



(RESEARCH ARTICLE)



## EVALUATION OF MICRORNA-451, VASCULAR CELL ADHESION MOLECULE-1, SOLUBLE P-SELECTIN, CD4 AND CD36 IN SICKLE CELL DISEASE SUBJECTS IN BAYELSA STATE, NIGERIA.

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### ABSTRACT

**Introduction:** Sickle cell disease (SCD) is an autosomal recessive inherited haemoglobin disorder caused by a  $\beta$ -globin gene mutation and characterised by haemolysis, inflammation, endothelial activation, abnormal cellular adhesion and vaso-occlusion. MicroRNA-451 (miRNA-451), vascular cell adhesion molecule-1 (VCAM-1), soluble P-selectin, CD4 and CD36 are involved in erythroid regulation, vascular adhesion and immune responses.

**Objective:** This study evaluated the relative expression of miRNA-451 and the levels of VCAM-1, soluble P-selectin, CD4 and CD36 among HbSS, HbAS and HbAA subjects in Bayelsa State, Nigeria and determined the relationships among these parameters in Bayelsa State, Nigeria.

**Study design:** This comparative cross-sectional study included 186 participants from different medical facilities across Bayelsa state with the eight (8) Local Government Area: 62 each with HbSS group as case study and HbAS and HbAA groups as control Subjects. Subjects were within the aged 2 - 51 years males and females subjects.

**Methodology:** Haemoglobin genotype was determined by alkaline haemoglobin electrophoresis. Plasma VCAM-1 and soluble P-selectin were measured by enzyme-linked immunosorbent assay, and CD4 and CD36 were measured by flow cytometry. Whole-blood miRNA-451 was quantified by reverse-transcription quantitative polymerase chain reaction.

**Statistical tools:** Data were analysed using SPSS version 21.0.

**Result:** Group means were compared using one-way analysis of variance followed by the Waller–Duncan multiple-range test. Correlation coefficients were calculated for the combined sample and separately within each genotype group.

Statistical significance was set at  $p < 0.05$ . Mean relative miRNA-451 expression was higher in HbSS participants than in HbAS and HbAA subjects. VCAM-1 concentration was higher in HbSS than in HbAS and HbAA subjects. Soluble P-selectin was higher in HbSS than in HbAS and HbAA subjects. CD4 counts did not differ significantly among groups. CD36 values were higher in HbSS than in HbAS and HbAA subjects. In the combined sample, miRNA-451 correlated positively with VCAM-1, soluble P-selectin and CD36. VCAM-1 correlated with soluble P-selectin and CD36 and soluble P-selectin correlated with CD36 at  $p < 0.01$ . No significant correlations were observed within individual genotype groups. Haemoglobin SS participants had higher miRNA-451, VCAM-1, soluble P-selectin and CD36 values than HbAS and HbAA participants, whereas CD4 counts were comparable. These findings describe differences in erythroid, endothelial and cellular adhesion-related parameters among the studied genotype groups but do not establish causal relationships.

**Keywords:** Sick Cell Disease; Mirna-451; VCAM-1; Soluble P-Selectin; CD4; CD36; Nigeria; Bayelsa State.

## 1 INTRODUCTION

Sickle cell disease is an autosomal recessive inherited haemoglobin disorder caused by a mutation in the  $\beta$ -globin gene. The resulting haemoglobin S polymerises when deoxygenated, reducing red-cell deformability and promoting sickling, haemolysis and microvascular obstruction [1,2]. SCD is common in sub-Saharan Africa and is characterised by interacting processes that include chronic anaemia, haemolysis, inflammation, endothelial activation, abnormal cellular adhesion and recurrent vaso-occlusion [1,2].

These processes may contribute to progressive organ complications [3]. Vaso-occlusion involves interactions among sickled erythrocytes, leucocytes, platelets and endothelial cells rather than erythrocyte sickling alone [4]. Endothelial activation and increased adhesion-molecule expression facilitate interactions between circulating cells and the vascular wall [4,5]. VCAM-1 is expressed on activated endothelial cells and mediates interactions with leucocytes. Increased concentrations of soluble endothelial adhesion molecules have been reported in SCD and associated with pulmonary hypertension, organ dysfunction and mortality [5].

