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# Plasma Soluble Progenitor Cell Receptors as Biomarkers for Severe Anemia Among Malaria-Infected Pediatrics: A Prospective Study in Ghana

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## ABSTRACT

**Background:** Soluble forms of progenitor cell receptors may be implicated in the delayed erythropoietic response during severe anemia. In this study, plasma levels of soluble erythropoietin receptor (sEPO-R) and soluble granulocyte, macrophage-colony stimulating factor receptor (sGM-CSFR) were assessed in *Plasmodium falciparum*-infected children in Ghana.

**Methods:** This case-control study was conducted at Tamale Teaching Hospital, Ghana. One hundred and twenty *P. falciparum*-infected, and 60 uninfected children aged 12–144 months were recruited from April to July, 2023. About 4 mL of blood was collected for malaria microscopy, full blood count using a haematology analyser, and sEPO-R, sGM-CSFR and erythropoietin (EPO) estimation using enzyme-linked immunosorbent assays. Data were analyzed using SPSS version 26.0.

**Results:** Plasma levels of sEPO-R were higher among participants with severe malarial anemia (SMA) than those in the non-SMA and control groups ($p < 0.001$). Plasma sGM-CSFR levels were higher in *P. falciparum*-infected children than in controls, but the levels were similar between the SMA and non-SMA groups. Hemoglobin ($r = -0.823$, $p < 0.001$), RBC ($r = -0.852$, $p < 0.001$), HCT ($r = -0.790$, $p < 0.001$) and platelets ($r = -0.810$, $p < 0.001$) negatively correlated with sEPO-R. There was a strong positive correlation between sEPO-R and EPO in *P. falciparum*-infected children ($r = 0.901$, $p < 0.001$). Plasma sEPO-R better predicted severe anemia among malaria-infected children (cut-off point: 161.5 pg/mL, sensitivity: 96.0%, specificity: 82.9%, AUC: 0.964, $p < 0.001$).

**Conclusion:** *P. falciparum*-infected children had higher plasma levels of sGM-CSFR, sEPO-R and EPO. Plasma sEPO-R correlated negatively with erythrocyte parameters, suggesting a possible contribution of the endogenous receptor to the

development of severe anemia in children with malaria. Further studies to investigate the neutralizing effects of sEPO-R on erythropoietic response during malaria are recommended.

## 1 | Introduction

Despite years of eradication efforts, malaria still contributes greatly to global morbidity and mortality, especially in underdeveloped countries, with children and pregnant women mostly affected [1]. Approximately two-thirds of the 249 million global malaria cases reported by the World Health Organization (WHO) in 2022 suffered from anemia, which contributed significantly to the death of 608,000 patients from the disease. The highest burden of malaria was felt in the WHO African region with 233 million malaria cases, and 580,000 malaria-associated mortalities reported, and approximately 80% of the deaths occurred in children [2]. Ghana is malaria-holoendemic country, ranked among top 15 countries heavily burdened by malaria, and contributes to 2.2% of global malaria morbidities and 2.0% global malaria-related mortalities [2]. The Ghana Statistical Service reported 8.6% prevalence of malaria in 2022, and this makes malaria the major contributor to out-patient department visits, and principal cause of hospitalization especially in children [3].

Severe anemia is common in pediatric malaria [4], and remains the most predominant hematological stress in *Plasmodium falciparum*-infected children in Ghana and other tropical areas where malaria is pervasive [2]. The dyserythropoiesis associated with malaria may result from disruption of resident progenitor cell receptors in the bone marrow into the bloodstream [5–7]. The severe anemia reduces the supply of oxygen to tissues [8], and the resulting hypoxia triggers the interstitial cells of the kidneys to release erythropoietin to facilitate erythropoiesis [8]. Erythropoiesis is effective in the presence of suitable conditions including viable stem and stromal cells, essential nutrients, growth factors/signaling molecules such as erythropoietin, interleukins (IL-3, IL-12 and IL-8), and transforming growth factor (TGF)-β, functional bone marrow, and the availability of unique progenitor cell receptors especially erythropoietin receptor (EPO-R) and granulocyte, macrophage-colony stimulating factor receptor (GM-CSFR) [9, 10].

Erythropoietin interacts with resident EPO-R in the bone marrow through the erythroblastic island, which promotes proliferation, differentiation and growth of erythroid progenitor cells, especially burst-forming unit-erythroid (BFU-E), colony-forming unit-erythroid (CFU-E) and pronormoblast, and prevents early apoptosis of the progenitor cells [11, 12]. In addition, in the presence of IL-3, IL-6 and erythropoietin, GM-CSF interacts with its resident receptor GM-CSFR in the bone marrow to induce the differentiation of multipotent haemopoietic stem cells to the myeloid progenitor lineage [13].

Despite the reported increase in plasma levels of erythropoietin during malaria in children [14–16], dyserythropoiesis remains a common occurrence during malaria progression [17]. Resident progenitor cell receptors in the bone marrow may be shed-off and migrate to the blood circulation in soluble forms following unfavorable conditions such as stress, inflammation and high altitude-induced hypoxia [17–19]. Earlier studies discovered alternative spliced mRNA variant of EPO-R in the bone marrow, enabling an increase in the soluble form of the receptor (sEPO-R) in the bloodstream. The soluble erythropoietin receptor lacks the transmembrane domain of the receptor, but has higher affinity for erythropoietin; it binds to erythropoietin in circulation, and prevents the cytokine from migrating to the bone marrow, thus functioning as a potential antagonist to erythropoietin [18–22].

It is unclear whether endogenous progenitor cell receptors (soluble erythropoietin receptor and sGM-CSFR) regulate the erythropoietic response to *P. falciparum* malaria-induced anemia. We hypothesized that the delayed erythropoietic response observed during malaria is associated with elevated plasma levels of sEPO-R and sGM-CSFR. This study assessed plasma levels of soluble progenitor cell receptors, and determined their relationship with severe anemia in malaria. The findings of this study will contribute to the pathophysiology of *P. falciparum* malarial anemia, and possibly direct therapeutic protocols.

