectin levels among individuals with different haemoglobin phenotypes

Correlation between the mean level of E-selectin and age with disease severity among patients with HbSS showed a positive relationship that didn't reach significance. However, there was significant negative correlation of disease severity with BMI (Table 3).

Table 3: Correlation of the mean level of E-selectin, BMI, and age with disease severity in subjects with sickle cell anemia (HbSS)

HbSS Disease severity	R	p-value
Disease severity vs E-selectin (ng/l)	0.155	0.414
Disease severity vs BMI (kg/m^2)	-0.375	0.041*
Disease severity vs Age (years)	0.029	0.881

***statistically significant**

Discussion

The findings of this study showed a significant difference in the mean level of E-selectin in the different hemoglobin (Hb) phenotype groups ($p<0.05$). There was an increase in the mean level of E-selectin in subjects with homozygous sickle cell anemia (HbSS) compared with the control groups (HbAS and HbAA) which reached statistical significance. This agrees with the findings of Proenca-Ferreira and Antwi-Boasiako et al [11,19] in which they showed a significant increase in E-selectin in subjects with homozygous sickle cell anemia (HbSS). Similarly, a study conducted in Ile Ife, Nigeria reported significantly higher level of E-selectin among children with sickle cell anaemia in Vaso-occlusive crisis compared to those in steady state as well as children with HbAA. E-selectin level was also found to be significantly related the frequency of VOC and haematological parameters [20]. The finding of significant relationship of E selectin to disease severity in their study compared to this study where this relationship didn't reach statistical significance, could be due to their study population which included SCA patients in VOC compared to those in steady state and HbAA while this study compared SCA patients in steady state with HbAS and HbAA. We also used a more robust objective score for disease severity

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which is a composite of many parameters. Also, Najjar *et al*. [9], reported a significant increase of E selectin in patients with sickle cell disease in Saudi Arabia ethnicity and non-Saudi patients. The increase in E-selectin level could be due to activation of endothelial cells. The hypercoagulable state in SCD is brought about by release of tissue factor from the activated endothelial cells leading to activation of the coagulation cascade that contributes to Vaso-occlusion [21]. Adhesion molecules (E-selectin, ICAM-1, VCAM-1) are crucial to the recruitment of leucocytes in sites of Vaso-occlusion and these molecules are up regulated on the activated endothelial cell during inflammation [22,23].

Hidalgo et al. [24] demonstrated that E-selectin also acts as a signal transducer that allows the capturing of circulating platelets and erythrocytes leading to acute vascular occlusions that may be lethal. They concluded that their results "indicate that endothelial selectins can influence neutrophil behavior beyond its canonical rolling step through delayed, organ-damaging, polarized activation." Therefore, inactivating, or modulating E-selectin activity may prevent tissue damage in SCA. Colchicine, a drug that is relatively cheap, safe, and generally available has been found to affect inflammation in a multimodal manner, including modulating the distribution, on endothelial cells and neutrophils, of E-selectin [25,26]. The authors therefore propose clinical trials using Colchicine as prophylactic therapy for SCD

Our study showed a significant decrease in the mean BMI of subjects with sickle cell anemia (HbSS) compared to the control groups (HbAA and HbAS). This agrees with the finding of Odetunde *et. al*. [16] who showed a significant decrease in body mass index in subjects with sickle cell anemia (HbSS). This may be accounted for by a high metabolic rate and decreased absorption. [27]. In sickle cell anemia, hypoxemia, and tissue hypoperfusion occur which can

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cause tissue impairment and therefore growth retardation [28]. This is reflected in impairment in various anthropometric measurements [29].

This study also revealed a significant correlation of BMI with disease severity in subjects with sickle cell anemia (HbSS). This contrasts with the findings of Hall *et al*. [30] who reported no correlation of BMI with disease severity. Mean levels of serum E-selectin and age in subjects with sickle cell anemia (HbSS), showed a positive correlation with disease severity, although this did not reach statistical significance.

Conclusion

Patients with HbSS have significantly higher levels of E-selectin, (making colchicine, a drug which affects qualitative expression of E-selectin and with multimodal action on inflammation potentially useful therapy in SCA) and significantly lower value of BMI compared to the control subjects (HbAA and HbAS). There was positive correlation, which did not reach statistical significance, of disease severity with E-selectin as well as age; but significant correlation with BMI in patients with HbSS. Further studies should be carried out on a wider scale to determine the actual effect of E-Selectin as a biomarker for early detection of disease severity in patients with HbSS, and a clinical trial using colchicine as prophylactic therapy for SCA.