Circulating activated endothelial cells have also been demonstrated in sickle cell anaemia [6]. Soluble P-selectin is expressed on activated platelets and endothelial cells and contributes to blood-cell adhesion. Increased whole-blood adhesion to Soluble P-selectin has been reported during vaso-occlusive events in SCD [7]. MicroRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally. miRNA-451 is associated with erythroid differentiation and red-cell biogenesis and is highly expressed in mature erythrocytes [8].

The miR-144/451 locus is important for erythroid homeostasis [9], and miRNA-451 has been associated with protection against oxidative stress in erythroid cells [10,11].

Altered miRNA-451 expression has been reported in haemoglobinopathies. Plasma miRNA-451 has been associated with haemolysis in  $\beta^0$ -thalassaemia/HbE disease [12]. Differences in miRNA-451 and P-selectin have also been reported among individuals with haemoglobin-S variants in Nigeria [13], and circulating miRNA-451a profiles have differed among sickle haemoglobin genotypes [14]. These findings are not directly comparable with the present study because sample types and laboratory methods differ.

CD36 is a membrane glycoprotein expressed on several blood-cell populations and has been implicated in cellular adhesion. Experimental studies have implicated CD36 in the adhesion of sickle erythrocytes to endothelial cells [15].

SCD is also associated with altered immune responses. Both innate and adaptive immune mechanisms may contribute to disease complications [16]. Absolute CD4 count represents one component of adaptive immunity but does not capture all immune-cell functions or subsets.

Nigeria has a substantial burden of SCD. A study in Calabar reported HbSS prevalence of 2.14% and an overall SCD prevalence of 2.28% among haemoglobin electrophoresis records examined [17]. However, limited information is available on the combined assessment of miRNA-451, VCAM-1, soluble P-selectin, CD4 and CD36 among HbSS, HbAS and HbAA individuals in Bayelsa State. This study therefore compared these parameters and examined their correlations among the three genotype groups.

### 1.1 Statement of problem

SCD remains an important public health problem in Nigeria and involves chronic haemolysis, inflammation, endothelial activation, cellular adhesion and recurrent vaso-occlusion [1,2]. Routine laboratory tests do not capture all of these processes. Additional molecular and cellular measurements may help describe biological differences among genotype groups. Information on the combined pattern of miRNA-451, VCAM-1, soluble P-selectin, CD4 and CD36 among HbSS, HbAS and HbAA individuals in Bayelsa State is limited. Further comparative laboratory studies are therefore warranted.

## 1.2 Justification of study

miRNA-451, VCAM-1, soluble P-selectin and CD36 represent different aspects of erythroid regulation, endothelial activation and cellular adhesion, while CD4 provides information on one component of adaptive immunity. Measuring these parameters in the same population may describe differences among HbSS, HbAS and HbAA individuals and provide data for future studies of clinical outcomes.

## 1.3 Aim of study

The aim was to compare miRNA-451, VCAM-1, soluble P-selectin, CD4 and CD36 among HbSS, HbAS and HbAA individuals in Bayelsa State, Nigeria.

### *Specific objectives*

The objectives were to:

- Compare relative miRNA-451 expression among HbSS, HbAS and HbAA participants;
- Compare blood VCAM-1 concentrations among the three genotype groups;
- Compare blood soluble P-selectin concentrations among the three genotype groups;
- Compare CD4 and CD36 measurements among the three genotype groups; and assess correlations among miRNA-451, VCAM-1, soluble P-selectin, CD4 and CD36 in the combined sample and within each genotype group.

### *Research questions*

- Does miRNA-451 expression differ among HbSS, HbAS and HbAA participants?
- Does VCAM-1 and soluble P-selectin concentrations differ among the three genotype groups?
- Does CD4 and CD36 measurements differ among the three genotype groups?
- Were there significant differences when the studied parameters were correlated in the combined sample and within individual genotype groups?

## 1.4 Research hypotheses

### 1.4.1 Null hypotheses

- $H_{01}$ : There is no statistically significant difference in miRNA-451 levels among HbSS, HbAS and HbAA participants and there is no statistically significant correlation between miRNA-451 and the other study parameters in the combined sample or within the individual genotype groups.
- $H_{02}$ : There is no statistically significant difference in VCAM-1 levels among HbSS, HbAS and HbAA participants and there is no statistically significant correlation between VCAM-1 and the other studied parameters in the combined groups or within the individual genotype groups.
- $H_{03}$ : There is no statistically significant difference in soluble P-selectin levels among HbSS, HbAS and HbAA participants and there is no statistically significant correlation between soluble P-selectin and the other studied parameters in the combined groups or within the individual genotype groups.
- $H_{04}$ : There is no statistically significant difference in CD4 cell counts among HbSS, HbAS and HbAA participants and there is no statistically significant correlation between CD4 cell counts and the other studied parameters in the combined groups or within the individual genotype groups.
- $H_{05}$ : There is no statistically significant difference in CD36 cell counts among HbSS, HbAS and HbAA participants and there is no statistically significant correlation between CD36 cell counts and the other study parameters in the combined sample or within the individual genotype groups.