## 2 | Materials and Methods

### 2.1 | Study Design and Duration

This was a hospital-based case-control study from April to July, 2023 in Tamale, Ghana.

### 2.2 | Study Site

This study was conducted at the Clinical Laboratory Department of Tamale Teaching Hospital (TTH). Tamale Teaching Hospital serves as a referral facility for the five northern regions of Ghana. Tamale is the capital of Northern region, with a population of about 371,351 and located at latitude 9.3930 N and longitude 0.8235 W. The hospital's digital address is NT-0101-5777 [23]. Malaria is holoendemic in Tamale, and the major cause of illness and death in the area. Malaria remains the principal cause of pediatric outpatient department (OPD) visits and hospitalization at the Tamale Teaching Hospital [3, 24]. Of the 13,125 children who visited the OPD in the Pediatric clinic of TTH in 2023, 1262 representing 9.62% tested positive during malaria microscopy (Hospital Records).

### 2.3 | Study Participants

The study comprised 180 children (120 *P. falciparum* confirmed cases, with 60 controls without malaria), aged from 12 to 144 months. Participants with malaria were recruited from the Clinical Laboratory Department of the Tamale Teaching

Hospital, and the controls were selected from a basic school around the hospital during a general health screening exercise.

## 2.4 | Sample Size

The Kelsey's formula for case-control studies was used to estimate the required sample size for this study [23]:

$$N_{\text{cases-Kelsey}} = \left[\frac{r+1}{r}\right]\frac{P(1-P)\left(Z_{\frac{\alpha}{2}}+Z_{\beta}\right)^2}{(p1-p2)^2}, \text{ and}$$

$$\mathrm{P} = \left[\frac{p1 + (r \quad \mathrm{X} \quad p2)}{r+1}\right]$$

Where "$N_{\text{cases-Kelsey}}$" is the required sample size for the cases.

$Z_{\frac{\alpha}{2}}$ represents the critical value of the normal dispersion at $\alpha/2$, at a confidence level of 95% = 1.96.

$Z_{\beta}$ represents the critical value of the normal distribution at $\beta$ (this study used a power of 80%, "$\beta$" is 0.2 and the critical value is 0.84).

$p1$ represents the prevalence of malaria-related anemia in children in Ghana, 76% [25].

$p2$ represents the prevalence of anemia among children without malaria in Ghana, 33.3% [26].

$p1-p2$ is the smallest difference in proportions that is clinically important.

"$r$" is the ratio of the *P. falciparum* malaria group to the group without malaria, which is 2:1 in this study.

The minimum number of participants with malaria-related anemia required for this study was 44, with the corresponding number of participants without malaria at 22.

However, to increase the statistical power, this study recruited a total of 180 participants (50 participants with severe malarial anemia [SMA], 70 with non-SMA, and 60 controls without malaria).

## 2.5 | Inclusion and Exclusion Criteria

This study included patients who were referred to the Clinical Laboratory Department and had malaria confirmed through microscopy during the study period, and whose parents/guardians gave their consents. Also, apparently healthy children without malaria nor anemia were selected from a nearby basic school after their parents/guardians had given consents.

Participants taking anti-malarial drugs, who were hospitalized, comorbid with any other disease that could induce inflammation or affect hematological indices, in coma or with cerebral malaria, with sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, helminth infections, and those whose consents were withheld by parents/guardians were excluded from the study.

## 2.6 | Specimen Collection and Processing

Approximately 4 mL of venous blood was collected aseptically and dispensed into dipotassium ethylenediaminetetraacetic acid ($K_2$EDTA) test tubes at the Clinical Laboratory before commencement of treatment. Whole blood was used for full blood count (FBC) measurements and blood film for malaria parasites. The samples were centrifuged, and the plasma obtained was stored in Eppendorf tubes at −20°C until erythropoietin, sEPO-R and sGM-CSFR analysis. The tubes were code-labeled to ensure the anonymity of participants. Venous blood specimen was collected by experienced Medical Laboratory Scientists who were licensed by Allied Health Professions Council, Ghana.

## 2.7 | Laboratory Investigations

About 6 and 2 μL of whole blood were used to prepare thick and thin films respectively; stained with 10% Giemsa working solution and examined under light microscope (Olympus, CX21, Japan) by two independent Microscopists. Parasite density for each participant was evaluated using the formula below as described by the WHO [27]:

$$\text{Parasites per } \mu\text{L of blood} = \frac{\text{Parasite counted x absolute WBC}}{\geq 200\text{WBCs}}$$

The presence and speciation of *P. falciparum* was confirmed when trophozoites appeared ringed and comma-shaped, usually occupied about 1/3rd to 1/5th the diameter of the parasitized RBC, with fine and regular cytoplasm, marginal or accolè forms seen, double chromatin dots, schizonts containing about 12–30 merozoites, and may contain Maurer's dots [23]. The participants were categorized into children under 59 months and ≥ 60–144 months, based on malaria reporting guidelines recommended by the National Malaria Control Program, Ghana Health Service, Ghana [28]. Immediately venous sample was taken, the FBC was measured using an automated haematology analyzer (URIT-5250, China). After the URIT-5250 haematology analyzer had undergone successful self-check and automatic start-up with background results showing blood cell counts of zero, quality control analysis was carried out daily on normal control blood samples for red cells, white cells and platelets to demonstrate that the machine was performing within the expected ranges of the quality control sample before any test done on participants' samples. Blood films for malaria parasites and FBC were performed on the same day of sample collection at the Haematology Laboratory of the TTH, Ghana. *P. falciparum*-infected children with hemoglobin (Hb) less than 5.0 g/dL were classified into the severe malarial anemia group, and participants with *P. falciparum* in peripheral blood, but Hb ≥ 5.0 g/dL belonged to the non-SMA group [27]. Soluble progenitor cell receptors (sEPO-R and sGM-CSFR) and erythropoietin were measured from the −20°C stored plasma using a sandwich enzyme-linked immunosorbent assay (ELISA)

(Poweam, China) at the University for Development Studies Laboratory. The assay range for sEPO-R, sGM-CSFR and erythropoietin ELISA were 15–900 pg/mL, 5–120 pg/mL, and 1–95 IU/L, respectively. All procedures were performed according to protocols recommended by the manufacturers.

