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# P-selectin level in haemoglobin-S variants steady-state subjects in Port Harcourt Nigeria

## Abstract

**Introduction**: P-selectin is a cell adhesion molecule that play key roles in vaso-occlusive crisis, atherosclerosis, inflammation and is highly implicated in sickle cell diseases. This cross-sectional study determines the level of P-selectin in haemoglobin-S variants steady state subjects in Port Harourt Nigeria.

**Materials and Methods**: Eighty (80) subjects comprising of haemoglobin-S variants in steady-state and apparently healthy haemoglobin A (HbAA) male and female aged within 5-60years were recruited for the study. Five millilitres (5mls) of venous blood were aseptically collected by venepuncture from each participant into plain tube and analysed using Elabsceince ELISA kits. Data obtained were analysed using SPSS version 24, and results considered to be significant at $p<0.05$.

**Results:** P-selectin levels were all within detectable normal reference ranges with highest value observed in HbSS subjects (10.98±2.72ng/ml) followed by HbSC subjects (9.58±3.51ng/ml), HbAA subjects (9.42±3.17ng/ml) and lowest value in HbAS subjects (8.01±3.19ng/ml) respectively ($p=0.037$). Comparison of P-selectin level based on gender showed no statistically significant difference across the various groups ($p>0.05$).

**Conclusion:** The study revealed a higher P-selectin level in HbSS and HbSC subjects although all within normal detectable reference ranges. It further demonstrated that gender has no effect on the expression of P-selectin in haemoglobin-S variant steady state subjects and normal haemoglobin A (HbAA). Measurement of P-selectin level in sickle cell diesease is recommended as an indirect way of investigating and monitoring red cell haemolysis, platelet aggregation and degree of inflammation in haemoglobin-S variants subjects.

**Keywords**: p-selectin, cell adhesion, haemoglobin-s variants, steady-state, sickle cell disease.

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## Introduction

P-selectin (CD62P) is a single chain glycoprotein adhesion molecule with molecular weight of 140kDa rapidly and chronically expressed on the surface of endothelial cells lining and blood vessels (specifically activated platelets) with a concentration of 100ng/ml$^{-1}$ in plasma of normal individuals.[1,2] These molecule have been found to specifically function in mediating the initial capture, rolling and recruitment of white blood cells on activated vascular endothelium and the interaction between activated platelets and leukocytes triggered by molecules such as histamine, thrombin.[2–4] P-selectin is also known to play important role in atherosclerosis and are found to circulate in plasma in a soluble form (sP-selectin) inducing procoagulant microparticle formation and causing vascular occlusive tendencies. Although on the molecular level, this vaso-occlusive teendencies can be initiated due hypoxia-induced polymerization of haemoglobin SS (HbSS) capable of causing membrane damage resulting in decrease red blood cell survival in peripheral circulation through intravascular haemolysis and accelerated phagocytosis in the spleen.[4–6] Further membrane damages following this haemolysis and accelerated haemolysis in the spleen results in calcium influx with efflux of potassium and water from sickle red blood cells leading to cell dehydration and sickling and the vaso-occlusive process is recurred.[4,7] Researches have shown that vascular occlusion remains as a major cause of the morbidity associated with subjects having haemoglobin-S variants haempglobin particularly those with the sickle cell (HbSS) variants or sickle cell disease.[8–10] The tendency of sickle red blood cells to adhere to the vascular endothelium is believed to be a major contributor and possibly primary cause of the vaso-occlusive process in this set of patients.[9,10] Other researches by Bennewitz et al.[11] and Vats et al.[10] further demonstrated that chronic P-selectin deficiency attenuates liver ischemia but fails to prevent hepatobiliary injury. Most recently, P-selectin antibody therapy has been approved by the US Food and Drug Administration for the prevention of vaso-occlusive pain episode (VOE) in patients with sickle cell disease[10,12] and has thus become a potential new target for pharmacological therapy to combat the complications of sickle cell disease. Haemaglobin-S variants constitute the mutant or abnormal form of haemoglobin-S in a population caused by genetic variations that changes the sequences and number of nucleotides within the globin gene/chain. They include the following: haemoglobin AS (HbAS), haemoglobin SC (HbSC), and haemoglobin SS (HbSS) and are responsible for the occurrence of blood disorders such as sickle cell disease, sickle cell anaemia and haemolytic anaemias.[13–15]