### 1.5 Alternative hypotheses

- $H_{01}$ : There is a statistically significant difference in miRNA-451 levels among HbSS, HbAS and HbAA participants and there is a statistically significant correlation between miRNA-451 and the other studied parameters in the combined groups or within the individual genotype groups.
- $H_{02}$ : There is a statistically significant difference in VCAM-1 levels among HbSS, HbAS and HbAA participants and there is a statistically significant correlation between VCAM-1 and the other study parameters in the combined sample or within the individual genotype groups.
- $H_{03}$ : There is a statistically significant difference in soluble P-selectin levels among HbSS, HbAS and HbAA participants and there is a statistically significant correlation between soluble P-selectin and the other study parameters in the combined sample or within the individual genotype groups.

- $H_{04}$ : There is a statistically significant difference in CD4 cell counts among HbSS, HbAS and HbAA participants and there is a statistically significant correlation between CD4 cell counts and the other studied parameters in the combined groups or within the individual genotype groups.
- $H_{05}$ : There is a statistically significant difference in CD36 cell counts among HbSS, HbAS and HbAA participants and there is a statistically significant correlation between CD36 and the other studied parameters in the combined groups or within the individual genotype groups.

## 2 MATERIALS AND METHODS

### 2.1 Study area

The study was conducted in Bayelsa State, in the Niger Delta region of Southern Nigeria. The state comprises of eight Local Government Areas: Brass, Ekeremor, Kolokuma/Opokuma, Nembe, Ogbia, Sagbama, Southern Ijaw and Yenagoa.

Participants were recruited from Niger Delta University Teaching Hospital, Okolobiri; Federal Medical Centre, Yenagoa; Diete Koki Memorial Hospital; Family Care Hospital, Yenagoa; and selected General hospitals and Comprehensive health centres in participating Local Government Areas.

### 2.2 Study design

A comparative cross-sectional design was used. Participants were classified as HbSS, HbAS or HbAA according to haemoglobin genotype.

### 2.3 Study population

The study included 186 participants aged 2 - 51 years: 62 HbSS, 62 HbAS and 62 HbAA participants.

### 2.4 Sample size determination

The sample size was calculated using the prevalence formula;

$$n = Z^2pq/d^2$$

where n is the required sample size, Z is the standard normal deviate at the 95% confidence level (1.96), p is the estimated prevalence (0.02), q is 1-p (0.98) and d is the allowable error (0.0349).

$$n = \{(1.96)^2(0.02)(0.98)\}$$

$$\{(0.0349)^2\}$$

$$=61.79$$

The calculated sample size was approximately 62 participants per group. The prevalence used was based on the HbSS prevalence reported in Calabar [17].

### 2.5 Sample allocation

Equal allocation produced three groups of 62 participants, for a total of 186 participants.

### 2.6 Inclusion criteria

Participants were eligible if they:

were aged 2– 51 years;

They were confirmed HbSS, HbAS or HbAA genotype;

They were available at a participating centre during the study period; and provided consent or assent as applicable.

### 2.7 Exclusion criteria

Participants were excluded if they had a condition likely to substantially affect the measured parameters or were outside the specified age range.

## 2.8 Ethical approval

Ethical approval was obtained from the relevant ethics committees with their respective approved numbers; Federal Medical Centre, Yenagoa; Bayelsa State Ministry of Health; and Madonna University Nigeria.

Written informed consent was obtained from adults. For minors, parental or legal-guardian consent and participant assent were obtained as required by the approved protocol. Participant confidentiality was maintained and samples were used only for the purposes approved by the ethics committees.

## 2.9 Sample collection

Five millilitres of venous blood were collected from each participant using standard procedure. Three millilitres were placed in EDTA tubes for VCAM-1, soluble P-selectin, CD4 and CD36 analyses. Two millilitres were placed in DNA/RNA Shield for miRNA-451 analysis.