### 2.8 | Data Analysis

Data were analyzed using IBM SPSS software version 26.0 (Armonk, NY, USA). After the normality test, normally distributed data were presented as mean ± standard deviation (SD), and skewed data were presented as median (interquartile ranges). One-way ANOVA or Kruskal–Wallis test was used to compare continuous data among participants in the SMA, non-SMA, and control groups. Post-hoc analysis to compare continuous data within multivariate variables was done using Tukey test (for parametric data) or Kruskal–Wallis pairwise comparisons (for non-parametric test). Spearman correlation test was used to determine the correlation between two numerical data. Receiver operating curve (ROC) analysis indicating the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), was used to determine the predictive abilities of the soluble progenitor cell receptors for severe malarial anemia. Statistical significance was set at $p < 0.05$.

### 2.9 | Ethical Consideration and Informed Consent

This study was performed in accordance with the Helsinki Declaration on research involving humans. The Institutional Review Board of the University for Development Studies, Ghana approved this case-control study (UDS/IRB/15/2023). Permission was attained from the managers of Tamale Teaching Hospital. Because participants were minors (aged 12 to 144 months), informed consent was obtained from their parents or guardians. A written informed consent was obtained from the parents or guardians of the participants by signing or thumb-printing the written informed consent form. Informed consents of the cases were obtained by the researchers at the Clinical Laboratory department. Also, the controls (children in the basic school) took the written consent forms home, and returned it the next day. Those whose parents/guardians signed/thumb-printed the consent forms were selected as controls in the study.

## 3 | Results

### 3.1 | Demographic and Clinical Characteristics of the Study Participants

A total of 180 children were included; comprising 120 infected with *P. falciparum* and 60 uninfected controls. Seventy participants (38.9%) with confirmed *P. falciparum* and hemoglobin concentration less than 5.0 g/dL were categorized into the severe malarial anemia group. Participants with non-severe malarial anemia had Hb ≥ 5.0 g/dL in addition to the presence of the asexual forms of *P. falciparum*. The median ages of the cases and controls were similar (24.0 [12.0–81.0] vs. 36.0 [12.0–84.0] months, $p = 0.800$]. Males (106/58.9%) and children aged less than 60 months (114/63.3%) were predominant in the study (Table 1).

### 3.2 | Blood Cell Indices Among the Participants

Participants with SMA had significantly lower RBC $\times 10^{12}$/L [1.5 (1.3–1.6) vs. 3.6 (3.5–3.9) vs. 4.0 (3.9–4.5), $p < 0.001$], Hb g/dL [4.5 (4.0–4.8) vs. 10.6 (9.7–11.1) vs. 11.9 (11.4–12.5), $p < 0.001$], HCT% [13.8 (12.8–15.3) vs. 31.2 (28.6–33.5) vs. 35.6 (34.6–36.8), $p < 0.001$] and platelets $\times 10^9$/L [133.0 (108.0–161.0)

**TABLE 1** | Demographic and clinical characteristics of the study participants.

| | Children aged 12–144 years | | | |
|---|---|---|---|---|
| **Variables** | **Combined ($n = 180$)** | **Participants with malaria ($n = 120$)** | **Controls ($n = 60$)** | ***p*-value** |
| Age category (months) | | | | 0.512 |
| 12–59 | 114 (63.3) | 74 (61.7) | 40 (66.7) | |
| 60–144 | 66 (36.7) | 46 (38.3) | 20 (33.3) | |
| Sex | | | | 0.391 |
| Males | 106 (58.9) | 68 (56.7) | 38 (63.6) | |
| Females | 74 (41.1) | 52 (43.3) | 22 (36.7) | |
| Malaria parasites | | | | |
| Present | — | 120 (66.7) | — | — |
| Absent | — | 60 (33.3) | — | — |
| Participants type | | | | |
| SMA | — | 70 (38.9) | — | — |
| Non-SMA | — | 50 (27.8) | — | — |
| Controls | — | 60 (33.3) | — | — |

*Note:* n, number of participants; SMA, Severe malarial anemia; Non-SMA, Non-severe malarial anemia. Categorical data are presented in frequencies with corresponding percentages in parentheses. Categorical data were compared using $\chi^2$ test. $p < 0.05$ was considered statistically significant.

vs. 274.5 (152.0–354.0) vs. 358.0 (297.0–386.0), $p < 0.001$] than those in the non-SMA and the control groups. Other red cell indices (MCH, MCHC and RDW-CV), absolute lymphocyte counts, and platelet parameters (PDW and PCT) differed significantly among SMA, non-SMA and control groups ($p < 0.05$). However, there were no significant differences in the total white cell counts, absolute neutrophils, monocytes, eosinophils, basophils, MCV, and MPV ($p > 0.05$) among the study participants (Table 2).

### 3.3 | Plasma Levels of Soluble Erythropoietin Receptor Among the Study Participants

Soluble erythropoietin receptor levels were higher in participants with *P. falciparum* malaria than in the uninfected controls [82.3 (59.0–90.7)]; however, the receptor was elevated in the severe malarial anemia group [402.0 (319.7–435.3)] when compared with those in the non-SMA group [110.5 (100.7–146.0)], and this was statistically significant ($p < 0.001$) (Figure 1).

### 3.4 | Plasma Levels of Soluble Granulocyte, Macrophage-Colony Stimulating Factor Receptor Among the Study Participants

The plasma levels of sGM-CSFR were significantly higher in *P. falciparum*-infected children than those in the control group: SMA (ng/L) [54.6 (42.0–73.0)]; non-SMA (ng/L) [53.0 (39.8–67.3)] and controls (ng/L) [34.0 (28.9–40.7)], $p < 0.001$. However, plasma levels of sGM-CSFR were comparable between participants with severe and non-severe malarial anemia ($p = 0.513$) (Figure 2).

