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Soluble P- and E-selectin levels as determinants of vaso-occlusive crises among sickle cell anemia patients in a tertiary hospital Northwestern Nigeria

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Abstract:

BACKGROUND: Vascular E- and P-selectins play important roles in sickle cell anemia (SCA), vaso-occlusive crises (VOC); however, the extent to which they determine VOC has not been studied.

OBJECTIVES: The aims of the study were to assess vascular selectin levels and the extent to which they determine VOC among SCA patients.

SUBJECTS AND METHODS: This was a cross-sectional comparative study conducted among patients with SCA in a tertiary hospital, Northwestern Nigeria. Eighty-eight participants were enrolled (44 each in VOC and steady state). Soluble E (sE) and P (sP) selectin levels were assessed using enzyme-linked immunosorbent assay technique. The frequency of blood transfusion and bone pains was collated.

RESULTS: Patients with VOC compared to those in steady state had higher levels of sP-selectin (5.5 ± 4.6 ng/ml vs. 3.2 ± 0.8 ng/ml, $P = 0.001$) and mean rank sE-selectin (53.2 vs. 35.8, $P = 0.001$). The odds ratio (OR) for sE-selectin levels and VOC was 1.135 ($P = 0.009$), while that of sP-selectin was 2.693 ($P = 0.002$). The adjusted ORs for sE-selectin and sP-selectin were 1.184 (95% confidence interval [CI]: 1.021, 1.373) and 3.748 (95% CI: 1.475, 9.524), respectively.

CONCLUSIONS: Patients in VOC have elevated sP-and E-selectin levels. sP-selectin level is a better predictor of VOC compared to sE-selectin.

Keywords:

Crises, sickle cell, vascular selectins

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Introduction

The understanding of the pathophysiological bases of vaso-occlusive crises (VOCs) has evolved over time. Initially thought of as a primary red blood cell disorder, studies have demonstrated multicellular, multifaceted, and multilevel interactions that lead to VOCs as well as complications in sickle cell anemia (SCA).[1,2] These involve white blood cells, platelets, vascular endothelium, and adhesion molecules, among others.[3]

The selectin family is an important class of adhesion molecules involved in the pathogenesis of SCA. These adhesion molecules are lectins situated on white blood cells, platelets, and endothelial cells: referred to as the L-, P-and E-selectins.[4] They are calcium-dependent adhesion molecules and are upregulated by diverse inflammatory stimuli.[5] The P- and E-selectins referred to as the vascular selectins[4] have been demonstrated to be elevated during VOCs compared to steady states.[6]

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However, the extent to which these selectins may, individually and collectively, determine whether an individual is in VOC or not has not been evaluated. This may be important because of the ongoing quest for a biomarker for VOCs. Hence, this study aimed to assess levels of vascular selectins and their relationships with clinical states (VOC and steady state) with a view to understanding how vascular selectins determine VOCs among SCA patients attending a tertiary health facility in Northwestern Nigeria.

Subjects and Methods

Study design and sampling technique

This was a comparative cross-sectional study in which two groups of SCA patients were enrolled; in VOC and steady state. Participants in the VOC study arm were enrolled as they presented consecutively. Age (age ± 2 years) and gender matching were utilized in enrolling those in steady state as the comparative arm.

Sample size determination

Power estimation for sample size was done using the sample size for the regression analysis feature of the Windows version of the Program for Epidemiologists (WINPEPI v11.39).[7] It was estimated that using four predictors, a minimum of 82 participants (41 per arm) will be required to have at least an 80% power at a significance level of 0.05 of detecting at least a medium effect size (R^2 of 0.13) in the variance of the clinical state (VOC versus steady state). However, this minimum sample size was inflated by 10% resulting in a final sample size of 91. Eventually, 88 participants (44 per arm) were enrolled for the study.

Ethical considerations

Ethical clearance was obtained from the Institutional Health and Research Ethics Committee of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria.

Study population

All patients with SCA aged ≥ 18 years who presented to the Hematology and Blood Transfusion Department of ABUTH Zaria, Kaduna State, Nigeria, were enrolled in the study after granting informed consent.