Samples for CD4 and CD36 were processed according to the flow-cytometry procedure. Plasma was obtained by centrifugation at 3,000 rpm for 10 minutes. Samples for miRNA-451 were stored at  $-80^{\circ}\text{C}$  until extraction.

## 2.10 Laboratory investigations

### 2.10.1 Haemoglobin genotype determination

Haemoglobin genotype was determined by alkaline haemoglobin electrophoresis using Tris-EDTA buffer at pH 8.5 and cellulose acetate. Electrophoresis was performed at 150 V for 15 minutes. Bands were interpreted as HbSS, HbAS or HbAA accordingly [31].

### 2.11 Determination of VCAM-1

Plasma VCAM-1 was measured by sandwich enzyme-linked immunosorbent assay using a commercial VCAM-1 kit according to the manufacturer's instructions [18].

Samples, Standards and Controls (100  $\mu\text{L}$ ) were added to the appropriate wells and incubated at  $37^{\circ}\text{C}$  for 90 minutes. Wells were washed three times, biotinylated detection antibody was added and the plate was incubated at  $37^{\circ}\text{C}$  for 60 minutes. After washing, Streptavidin–horseradish peroxidase was added and incubated at  $37^{\circ}\text{C}$  for 30 minutes. Wells were washed five times, tetramethylbenzidine was added and the plate was incubated in the dark at  $37^{\circ}\text{C}$  for 20 minutes. The reaction was stopped with 50  $\mu\text{L}$  of 2 M sulphuric acid. Absorbance was read at 450 nm and concentrations were calculated from the standard curve [7].

### 2.12 Determination of soluble P-selectin

Plasma soluble P-selectin was measured by enzyme-linked immunosorbent assay. Samples, Standards and Controls (50  $\mu\text{L}$ ) were added to the appropriate wells and incubated at room temperature for two hours. Wells were washed three times, anti-P-selectin antibody was added and the plate was incubated at room temperature for 60 minutes. After washing, 50  $\mu\text{L}$  of Streptavidin–horseradish peroxidase were added and incubated at  $37^{\circ}\text{C}$  for 60 minutes.

Wells were washed three times with 350  $\mu\text{L}$  of wash buffer per well. Tetramethylbenzidine (50  $\mu\text{L}$ ) was added, and the plate was incubated at  $37^{\circ}\text{C}$  for 15 minutes in the dark. The reaction was stopped with 50  $\mu\text{L}$  of 2 M sulphuric acid and absorbance was read at 450 nm [5].

### 2.13 Determination of CD4

CD4 was measured by flow cytometry. Whole blood was stained with fluorochrome-conjugated monoclonal antibodies, followed by red-cell lysis and flow-cytometric analysis.

Twenty microlitres of the appropriate antibody were added to 20  $\mu\text{L}$  of well-mixed whole blood. The mixture was vortexed and incubated in the dark for 15 minutes. Lysing buffer (800  $\mu\text{L}$ ) was added and the preparation was analysed by flow cytometry. Results were expressed as cells/ $\mu\text{L}$  [21].

### 2.14 Determination of CD36

CD36 was measured by flow cytometry. Whole blood was stained with fluorochrome-conjugated monoclonal antibodies. Briefly, 20  $\mu\text{L}$  of the appropriate antibody was added to 20  $\mu\text{L}$  of well-mixed whole blood. The mixture was vortexed and incubated in the dark for 15 minutes. Thereafter, 800  $\mu\text{L}$  of lysing buffer was added to lyse the red blood cells, and the preparation was analysed by flow cytometry. The results were expressed as cells/ $\mu\text{L}$  [25].

### 2.15 Extraction of miRNA-451

miRNA-451 was measured in whole blood. It was extracted using a combined TRIzol and Direct-zol microRNA procedure.

Blood preserved in DNA/RNA Shield was thawed at room temperature and vortexed for 15 seconds. Two hundred microlitres of the blood–preservative mixture, corresponding to approximately 100 µL of blood, were transferred to a clean tube. TRIzol (600 µL) was added, mixed and incubated at room temperature for five minutes [13].

Ethanol (800 µL, 100%) was added and mixed. The preparation was loaded in two portions onto a Zymo-Spin IIC column and centrifuged at  $12,000 \times g$  for 30 seconds. The column was washed with 400 µL of RNA Wash Buffer.