### 3.5 | Correlation Between Erythroid Progenitor Cell Receptors and Blood Cell Parameters of *P. falciparum*-Infected Participants

There was a strong negative correlation between plasma soluble erythropoietin receptor, and selected blood cell parameters (RBC, Hb, HCT, and platelets). Again, red

**TABLE 2** | Blood cell indices among the participants.

| Blood cell indices | Combined ($n = 180$) | Study Participants: Non-SMA ($n = 70$; Hb ≥ 5.0 g/dL)[a] | SMA ($n = 50$; Hb < 5.0 g/dL)[b] | Controls ($n = 60$; No MPs)[c] | *p*-value | Pairwise comparisons |
|---|---|---|---|---|---|---|
| RBC × $10^{12}$/L | 3.6 (1.7–4.0) | 3.6 (3.5–3.9) | 1.5 (1.3–1.6) | 4.0 (3.9–4.5) | < 0.001 | a&b, b&c, a&c |
| Hb (g/dL) | 10.7 (4.9–11.7) | 10.6 (9.7–11.1) | 4.5 (4.0–4.8) | 11.9 (11.4–12.5) | < 0.001 | a&b, b&c, a&c |
| HCT% | 31.7 (16.3–34.9) | 31.2 (28.6–33.5) | 13.8 (12.8–15.3) | 35.6 (34.6–36.8) | < 0.001 | a&b, b&c, a&c |
| MCV (fL) | 77.6 (68.4–81.7) | 79.5 (69.6–81.9) | 75.0 (65.2–79.9) | 77.0 (66.2–83.9) | 0.170 | N/A |
| MCH (pg) | 26.2 ± 4.5 | 27.4 ± 4.4 | 24.9 ± 4.1 | 26.1 ± 4.8 | 0.012 | a&b |
| MCHC (g/dL) | 34.2 ± 3.0 | 35.2 ± 2.9 | 32.8 ± 3.4 | 34.0 ± 2.1 | < 0.001 | a&b, a&c |
| RDW-CV% | 10.0 (8.3–11.7) | 9.4 (8.3–11.0) | 11.4 (10.1–12.8) | 9.0 (8.1–11.5) | 0.001 | a&b, b&c |
| WBC × $10^{9}$/L | 9.2 (6.6–12.9) | 9.4 (7.1–11.4) | 7.6 (5.9–14.5) | 9.9 (7.0–13.9) | 0.445 | N/A |
| Neutrophil% | 51.9 (39.5–65.9) | 55.1 (42.5–68.0) | 57.4 (41.2–67.7) | 44.1 (36.2–63.1) | 0.058 | N/A |
| Lymphocyte% | 33.7 (21.8–48.1) | 31.3 (21.8–45.1) | 26.8 (16.8–39.8) | 41.5 (23.1–53.7) | 0.007 | a&b, b&c, |
| Monocyte% | 7.3 (5.5–11.1) | 7.4 (6.0–11.1) | 7.3 (4.6–11.7) | 7.3 (5.0–9.0) | 0.629 | N/A |
| Eosinophil% | 1.7 (0.7–3.9) | 1.9 (0.7–3.3) | 1.6 (0.6–6.8) | 1.5 (0.7–4.8) | 0.852 | N/A |
| Basophil% | 0.1 (0.1–0.1) | 0.1 (0.1–0.1) | 0.1 (0.1–0.2) | 0.1 (0.1–0.1) | 0.022 | a&b |
| Platelet × $10^{9}$/L | 274.5 (152.0–354.0) | 259.0 (200.5–344.3) | 133.0 (108.0–161.0) | 358.0 (297.0–386.0) | < 0.001 | a&b, b&c, a&c |
| MPV (fL) | 5.8 (5.4–6.5) | 5.8 (5.4–6.4) | 6.0 (5.6–6.7) | 5.8 (5.2–6.3) | 0.057 | N/A |
| PDW% | 7.0 (6.1–8.3) | 6.7 (6.1–8.0) | 8.6 (7.3–9.5) | 6.6 (6.1–7.4) | < 0.001 | a&b, b&c |
| PCT% | 0.2 (0.1–0.3) | 0.2 (0.2–0.3) | 0.1 (0.1–0.2) | 0.2 (0.2–0.4) | < 0.001 | a&b, b&c, a&c |

*Note:* $n$ = Number of participants, a = Represents participants in non-severe malarial anemia group, b = Represents participants in severe malarial anemia group, c = Represents control participants, N/A = Not applicable, SMA = Severe malarial anemia, RBC = Absolute red blood cell count, Hb = Hemoglobin concentration, g/dL = Grams per deciliter, HCT = Hematocrit, MCV = Mean cell volume, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concentration, RDW-CV = Red blood cell distribution width-coefficient of variation, TWBC = Total white blood cell count, MPV = Mean platelet volume, PDW = Platelet Distribution width, PCT = Plateletcrit. Parametric data (MCH and MCHC) presented as mean ± standard deviation were compared using One Way ANOVA, and non-parametric data presented as median (IQR = Interquartile ranges) were compared using Kruskal–Wallis test. $p < 0.05$ was considered statistically significant.