Although P-selectin as an adhesion molecule has been shown to prevent vaso-occlusive event, causes atherosclerosis in patients with sickle cell disease,[10] there remains paucity of information on the level of P-selectin in subjects with haemoglobin-S variants such as Haemoglobin (AS, SC and SS); thus the understanding of the pathophysiology of vaso-oclusion, management vis a vis clinical diagnosis in haemoglobin-S variant subject remains a complex mechanism. This research is aimed at accessing the level of P-selectin in subject with haemoglobin-S variants in steady-state in Port Harcourt Nigeria.

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## Materials and methods

### Study design and population

This cross-sectional study was conducted amongst eighty (80) subjects with haemoglobin-S variants (HbSS, HbSC and HbAS) in steady-state and apparently healthy haemoglobin A (HbAA) male and female within the ages of 5-60years of age attending tertiary hospitals within Port Harcourt Rivers State Nigeria. The medical history as well as the biodata, socio-demographic and lifestyle of all subject in the study was obtained with the use of a well-structured questionnaire

### Ethical clearance/Informed consent

Formal approval was obtained from the Rivers State Ministry of Health Port Harcourt and verbal and/or written consent obtained from each participant.

### Blood sample collection and processing

Five millilitres (5mls) of venous blood was aseptically collected through venepuncture from each participant into a plain tube. The blood sample was transported to the laboratory at room temperature and stored at $4^0$C prior to analysis using human P-selectin Elabscience ELISA kits.

### Determination of P-selectin level using Elabscience ELISA kit as described by Elabscicnce Biotech Co., Ltd, China

Samples were assayed using Elabscience ELISA kits that utilizes the Sandwich-ELISA as method. The ELISA-micro plates provided with the kit are pre-coated with antibody specific to Human P-selectin and when the standard samples are added to the micro-ELISA plate wells in combination with the specific antibody, and a biotinylated detection antibody specific for Human P-selectin and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each microplate well successively and incubated; then followed by the washing away of free components, and the addition of thr substrate solution that causes the Avidin-HRP conjugate to appear blue in colour. The series of enzyme-substrate reaction taking place is stopped by the addition of stop solution with end product resulting in a yellow colouration. The optical density (OD) is then measured spectrophotometrically at a wavelength 450nm. The optical density (OD) value obtained from the reading is directly proportional to the concentration of Human P-selectin in the sample.

### Statistical analysis

Statistical package for social science (SPSS) version 24 was used for data analysis. Descriptive statistical tools such as mean and standard deviation (SD) was used. Analysis of variance (ANOVA) was used to compare means of more than two groups for inferential evaluation, with Turkey's multiple comparison test to check for mean difference between multiple groups. Student t-test was used for comparison of means.

## Results

### Social demographic data of studied population

A total of eighty (80) subjects consisting of 37 (46.25%) females and 43 (53.75%) males of which 20 (25%) haemoglobin AA (HbAA) (nine (9) males and eleven (11) females); 20 (25%) Haemoglobin AS (HbAS) (five (5) males and fifteen (15) females); 20 (25) Haemoglobin SS (HbSS) (ten (10) male and females each), and 20 (25%) haemoglobin SC (HbSC) (thirteen (13) males and seven (7) females) all age between 5-60years of age as shown in Table 1.\