For DNase treatment, 5 µL of DNase I and 75 µL of DNA Digestion Buffer were combined and 80 µL of the mixture were applied to the column. The column was incubated at 30°C for 15 minutes.

The column was washed twice with 400 µL of Direct-zol RNA Pre-Wash and once with 700 µL of RNA Wash Buffer, followed by centrifugation at  $12,000 \times g$  for two minutes. RNA was eluted with 35 µL of DNase/RNase-free water and centrifuged at  $12,000 \times g$  for 30 seconds. Extracted RNA was stored at –80°C [27].

### 2.16 Complementary DNA synthesis

- Complementary DNA was synthesised by reverse transcription using M-MuLV reverse transcriptase and random hexamer priming.
- The miRNA-451 primers were:
- Forward: 5'-AAACCGTTACCATTACTGAGTT-3'
- Reverse: 5'-GTGCAGGGTCCGAGG-3'
- U6 was used as the reference control:
- Forward: 5'-CTCGCTTCGGCAGCACA-3'
- Reverse: 5'-AACGCTTCACGAATTTGCGT-3'
- RNA was incubated at 65°C for 10 minutes before addition of the reverse-transcription mixture. The reaction was incubated at 37°C for two hours and then at 65°C for 10 minutes. cDNA was stored appropriately until quantitative PCR [13].

### 2.17 Quantitative real-time PCR

Quantitative real-time PCR was performed in a 48-well plate. Each 25-µL reaction contained 12.5 µL of 2× Real-Time Probe Master Mix, 7.5 µL of miRNA-451 primer mix and 5 µL of cDNA. Amplification was performed according to the manufacturer's instructions and the laboratory protocol. Runs were analysed using StepOne software version 2.2 [27].

### 2.18 Statistical analysis

Analyses were performed using SPSS version 21.0. Continuous variables were summarised as mean  $\pm$  standard deviation. One-way analysis of variance compared the three genotype groups, followed by the Waller-Duncan multiple-range test. Correlation coefficients were calculated for the combined sample and separately within each genotype group. Statistical significance was set at  $p < 0.05$ .

### 3 RESULTS

#### 3.1 Comparison of studied parameters among HbSS, HbAS and HbAA participants.

**Table 1: Mean  $\pm$  standard deviation of miRNA-451, VCAM-1, Soluble P-Selectin, CD4 and CD36 across the different groups.**

| Parameter/unit             | HbSS                                 | HbAS                               | HbAA                              |
|----------------------------|--------------------------------------|------------------------------------|-----------------------------------|
| microRNA-451               | 11.86 $\pm$ 1.22 <sup>b</sup>        | 1.42 $\pm$ 0.29 <sup>a</sup>       | 1.42 $\pm$ 0.31 <sup>a</sup>      |
| Relative Expression        |                                      |                                    |                                   |
| VCAM-1 (ng/mL)             | 678.81 $\pm$ 97.32 <sup>c</sup>      | 385.92 $\pm$ 85.38 <sup>b</sup>    | 302.42 $\pm$ 87.85 <sup>a</sup>   |
| Soluble P-Selectin (pg/mL) | 10905.00 $\pm$ 422.51 <sup>c</sup>   | 9162.73 $\pm$ 1232.01 <sup>b</sup> | 7969.23 $\pm$ 386.81 <sup>a</sup> |
| CD4 (cells/ $\mu$ L)       | 632.26 $\pm$ 119.85 <sup>a</sup>     | 605.86 $\pm$ 133.17 <sup>a</sup>   | 631.05 $\pm$ 140.92 <sup>a</sup>  |
| CD36 (cells/ $\mu$ L)      | 1,426.75 $\pm$ 9,680.40 <sup>b</sup> | 503.75 $\pm$ 6471.78 <sup>a</sup>  | 517.55 $\pm$ 5871.37 <sup>a</sup> |

Note: Means within the same column bearing different superscript letters (a, b, c) differ significantly at  $p < 0.05$ ; Values are presented in mean  $\pm$  standard deviation. Means with different superscript letters within a row differ significantly at  $p < 0.05$ . miRNA-451 expression was higher in HbSS than in HbAS and HbAA participants; HbAS and HbAA did not differ significantly. VCAM-1 was higher in HbSS than in HbAS and HbAA participants and was higher in HbAS than in HbAA participants. Soluble P-selectin was higher in HbSS than in HbAS and HbAA participants and was higher in HbAS than in HbAA participants. CD4 did not differ significantly among the three groups and CD36 was higher in HbSS than in HbAS and HbAA participants; HbAS and HbAA did not differ significantly.