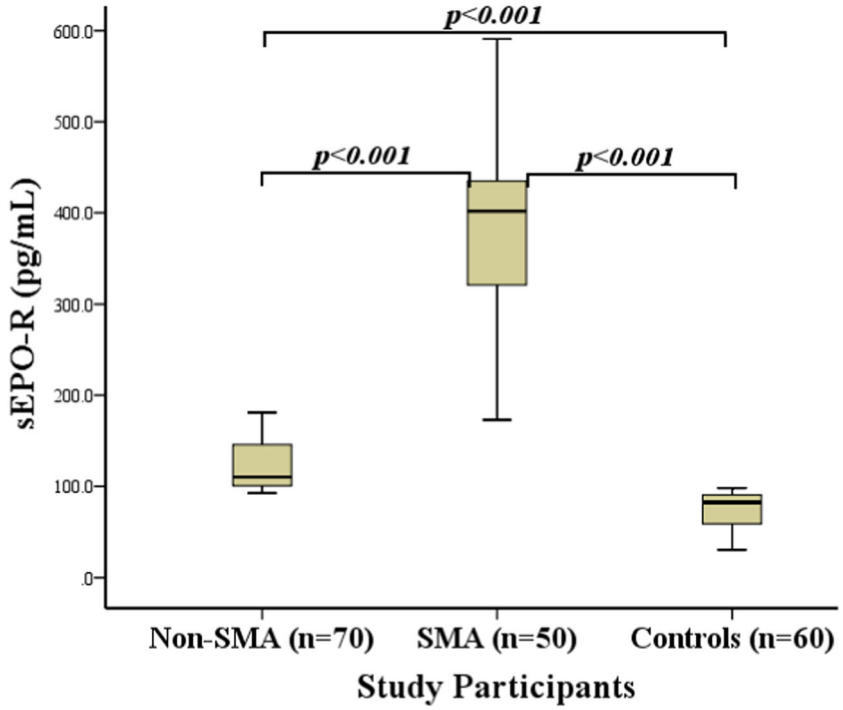


**FIGURE 1** | Plasma levels of soluble erythropoietin receptor among the study participants. n, number of participants; sEPO-R, soluble erythropoietin receptor; SMA, severe malarial anemia; Non-SMA, non-severe malarial anemia; pg/mL,picogram per milliliter. Data were presented as median (IQR = Interquartile ranges). $p < 0.05$ was considered statistically significant.

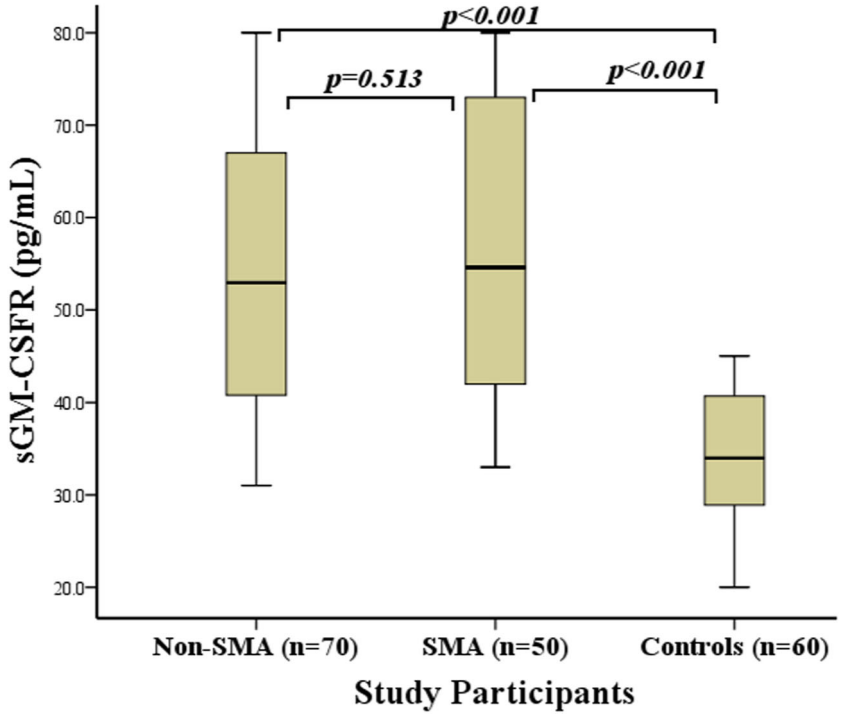


**FIGURE 2** | Plasma levels of soluble granulocyte, macrophage-colony stimulating factor receptor among the study participants. sGM-CSFR, soluble granulocyte, macrophage-colony stimulating factor receptor; SMA, severe malarial anemia; Non-SMA,non-severe malarial anemia; ng/L, nanogram per litre. Data were presented as median (IQR = Interquartile ranges). Statistical significance was set at $p < 0.05$.

cell distribution width, platelet distribution width and basophils, weakly positively correlated with sEPO-R. On the other hand, of all the blood cell parameters, only MCHC (very weak negative correlation), and PDW (very weak positive correlation) were associated with plasma soluble granulocyte, microphage-colony stimulating factor receptor among *P. falciparum*-infected children (Table 3).

**TABLE 3** | Correlation between erythroid progenitor cell receptors and haemogram of *P. falciparum*-infected participants.

| | Progenitor cell receptors | | | |
|---|---|---|---|---|
| | sEPO-R | | sGM-CSF | |
| **Blood cell indices** | ***r*** | ***p*-value** | ***r*** | ***p*-value** |
| RBC $\times 10^{12}$/L | −0.823 | < 0.001** | −0.121 | 0.188 |
| Hemoglobin (g/dL) | −0.852 | < 0.001** | −0.148 | 0.107 |
| Hematocrit% | −0.790 | < 0.001** | −0.139 | 0.129 |
| MCV (fL) | −0.113 | 0.221 | −0.031 | 0.741 |
| MCH (pg) | −0.130 | 0.156 | −0.145 | 0.114 |
| MCHC (g/dL) | −0.249 | 0.006** | −0.190 | 0.038* |
| RDW-CV% | 0.294 | 0.001** | −0.161 | 0.080 |
| WBC $\times 10^{9}$/L | −0.005 | 0.956 | 0.019 | 0.840 |
| Neutrophil% | 0.073 | 0.428 | 0.037 | 0.692 |
| Lymphocyte% | −0.119 | 0.197 | 0.010 | 0.918 |
| Monocyte% | −0.054 | 0.557 | −0.060 | 0.517 |
| Eosinophil% | −0.011 | 0.902 | −0.048 | 0.604 |
| Basophil% | 0.331 | < 0.001** | 0.049 | 0.596 |
| Platelet $\times 10^{9}$/L | −0.810 | < 0.001** | −0.116 | 0.206 |
| MPV (fL) | 0.302 | 0.001** | 0.116 | 0.209 |
| PDW% | 0.576 | < 0.001** | 0.190 | 0.037* |
| Plateletcrit% | −0.464 | < 0.001** | −0.063 | 0.495 |

*Note:* sEPO-R, soluble erythropoietin receptor; sGM-CSFR, soluble granulocyte, macrophage-colony stimulating factor receptor; $r$ = correlation coefficient; RBC = Absolute red blood cell count, g/dL = grams per deciliter; HCT = Hematocrit, MCV = Mean cell volume, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concentration, RDW-CV = Red blood cell distribution width-coefficient of variation, WBC = White blood cell count, MPV = Mean platelet volume, PDW = Platelet Distribution width, PCT = Plateletcrit. Pearson correlation test was used to determine the correlation between progenitor cell receptors and pparametric continuous variables (MCH and MCHC). The correlation between progenitor cell receptors and non-parametric variables (RBC, Hb, HCT, MCV, RDW-CV, WBC, neut%, lymph%, mono%, eos%, baso%, platelet, MPV, PDW and PCT) were determined using Spearman correlation test. Statistical significance was set at $p < 0.05$.
*Correlation was significant at 0.05.
**Correlation was significant at 0.01.