Ethical Approval and Consent

The ethical approval for this study NAUTH/CS/66/VOL.14/VER 3/130/2021/095 was obtained from the Research and Ethics Committee of the tertiary health center in accordance with the Helsinki declaration by the World Medical Association (WMA) on the ethical principles for medical research involving human subjects [18]. Informed written consent was obtained from each participant before being recruited into the study.

Limitations of the study

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Determination of β-globin gene haplotypes was out of the scope of this work and so were not done.

Other factors that may affect disease severity such as haemoglobin F level was not assessed. A longitudinal study that will assess E-selectin in individual subjects during steady state and crises would also increase our understanding of the role of E-selectin.

References

1. Ware, R. E., de Montalembert, M., Tshilolo, L., & Abboud, M. R. (2017). Sickle cell disease. *The Lancet*, *390*(10091), 311-323.

2. Tegha, G., Topazian, H., Kamthunzi, P., Howard, T., Tembo, Z., Mvalo, Tawonga M, Arielle H, Kenneth I. A, Irving F. H and Russell E. W. (2021). Prospective newborn screening for sickle cell disease and other inherited blood disorders in Central Malawi. *International Journal of Public Health*, 60.

3. Akingbola, T. S., Aworanti, O. W., & Ogundeji, S. P. (2022). Audit of clinical and laboratory parameters of hemoglobin SS patients in a Nigerian teaching hospital. *Annals of Medicine*, *54*(1), 2921-2928.

4. Okocha C., Manafa, P., Anowi, J., Manafa, V. and Efobi, C. GDF -15 and Severity Scores in Sickle Cell Disease Patients Attending Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. *Journal of Advances in Medicine and Medical Research*. 2020, **32** (1): 31- 37.

5. Korotkova, N. V., Kalinin, R. E., Suchkov, I. A., Mzhavanadze, N. D., Nikiforov, A. A., Romanov, B. K., & Bodrova, O. V. (2022). Assessment of the selectins and their ligand PSGL-1 level in patients with acute venous thrombosis. *Problems of Biological Medical and Pharmaceutical Chemistry*, *25*(5), 22-27.

6. Barthel SR, Gavino JD, Descheny L, Dimitroff CJ. Targeting selectins and selectin ligands in inflammation and cancer. *Expert OpinTher Targets* (2007) 11(11):1473–91. doi:10.1517/14728222.11.11.1473

7. Silva, M., Videira, P. A. and Sackstein, R. E-selectin ligands in the human mononuclear phagocyte system: Implications for infection, inflammation, and immunotherapy. *Frontiers in Immunology.*2017, **8**:1878.

8. McEver, R. P. Selectins: Initiators of leucocyte adhesion and signaling at the vascular wall. *Cardiovascular Research.* 2015, **107**:331-339.

9. Al Najjar, S., Adam, S., Ahmed, N. and Qari, M. Markers of endothelial dysfunction and leucocyte activation in Saudi and non-Saudi haplotypes of sickle cell disease. *Annals of Hematology. 2017,* **96**:141-146

10. Janeway, C. Immunobiology: the immune system in health and disease. New York: *Garland Science*, 2015, ISBN 0-8153-4101-6.

11. Antwi-Boasiako, C., Donkor. E.E., Sey, F., Dzudzor, B., Dankwah, G.B., Otu, K. H. Doku A., Dale C.A., Ekem I. Levels of Soluble Endothelium Adhesion Molecules and Complications among Sickle Cell Disease Patients in Ghana. *Diseases.* 2018, **6**(2):29.

12. Ajayi, O. I., Akinbo, D. B. and Okafor, A. M. Correlation between Body Mass Index and Hematological Indices in Young Adult Nigerians with Different Hemoglobin Genotypes. *American Journal of Biomedical Sciences*. 2017, **9**: 38-46.

13. Okocha, E. C., Manafa, O. P., Aneke, C. J., Onwuzuruike, E. C., Ibeh, C. N., & Chukwuma, O. G. Serum superoxide dismutase activity: A predictor of disease severity in Nigerian sickle cell anemia patients in steady state. *Medical Journal of Dr. DY Patil University*, 2017, 10(5), 406.