Inclusion and exclusion criteria

The absence of blood transfusions, painful crises, and fever for at least 3 months, 4 weeks, and 2 weeks, respectively, was considered indicative of being in steady state.[8] Patients taking hydroxyurea and anticoagulants were excluded from the study.

Data collected

Data on age, gender, and frequency of bone pains and blood transfusions in the previous 12 months were taken as indices of disease severity in the previous 12 months for all participants. In addition, a history of prehospital intake of analgesics for the VOC arm was collated.

Laboratory tests

Sandwich enzyme-linked immunosorbent assay (ELISA) for soluble E (sE) (Human E-Selectin ELISA Kit, CAT. NO.: EKHU-0176, Melsin Medical Co., Limited China) and soluble P (sP) (Human sP-selectin ELISA Kit, CAT NO; SL1618Hu, Sunlong Biotech Co., China) selectins was conducted on sera obtained from venous blood samples of all participants.

Statistical analysis

Data were analyzed using SPSS version 23 (IBM Corp, Armonk, NY, USA). Qualitative variables were summarized as frequencies and proportions. The distribution of all continuous variables was assessed using the Shapiro–Wilk test and these were reported as mean ± standard deviation or median and interquartile ranges (IQR) for normal and nonnormally distributed variables, respectively. Independent samples *t*-test or Mann–Whitney U (MWU)-tests were computed as appropriate. Simple binary logistic regression analyses were conducted with sE-selectin and sP-selectin levels as individual predictors, while the clinical state (VOC or steady state) was treated as the outcome variable. Thereafter, multiple logistic regression was done to adjust for the frequency of VOC and blood transfusions. The level of statistical significance was set at $P \leq 0.05$.

Results

The median (IQR) ages of the VOC and asymptomatic steady-state arms were 22.0 (19.0–24.0) years and 22 (19.8–24.0) years, respectively. Females constituted majority of each arm 26/44 (59.1%). A summary of education, occupation, and disease severity in previous 12 months is provided in Table 1.

Most patients 31/44 (70.5%) with VOC had taken analgesics at home before presenting to the clinic; a summary of the analgesics is provided in Figure 1. Patients with VOC had higher levels of sP-selectin and sE-selectin compared to those in steady state (5.5 ± 4.6 ng/ml vs. 3.2 ± 0.8 ng/ml, $t = 3.312$, $P = 0.001$) and mean rank sE-selectin (53.2 vs. 35.8, MWU = 584.500, $P = 0.001$), respectively. All binary logistic regression models were statistically significant. The variance in the outcome (VOC, steady state) explained by each model is summarized in Table 2.

Taken individually, binary logistic regression analyses revealed that sE-selectin and sP-selectin could correctly classify 59.1% and 72.7% of clinical states (VOC versus steady state), respectively. For each unit increase in sE-selectin the odds of a participant being in VOC rather than steady state was 1.135 (95% confidence interval [CI]-1.032, 1.248; P = 0.009). However, each unit increase in sP-selectin demonstrated a higher OR of 2.693 (95% CI: 1.433, 5.063; P = 0.002) of a participant being in VOC. A multiple logistic regression model with sE selectin, sP selectin, number of VOCs, and blood transfusions in the last 12 months as predictors correctly classified 72.0% of all clinical states with adjusted OR for sE-selectin of 1.184 (95% CI: 1.021, 1.373; P = 0.026) and sP-selectin of 3.748 (95% CI: 1.475, 9.524; P = 0.005).

Discussion

The higher sE-and P-selectin levels during VOC compared to asymptomatic steady state among patients with SCA in this study are similar to what Al Najjar *et al*.[6] reported among SCA patients in Saudi Arabia. However, the magnitude of increase differs with our findings being relatively lower than reported. This could be due to the prehospital intake of nonsteroidal analgesic drugs among the VOC arm in our study. Nonsteroidal anti-inflammatory agents have the potential to dampen inflammatory response,[9,10] which is an important driver of selectin expression and upregulation.[11]

In addition, the difference in the magnitude of vascular selectin levels in this study compared to those reported by Al Najjar *et al*. may be due to population-specific genetic variations responsible for the expression of these vascular selectins. Differences in sP selectin concentrations among selected Chinese ethnicities have been linked to polymorphisms involving the rs1800807 and rs1800808 genotypes.[12] In addition, the single-nucleotide polymorphisms, rs644234[13,14] and rs651007,[15] have been demonstrated to be related to increased sE-selectin levels.