### 3.6 | Correlation Between Erythroid Progenitor Cell Receptors and Erythropoietin of *P. falciparum*-Infected Participants

A strong positive correlation was found between plasma levels of soluble erythropoietin receptor and erythropoietin ($r = 0.901$, $p < 0.001$). However, there was no significant correlation between sGM-CSFR and erythropoietin among *P. falciparum*-infected Ghanaian children ($r = 0.073$, $p = 0.431$) (Figure 3).

### 3.7 | Predictive Values of Progenitor Cell Receptors for Severe Malarial Anemia Among *P. falciparum*-Infected Ghanaian Children

At an optimum cut-off point of sEPO-R at 161.5 pg/mL, sensitivity and specificity were 96.0% and 82.9%, respectively. The positive and negative predictive values were 80.0% and 96.7%, respectively, with a standard error of 0.017. Plasma soluble erythropoietin receptor was associated with severe malarial anemia in Ghanaian children infected with *P. falciparum* [AUC: 0.964 (95% CI: 0.930–0.998), $p < 0.001$] (Figure 4a). However, plasma soluble granulocyte macrophage-colony stimulating factor receptor was not related to severe *P. falciparum* malarial anemia (Cut-off point: 71.4 ng/L, sensitivity: 26.0%, specificity: 84.3%, AUC: 0.537, 95% CI: 0.432–0.643, $p = 0.486$, SE: 0.054, PPV: 54.2%, NPV: 61.5%) (Figure 4b).

## 4 | Discussion

Severe anemia remains the principal complication of *P. falciparum* malaria in children in underdeveloped countries including Ghana [5, 29]. Soluble forms of EPO-R and GM-CSFR in peripheral blood may inhibit the signaling properties of erythropoietin and GM-CSF respectively, and contribute significantly to severe malarial anemia pathogenesis. This study assessed plasma levels of soluble progenitor cell receptors and determined their relationship with severe anemia in children with malaria.

In this study, severe malarial anemic children had lower red cell parameters (Hb, RBC and HCT) than the participants in the non-SMA and control groups. This finding is consistent with earlier findings in Ghana [15, 30–32], Malawi [33], Cameroon [34, 35], Kenya [36–40] and Nigeria [41]. *P. falciparum* malaria-induced anemia results from the associated lyses of infected and uninfected erythrocytes, excessive sequestration of parasitized and non-parasitized erythrocytes in vital tissues, dyserythropoiesis, complement-mediated destruction of erythrocytes, the involvement of inflammatory mediators such as IL-6, IL-1, IFN-ɣ, and TNF-α that downregulate erythropoiesis, and direct

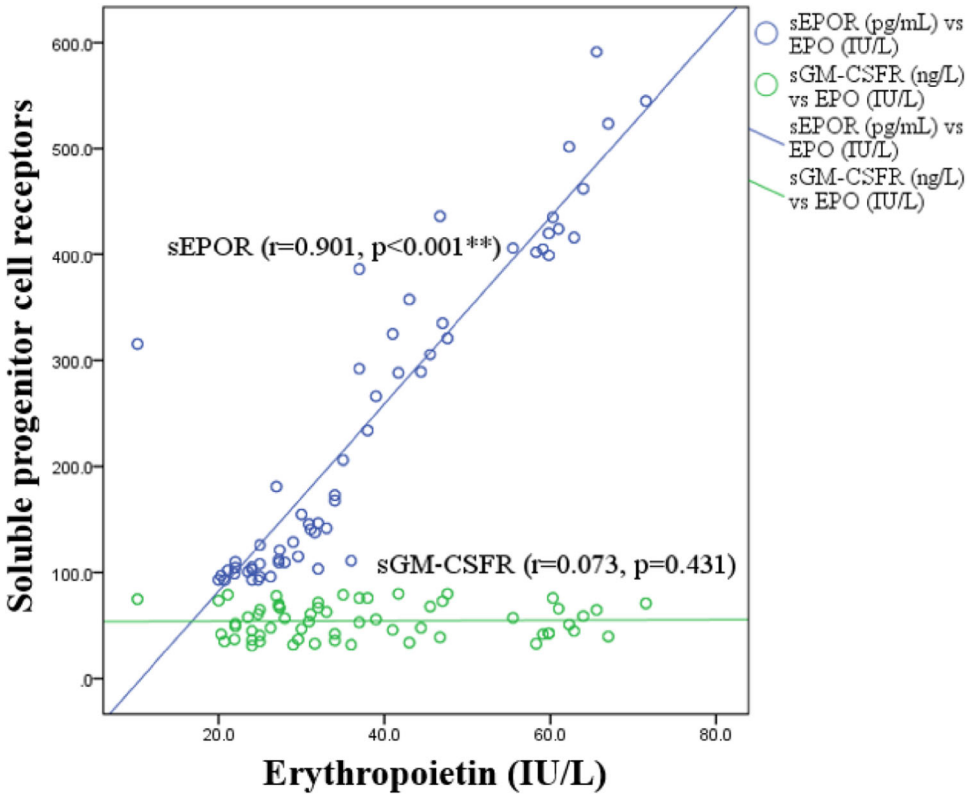


**FIGURE 3** | Correlation between erythroid progenitor cell receptors and erythropoietin of *P. falciparum*-infected participants. **Correlation was significant at 0.01. sEPO-R, soluble erythropoietin receptor; sGM-CSFR, soluble granulocyte, macrophage-colony stimulating factor receptor; EPO = erythropoietin; $r$ = correlation coefficient; IU/L = international unit per litre; pg/mL, picogram per milliliter; ng/L, Nanogram per litre. The correlation between progenitor cell receptors (sEPO-R and sGM-CSFR) and erythropoietin was determined using Spearman correlation test. Statistical significance was set at $p < 0.05$.

interaction with parasites variant surface antigens [5, 6, 23, 42–44]. Previous studies have reported the occurrence of thrombocytopenia in severe *P. falciparum* malaria [26, 45–47]. Thrombocytopenia secondary to malaria is suggested to occur through the splenic sequestration of platelets, ineffective bone marrow haemopoietic activities and the related antibody-mediated destruction of platelets [23, 26, 45–48]. Findings from the current study agree with the earlier findings [26, 45–47].