**Table 2 Correlation matrix of miRNA-451, VCAM-1, soluble P-selectin, CD4 and CD36 in the various groups.**

| Variables  | miRNA-451 | VCAM-1  | sP-Selectin | CD4   | CD36 |
|------------|-----------|---------|-------------|-------|------|
| miRNA-451  | 1         |         |             |       |      |
| VCAM-1     | 0.842**   | 1       |             |       |      |
| P-Selectin | 0.755**   | 0.602** | 1           |       |      |
| CD4        | 0.043     | 0.017   | 0.004       | 1     |      |
| CD36       | 0.975**   | 0.835** | 0.756**     | 0.045 | 1    |

Note: Values represent Pearson correlation coefficients (r).

\*\* Correlation is significant at  $p < 0.01$  (2-tailed).

In the combined sample, miRNA-451 correlated positively with VCAM-1, soluble P-selectin and CD36. VCAM-1 correlated positively with soluble P-selectin and CD36, and soluble P-selectin correlated positively with CD36. CD4 did not correlate significantly with any other parameter.

**Table 3: Correlation Matrix Among HbSS Subjects**

| Variables  | miRNA-451 | VCAM-1 | P-Selectin | CD4    | CD36 |
|------------|-----------|--------|------------|--------|------|
| miRNA-451  | 1         |        |            |        |      |
| VCAM-1     | -0.079    | 1      |            |        |      |
| P-Selectin | -0.168    | 0.150  | 1          |        |      |
| CD4        | -0.013    | 0.127  | 0.053      | 1      |      |
| CD36       | 0.055     | -0.034 | -0.144     | -0.083 | 1    |

No statistically significant correlations were observed among the parameters in the HbSS group ( $p > 0.05$ ).

**Table 4: Correlation Matrix Among HbAS Subjects**

| Variables  | miRNA-451 | VCAM-1 | P-Selectin | CD4    | CD36 |
|------------|-----------|--------|------------|--------|------|
| miRNA-451  | 1         |        |            |        |      |
| VCAM-1     | -0.061    | 1      |            |        |      |
| P-Selectin | -0.046    | 0.052  | 1          |        |      |
| CD4        | -0.003    | 0.068  | -0.054     | 1      |      |
| CD36       | 0.117     | 0.053  | 0.070      | -0.074 | 1    |

No statistically significant correlations were observed among the parameters in the HbAS group. ( $p > 0.05$ ).

**Table 5: Correlation Matrix Among HbAA Subjects**

| Variables  | miRNA-451 | VCAM-1 | sP-Selectin | CD4   | CD36 |
|------------|-----------|--------|-------------|-------|------|
| miRNA-451  | 1         |        |             |       |      |
| VCAM-1     | 0.025     | 1      |             |       |      |
| P-Selectin | 0.111     | 0.001  | 1           |       |      |
| CD4        | -0.189    | -0.239 | -0.195      | 1     |      |
| CD36       | -0.214    | -0.242 | -0.025      | 0.098 | 1    |

No statistically significant correlations were observed among the parameters in the HbAA group. ( $p > 0.05$ ).

## 4 DISCUSSION

This study compared miRNA-451, VCAM-1, soluble P-selectin, CD4 and CD36 among HbSS, HbAS and HbAA participants in Bayelsa State. Differences were observed in miRNA-451, VCAM-1, soluble P-selectin and CD36, whereas CD4 counts were similar among groups.

miRNA-451 expression was higher in HbSS than in HbAS and HbAA participants. miRNA-451 is associated with erythroid differentiation and is highly expressed in red blood cells [8–11]. Previous studies have reported altered miRNA-451 in haemoglobin disorders, including  $\beta^0$ -thalassaemia/HbE disease and sickle haemoglobin variants [12–14]. The present study measured the whole-blood miRNA-451 as compared to some previous studies who also measured plasma or circulating exosomes.

VCAM-1 was higher in HbSS than in HbAS and HbAA participants and higher in HbAS than in HbAA participants. VCAM-1 is involved in leucocyte adhesion to activated endothelium and increased molecules have been reported in SCD [5,6]. The present findings are consistent with differences in endothelial-activation markers among the genotype groups.