This study also demonstrates that higher sP selectin levels are more likely than sE selectin levels to determine VOC in patients with SCA. This may be because sP selectin has a dual source, as it is expressed by activated vascular endothelial cells and platelets.[4] The presence of activated platelets and endothelial cells has been well documented in sickle cell disease.[16-18] This is in contrast to sE selectin which is solely found on the endothelium.[4] This may also explain the success of the P selectin inhibitor (Crizanlizumab®) in clinical trials for SCA patients.[19,20] Crizanlizumab has been approved by the Food and Drug Administration for VOC prophylaxis.[19] Despite this relative success of P-selectin, it can be deduced from this study that there are other factors which account for VOC among SCA that cannot be explained by this study. These unexplained factors are consistent with the concept that VOCs are caused by several factors.[21,22] A limitation of this study is that it was conducted among a small group of SCA patients. Hence, it will be important to determine population-specific clinical decision limits of sP-selectin levels for the prediction of VOC in future studies. These may serve as monitoring tools during routine follow-up

Table 1: Educational, occupational status and disease severity of participants

	Arm of study		Total
	VOC	Steady state	
Education			
Primary	0	11	11
Secondary	21	14	35
Tertiary	23	19	42
Total	44	44	88
Occupation			
Professional/managerial/technical	4	0	4
Sales/services	1	8	9
Students	37	33	70
House wives	2	1	3
Unemployed	0	2	2
Total	44	44	88
Disease severity in previous 12 months, median (IQR)			
Number of blood transfusions	0 (0-1)	0 (0-1)	
Number of VOC	3 (1-5)	2 (1-4)	

VOC=Vaso-occlusive crises, IQR=Interquartile range

Table 2: Properties of binary logistic models for outcome variable (vaso-occlusive crises versus steady state)

Predictor(s)	χ^2 - statistic	df	P	Nagelkerke R^2
sE-selectin levels alone	15.551	1	<0.001	0.216
sP-selectin levels alone	22.039	1	<0.001	0.295
sE-, sP- selectin levels, blood transfusion and VOC in previous twelve months	36.709	4	<0.001	0.482

df=Degree of freedom, sE=Soluble E, sP=Soluble P, VOC=Vaso-occlusive crises

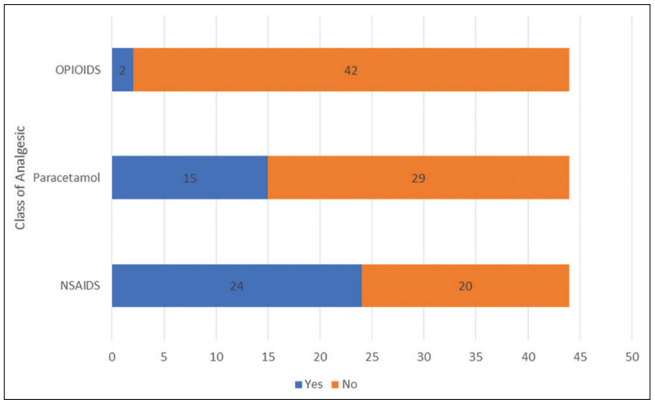

Figure 1: Type of analgesics taken before presentation

and possibly for monitoring therapy with crizanlizumab in conjunction with clinical parameters. Efforts along these lines have been successful with the microfluidic assay for sickle cell disease (SCD) biochip.[23]

Conclusion

The current study concluded that patients in VOC have elevated sP-selectin and E-selectin levels, and sP-selectin levels are better determinants of VOC compared to sE-selectin, so it is recommended that future studies consider assessing sP-selectin as a marker of disease severity.

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Conflicts of interest

There are no conflicts of interest.

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