In the current study, soluble erythropoietin receptor levels were higher in participants with *P. falciparum* malaria than the uninfected controls; and the receptor was elevated in participants with severe malarial anemia when compared with those in the non-SMA group. Resident erythropoietin receptor in the bone marrow is essential during erythropoietin-induced signaling of erythropoiesis. Erythropoietin interacts with the resident EPOR in the bone marrow via the erythroblastic island on the receptor, and this enhances proliferation, differentiation and growth of erythroid progenitor cells, especially BFU-E, CFU-E and pronormoblast, and averts premature apoptosis of the progenitor cells [11, 12]. However, extreme stress, inflammation and hypoxia may induce the shedding of the bone marrow resident erythropoietin receptor into peripheral blood forming a sEPO-R. The soluble erythropoietin receptor has higher affinity for erythropoietin; binds to erythropoietin in the bloodstream forming erythropoietin-soluble erythropoietin complex. The resulting complex prevents erythropoietin's migration to the bone marrow, reducing the proliferation and differentiation of progenitor cells and consequently retards erythropoiesis [18–22]. Previous studies have reported increased endogenous soluble erythropoietin receptor levels in the plasma in disease conditions including kidney failure, resulting in suppressed erythropoiesis despite increase in plasma levels of erythropoietin [19, 20, 49, 50]. During *P. falciparum* malaria, parasitized red cells express surface antigens mostly *Plasmodium falciparum* erythrocyte membrane protein-1 which mediates cytoadherence to endothelium, resulting in enhanced inflammation in sequestrated tissues [4, 7]. Extensive sequestration of *P. falciparum* in the bone marrow may induce a pro-inflammatory environment that could trigger the release of soluble erythropoietin receptor into peripheral blood and subsequently suppress terminal erythroid proliferation and differentiation [7]. This study observed a strong negative correlation between plasma soluble erythropoietin receptor and red cell parameters (Hb, RBC and HCT), and this may suggest negative effects of sEPO-R on erythropoietic response to severe *P. falciparum* malaria in children. The possible binding of erythropoietin to soluble erythropoietin receptor limits erythropoietin to the general circulation and prevents the cytokine's movement to the bone marrow to direct erythropoiesis. The associated dyserythropoiesis will trigger hypoxia-induced expression of erythropoietin from the peritubular interstitial cells of the kidneys, resulting in elevated plasma levels of erythropoietin [8]. This may account for the strong positive correlation observed between plasma soluble erythropoietin receptor and erythropoietin among *P. falciparum*-infected children in this study.

In the present study, plasma levels of soluble GM-CSFR were significantly raised in *P. falciparum*-infected children