Soluble P-selectin was higher in HbSS than in HbAS and HbAA participants and higher in HbAS than in HbAA participants. P-selectin contributes to interactions among blood cells, platelets and activated endothelium and P-selectin-dependent adhesion has been studied in SCD [7]. Because vaso-occlusive events were not assessed in this study, the results should not be interpreted as evidence that soluble P-selectin predicts vaso-occlusive crisis.

CD4 counts did not differ significantly among the groups. SCD may involve changes in immune-cell phenotype, activation and function that are not reflected by absolute CD4 count alone [16]. The present result therefore indicates only that absolute CD4 counts were comparable in the studied groups.

CD36 values were higher in HbSS than in HbAS and HbAA participants. CD36 has been implicated in sickle erythrocyte adhesion in experimental models [15]. Interpretation of the present finding depends on the cell population measured; the exact population, antibody and gating strategy must be reported from the laboratory record.

In the combined sample, miRNA-451, VCAM-1, soluble P-selectin and CD36 showed positive correlations. These associations may partly reflect differences in mean values between genotype groups. The correlations were not observed within the groups were analysed separately.

CD4 did not correlate with the other parameters. The overall findings describe group differences and pooled associations but do not establish causality, temporal relationships or clinical predictive value.

## 5 CONCLUSION

HbSS participants had higher miRNA-451, VCAM-1, soluble P-selectin and CD36 values than HbAS and HbAA participants, whereas CD4 counts did not differ significantly. Positive correlations among miRNA-451, VCAM-1, soluble P-selectin and CD36 were observed in the combined sample but not within individual genotype groups. These findings provide laboratory data on erythroid, endothelial and cellular adhesion-related parameters in the studied population and demonstrate significant positive correlations among several parameters in the combined sample. However, the absence of significant correlations within the individual genotype groups and the cross-sectional study design limit interpretation of these associations and do not establish causal relationships.

### *Recommendations*

- Future studies should examine miRNA-451 alongside haemolysis and erythropoietic markers.
- VCAM-1, soluble P-selectin and CD36 should be assessed in relation to documented clinical complications.
- Longitudinal studies should evaluate temporal changes and clinical outcomes.
- Future studies should include bilirubin, lactate dehydrogenase and reticulocyte count.
- Studies should compare steady-state participants with those experiencing vaso-occlusive crisis.
- Treatment effects, including hydroxyurea use, should be recorded and analysed.
- Immunological studies should assess CD4 T-cell subsets and functional markers.
- Larger multicentre studies should assess reproducibility in other Nigerian populations.

### *Limitations of study*

- The cross-sectional design prevents assessment of temporal or causal relationships.
- Clinical complications, including vaso-occlusive crisis, acute chest syndrome, stroke and organ dysfunction, were not directly assessed.
- Pooled correlations were not observed after genotype stratification, indicating that between-group differences may have contributed to the combined-sample associations.
- The study was conducted in selected health facilities in Bayelsa State and may not represent all individuals with SCD in the state or Nigeria.

## STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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### *Disclosure of conflict of interest*

The authors declare no conflicts of interest regarding this manuscript.

### *Statement of ethical approval*

Ethical approval was obtained from the relevant ethics committees with their respective approval numbers; the Federal Medical Centre, Yenagoa with Application form number: FMCY/REC/ECC/2026/FEB/0708 and Protocol No:1127; Bayelsa State Ministry of Health with Approval Number BSHREC/Vol. 1/25/07/003; and Madonna University Nigeria with Application number MUN/VC/REC/MUN/PG/MLS/Ph.D/23/004 Approved as R1.

### Statement of informed consent

Written informed consent was obtained from participants or their parents or legal guardians as applicable. Assent was obtained from minors where required by the approved protocol. Participant confidentiality was maintained and samples were used only for the purposes approved by the ethics committees.

### Contribution to knowledge

This study provides data on whole-blood miRNA-451, plasma VCAM-1, plasma soluble P-selectin, CD4 and CD36 among HbSS, HbAS and HbAA subjects in Bayelsa State.

HbSS participants had higher miRNA-451, VCAM-1, soluble P-selectin and CD36 values than the comparison groups, while CD4 counts were similar.

The study also shows that strong correlations observed in the combined sample were absent within genotype groups, highlighting the importance of genotype stratification when interpreting pooled associations.

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