14. Charan, J. and Biswas. T. How to Calculate Sample Size for Different Study Designs in Medical Research? *Indian Journal of Psychological Medicine*, 2013, **35**: 121-126.

15. Kohn, J. A cellulose acetate supporting medium for zone electrophoresis. *Clinica Chimita Acta*. 1957, **2** (4): 297 – 303.

16. Buttarello, M., Plebani, M. Automated blood cell counts: state of the art. *American Journal of Clinical Pathology*. 2008, **130** (1): 104 – 116.

17. Odetunde, O. I., Chinawa, J. M., Achigbu, K. I., and Achigbu, E. O. Body mass index and other anthropometric variables in children with sickle cell anaemia. *Pakistan journal of medical sciences*, 2016, **32**(2), 341–346.

18. Shrestha, B., Dunn, L. The declaration of Helsinki on Medical Research involving Human subjects: A Review of seventh Revision. *Journal Nepal Health Resource Counc*, 2020, **17**(4): 548 – 552.

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19. Proenca-Ferreira, R., Brugnerotto, A. F., Garrido, V. T., Ribeiro, M. R., Traina, F., Saad, S. T. O., Costa, F. F. and Conran, N. Platelets from Sickle Cell Disease Individuals Induce Endothelial Activation, Demonstrating ICAM-1 and E-Selectin Adhesion Molecule Expression, Inflammatory Cytokine Production and Activation of NFκB Transcription Factor Gene Expression. *Blood*. 2012, **120** (21): 2114.

20. Smith, O.S., Salawu, L., Adegoke, S.A., Ajose, O.A., Bolarinwa, R.A., Adedeji, T., Adeniyi, A. T. and Oderinu, A. K. High Levels of Serum Soluble Selectin-E Is Associated with Frequency of Vaso-Occlusive Crises and Haematological Parameters in Children with Sickle Cell Anaemia. Acad J Ped Neonatol. 2020, **10**(1): 555835.

21. Manwani, D., & Frenette, P. S. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. *Blood*, 2013, **122**(24), 3892–3898. https://doi.org/10.1182/blood-2013-05-498311.

22. Hebbel, R. P., Osarogiagbon, R. and Kaul, D. The endothelial biology of sickle cell disease: Inflammation and a chronic vasculopathy. *Microcirculation*. 2004, **11**(2):129-151.

23. Zhang, J., DeFelice, A. F., Hanig, J. P., & Colatsky, T. Biomarkers of endothelial cell activation serve as potential surrogate markers for drug-induced vascular injury. *Toxicologic pathology*, 2010, **38**(6), 856-871.

24. Hidalgo, A., Chang, J., Jang, J. E., Peired, A. J., Chiang, E. Y., & Frenette, P. S. Heterotypic interactions enabled by polarized neutrophil microdomains mediate thrombo inflammatory injury. *Nature medicine*, 2009, **15**(4), 384–391. https://doi.org/10.1038/nm.1939

25. Cronstein, B. N., Molad, Y., Reibman, J., Balakhane, E., Levin, R. I., & Weissmann, G. Colchicine alters the quantitative and qualitative display of selectins on endothelial cells and neutrophils. *The Journal of clinical investigation*, 1995, **96**(2), 994–1002. https://doi.org/10.1172/JCI118147

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26. Dalbeth, N., Lauterio, T. J., & Wolfe, H. R. Mechanism of action of colchicine in the treatment of gout. *Clinical therapeutics*, 2014, **36**(10), 1465–1479. https://doi.org/10.1016/j.clinthera.2014.07.017

27. Jesus, A. C. S., Konstantyner, T., Lobo, I. K. V. and Braga, J. A. P. Socioeconomic and nutritional characteristics of children and adolescents with sickle cell anemia: a systematic review. *RevistaPaulista de Pediatria.* 2018, **36**(4): 491- 499.

28. Beker, L. and Cheng, T. L. Principles of growth assessment. *Pediatrics in Review.* 2006, **27**(5):196–198.

29. Nandanwar, R. A. and Kamdi, N. Y. Sickle cell disease affects physical growth. *International Journal of Pharma and Biosciences*. 2013, **4**(2): 784- 789.

30. Hall, R., Gardner, K., Rees, D. C. and Chakravorty, S. High body mass index in children with sickle cell disease: a retrospective single-centre audit. *BMJ paediatrics open*, 2018, ***2***(1): e000302.

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