**Table 1** Social demographic data of studied population

| Study | Parameters | | | | | |
|---|---|---|---|---|---|---|
| **Groups** | **HbAA** | **HbAS** | **HbSS** | **HbSC** | **Number(n)** | **Frequency (%)** |
| **Age (years)** | | | | | | |
| 15-19 | - | 1 | 9 | 16 | 26 | 32.5 |
| 20-34 | 15 | 17 | 11 | 4 | 47 | 58.75 |
| 35-49 | 4 | 1 | - | - | 5 | 6.25 |
| 50-64 | 1 | 1 | - | - | 2 | 2.5 |
| Total | 20 | 20 | 20 | 20 | 80 | 100 |
| **Gender** | | | | | | |
| Male | 9 | 5 | 10 | 13 | 37 | 46.25 |
| Female | 11 | 15 | 10 | 7 | 43 | 53.75 |
| Total | 20 | 20 | 20 | 20 | 80 | 100 |

**Key:** HbAA, haemoglobin genotype AA; HbAS, haemoglobin genotype AS; HbSS, haemoglobin genotype SS; HbSC, Haemoglobin genotype SC; n, number of subjects.

### Comparative results of P-selectin levels in study population

Mean±SD values of P-selectin were all within detectable normal reference ranges with significantly higher values observed in HbSS subjects (10.98±2.72ng/ml) followed by HbSC subjects (9.58±3.51ng/ml), HbAA subjects (9.42±3.17ng/ml) and lowest value in HbAS subjects (8.01±3.19ng/ml) respectively (p=0.037) as shown in Table 2.

**Table 2** Comparative results of P-selectin levels in study population

| Parameters | HbAA (n=20) | HbAS (n=20) | HbSS (n=20) | HbSC (n=20) | P-value | F-value | Remarks |
|---|---|---|---|---|---|---|---|
| P-selectin (ng/ml) | 9.42±3.17 a | 8.01±3.19 b | 10.98±2.72 c | 9.58±3.51 a | 0.037 | 2.962 | S |

**Key:** Values in the same row with different superscripts (a, b, c) differ significantly when compared at p<0.05. S, Significant; NS, Not Significant; HbAA, haemoglobin genotype AA; HbAS, haemoglobin genotype AS; HbSS, haemoglobin genotype SS; HbSC, Haemoglobin genotype SC

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### Comparative results of P-selectin levels in studied population base on gender

A comparison of P-selectin levels based on gender in study population in haemoglobin A (HbAA) variants shows P-selectin level as 9.97±2.68ng/ml in males and 8.98±3.59ng/ml in females (p=0.494). Haemoglobin AS (HbAS) variants shows P-selectin level as 7.41±3.41ng/ml in males and 8.21±3.21ng/ml in females (p=0.641). Haemoglobin S (HbSS) variants shows P-selectin level as 10.07±1.26ng/ml in males and 11.89±3.49ng/ml in females (p=0.140) while Haemoglobin SC (HbSC) variants shows P-selectin level as 9.95±3.75ng/ml in males and 8.91±3.16ng/ml in females (p=0.541) as shown in Table 3.

**Table 3** Comparative results of P-selectin levels in studied population base on gender

| Haemoglobin variants | P selectin (ng/ml) | P value | Remark |
|---|---|---|---|
| **HbAA** | | | |
| Male (n=11) | 9.97±2.68 | 0.494 | NS |
| Female (n=9) | 8.98±3.59 | | |
| **HbAS** | | | |
| Male (n=5) | 7.41±3.41 | 0.641 | NS |
| Female (n=15 | 8.21±3.21 | | |
| **HbSS** | | | |
| Male (n=10) | 10.07±1.26 | 0.14 | NS |
| Female (n=10) | 11.89±3.49 | | |
| **HbSC** | | | |
| Male (n=13) | 9.95±3.75 | 0.541 | NS |
| Female (n=7) | 8.91±3.16 | | |

**Key:** S=Significant when compared at p<0.05, NS= Not Significant when compared at p<0.05. HbAA, haemoglobin genotype AA; HbAS, haemoglobin genotype AS; HbSS, haemoglobin genotype SS; HbSC, haemoglobin genotype SC