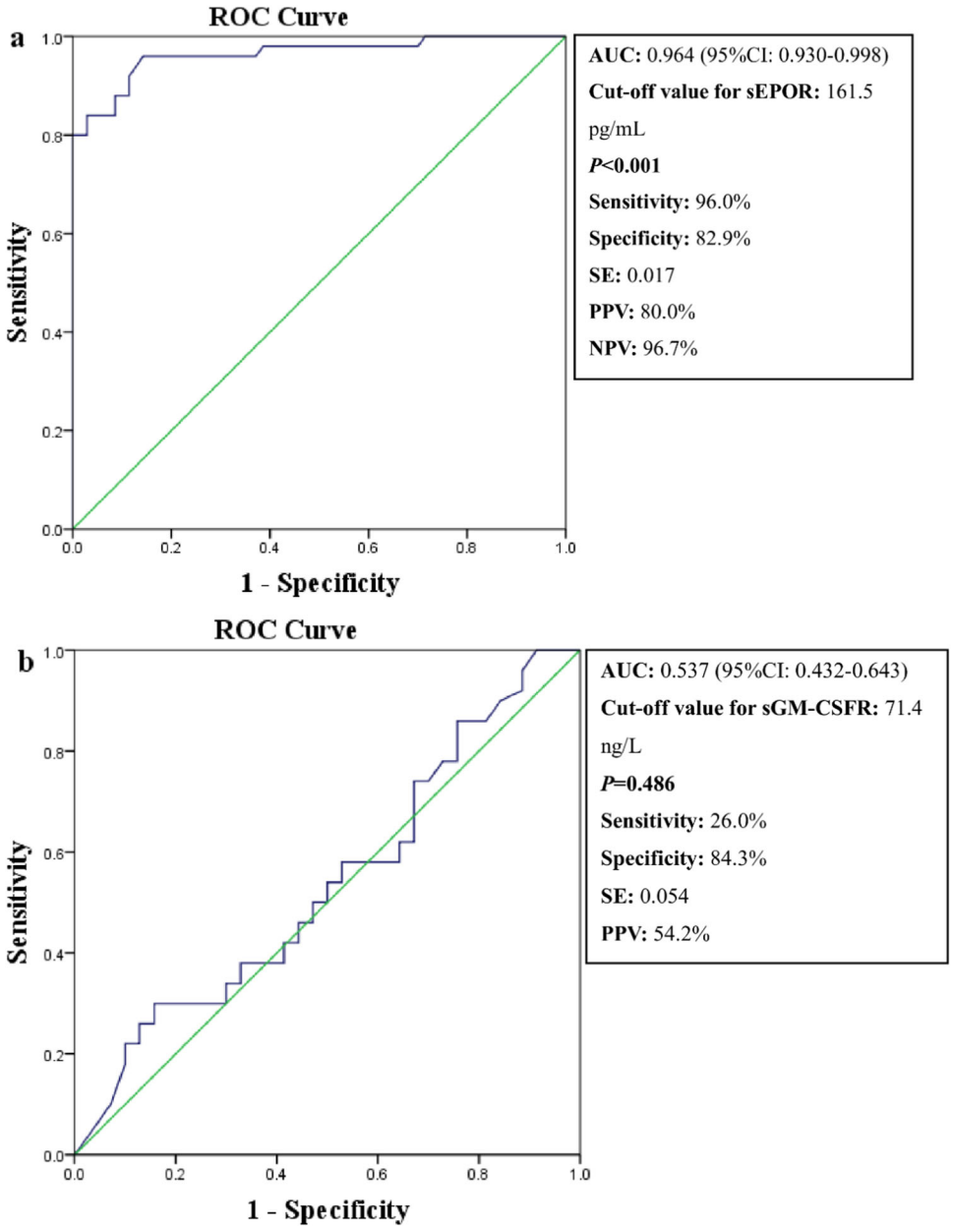


**FIGURE 4** | Predictive values of progenitor cell receptors for severe malarial anemia among *P. falciparum*-infected Ghanaian children. ROC, Receive operating curve; AUC, Area under the curve, SE, Standard error; sEPOR, soluble erythropoietin receptor; sGM-CSFR, soluble granulocyte, macrophage-colony stimulating factor receptor.

compared to those in the control group, but the receptor levels were similar between severe malarial anemia and the non-SMA groups. In unison with IL-3, IL-6 and erythropoietin, GM-CSF interacts with its resident receptor GM-CSFR in the bone marrow to facilitate the differentiation of multipotent haemopoietic stem cells to the myeloid progenitor lineage [13]. The present study did not find any correlation between plasma soluble GM-CSFR and blood cell parameters among Ghanaian children with *P. falciparum* malaria. Similarly, there was no correlation between soluble GM-CSFR and erythropoietin in *P. falciparum*-infected children in this study. This may suggest that endogenous soluble GM-CSFR may not have influence on erythropoietic response to childhood severe *P. falciparum* malaria-induced anemia.

This study further assessed the association between soluble forms of the progenitor cells and severe malarial anemia. Plasma soluble erythropoietin receptor was associated with severe malarial anemia in Ghanaian children. On the other hand, plasma soluble granulocyte macrophage-colony stimulating factor receptor did not associate with severe *P. falciparum* malarial anemia in the participants.

### 4.1 | Strengths and Limitations of the Study

The study identified the association between *P. falciparum* malaria and soluble forms of progenitor cell receptors in children. The negative influence of plasma soluble erythropoietin receptor on

erythropoietic response to severe malarial anemia was observed in this study.

However, this study had limitations. The study did not consider signs and symptoms of malaria experienced by the participants. The study did not assess possible confounders such as nutritional status, iron profile, and inflammatory cytokines which could influence erythrocytic response to severe malarial anemia. The case-control study design could not determine causality in the study. Again, this study could not assess the neutralizing effect of soluble erythropoietin receptor on erythropoietin during pediatric malaria-induced anemia.

## 5 | Conclusion

Plasma levels of progenitor cell receptors (sGM-CSFR and sEPO-R) were high among Ghanaian children with *P. falciparum* malaria. Plasma soluble erythropoietin receptor correlated negatively with red cell parameters, suggesting a possible contribution of the endogenous receptor to the development of severe malarial anemia in children. Further studies to investigate the neutralizing effects of plasma soluble erythropoietin receptor on erythropoietic response during malaria in children are recommended.

### Author Contributions

**Charles Nkansah:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, software, formal analysis, supervision. **Samuel K. Appiah:** investigation, writing – original draft, methodology, writing – review and editing, validation. **Felix Osei-Boakye:** writing – original draft, writing – review and editing, visualization, formal analysis. **Emmanuel Appiah-Kubi:** conceptualization, investigation, writing – original draft, writing – review and editing, visualization, methodology, validation, software, formal analysis, resources, data curation. **Gabriel Abbam:** writing – original draft, writing – review and editing, validation. **Samira Daud:** writing – original draft, validation, writing – review and editing. **Charles A. Derigubah:** writing – original draft, validation, writing – review and editing. **Simon B. Bani:** writing – original draft, validation, writing – review and editing. **Moses Banyeh:** writing – original draft, writing – review and editing, visualization, validation, formal analysis. **Kofi Mensah:** writing – original draft, writing – review and editing. **Ruby Tater:** conceptualization, investigation, writing – original draft, writing – review and editing, methodology, resources, data curation. **Jennifer Obeng Mensah:** resources, data curation, investigation, conceptualization, writing – original draft, writing – review and editing, methodology. **Anne Natornaa:** conceptualization, investigation, writing – original draft, methodology, writing – review and editing, data curation, resources. **Isaac Adjei:** investigation, writing – original draft, methodology, writing – review and editing; data curation, validation. **Muniru M. Tanko:** investigation, writing – original draft, methodology, writing – review and editing. **Gilbert Amankwaa:** conceptualization, investigation, writing – original draft, writing – review and editing, methodology, data curation, resources. **Peter K. Selleh:** writing – original draft, writing – review and editing, validation. **Samuel B. Aboagye:** conceptualization, investigation, writing – original draft, methodology, writing – review and editing, data curation, resources. **Onwuka K. Chima:** writing – original draft, writing – review and editing. **Sylvanus M. Kpangkpari:** writing – original draft, investigation, writing – review and editing, data curation. **Prince Ottah:** investigation, writing – original draft, writing – review and editing, data curation. **Enoch Boadi:** writing – original draft, writing – review and editing, validation. **Yeduah Quansah:** writing – original draft, writing – review and editing, validation, methodology. **Ejike F. Chukwurah:** conceptualization, writing – original draft, writing – review and editing, supervision, validation, methodology. **Boniface N. Ukwah:** writing – review and editing. **Victor U. Usanga:** Writing – review and editing.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

All relevant data are within the article. The original data used to support the findings of this study are available from the corresponding author upon reasonable request.

### Transparency Statement

The lead author Charles Nkansah affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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