## Discussion

Results of P-selectin in this study were all within detectable normal reference ranges with highest value observed in HbSS subjects, followed by HbSC subjects, HbAA subjects and the lowest value in HbAS subjects respectively (p=0.037). This implies that subjects with haemoglobin SS (HbSS) variant in steady state have high expression of P-selectin when compared to subjects with haemoglobin SC (HbSC), haemoglobin AA (HbAA) and haemoglobin AS (HbAS) respectively. Also, A comparsion of the mean values of haemoglobin AA (HbAA) and haemoglobin SC (HbSC) shows no statistically significant difference (p>0.05) implying that both HbAA and HbSC subjects have similar pattern for the expression of P-selectin. The higher values of P-selectin in the HbSS group in this study will not be unconnected with the fact that subjects with HbSS variants of haemoglobin are easily prone to red cell destruction due to the shortened life-span as a result of their deformed sickled shaped. The constant destruction (haemolysis) and rupturing of these red blood cell and release of fragments such as free haemoglobin, heme and reactive oxygen species which can together with leukocytes and platelets activate the vascular endothelium causing the obstruction of blood vessels and triggering inflammation and vaso-occlusive crisis that is capable of activating the release of P-selectin and thus the high P-selectin values in haemoglobin SS (HbSS) subjects in this study. Similarly, the higher values in P-selectin recorded in the HbSS group in this study could be attributed to fact that all subjects were in steady state and not on any Nonsteroidal anti-inflammatory agents that have the potential to dampen inflammatory response and/or selectin expression/upregulation.

Finding of this research are at deviant in terms of the values gotten from the findings of Uche et al.[16] who reported higher values of 14.19ng/ml for P-selectin in sickle cell anaemia (HbSS) subjects during crisis and lower values of 6.32ng/ml in normal haemoglobin AA (HbAA) subjects in their study carried out on sickle cell anaemia subjects in Lagos State. They concluded that serum P-selectin are significantly higher in HbSS subjects compared with HbAA controls. P-selectin values in this study is also higher than the values reported by Awwalu et al.[17] in subjects with sickle cell anaemia with vaso-occlusive crisis and in steady state. They reported 5.5 ng/ml for subjects with vaso occlusive crisis and 3.2 ng/ml in steady state subjects and attributed their findings to be due to the the prehospital intake of nonsteroidal analgesic drugs among the VOC subjects in the study. Research by Gunaydin and Bilge[18] and Osafo et al.[19] shows that Nonsteroidal anti-inflammatory agents have the potential to dampen inflammatory response, which is an important driver of selectin expression and upregulation.[20]

Comparison of P-selectin values in HbSS subjects with HbAS subjects shows that HbSS subjects have higher P-selectin values than HbAS subjects implying that P-selectin is more express in subjects with haemoglobin S (HbSS) variants than Haemoglobin AS (HbAS). Furthermore, there was no significant difference in the values of P-selectin between the HbAA and HbSC although higher values were seen in HbSC group (p>0.05) implying that both HbAA and HbSC subjects could possibly have similar pattern for the activation, expression and regulation of P-selectin levels in the blood. Comparison of P-selectin in the study show that male and female have similar pathway in response to vascular injury and the recruitmentt of leukocytes in the blood thus the level of P-selectin in both gender follow the same pattern or pathways.

## Conclusion

The study revealed a higher P-selectin level in HbSS and HbSC subjects although all within normal detectable reference ranges. It further demonstrated that gender has no effect on the expression of P-selectin in haemoglobin-S variant steady state subjects and normal haemoglobin A (HbAA). It is therefore recommended that P-selectin therapy be avoided in subjects with HbSS and HbSS genotype in order not to worsen their clinical state especially during crisis.

## Recommendations

Measurement of P-selectin level in sickle cell disease is recommended as an indirect way of investigating and monitoring red cell haemolysis, platelet aggregation and degree of inflammation in haemoglobin-S variants subjects.

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## Conflicts of interest

The author declares that there is no conflict of interest.

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