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Full Length Research Article

PREDICTORS OF HAEMOGLOBIN CONCENTRATION AMONG SCHOOL CHILDREN IN DELTA STATE, NIGERIA: THE ROLE OF SERUM VITAMINS A, C AND E

*[1]John Esimaje Moyegbone, [2]Prisca Onyema, [2]Eghonghon Ehianata Oronsaye, [3]Mary Kehinde Ayanlere, [2]Obiageli U. Oketta, [4]Edafe Oghenegangan Emurotu, [5]Ezekiel Uba Nwose

[1]Department of Environmental and Public Health, Faculty of Optometry, University of Benin, Benin City, Edo State, Nigeria
[2]Department of Optometry, Faculty of Allied Health Sciences, Bingham University, Karu, Nasarawa State, Nigeria
[3]Faculty of Medical Laboratory Science, College of Health Sciences, Wellspring University, Benin City, Edo State, Nigeria
[4]Department of Optometry, Faculty of Allied Medical Sciences, Dennis Osadebay University, Asaba, Delta State, Nigeria
[5]School of Health and Medical Sciences, University of Southern Queensland, Toowoomba, Australia

*Corresponding Author Email Address: john.moyegbone@uniben.edu

ABSTRACT
Anaemia remains a top nutritional concern among school-aged children worldwide, with profound consequences for physical growth, cognitive development, educational achievement, and immune function. Our study examined the relationships between haemoglobin concentration (HC) and serum vitamins A, C, and E, and identified independent predictors of HC among schoolchildren in Delta State, Nigeria. Overall, 201 school-aged children (6–17 years) participated in a cross-sectional survey in Delta State, Nigeria. The study employed a multistage sampling process to recruit participants, and data on sociodemographic characteristics and dietary habits were collected via interviewer-administered questionnaires. Body mass index (BMI) was calculated using WHO standard protocols, while serum vitamins A, C and E and HC were determined using standard laboratory methods. SPSS version 23 was used for data analysis at a 95% confidence level. Mean HC was 115.32 ± 41.15 g/L, and 38.3% of participants were anaemic. Pearson's correlation demonstrated no statistically relevant relationships between HC and serum vitamins A, C and E. Multiple linear regression analysis showed that age ($\beta = 0.152$, $p = 0.040$) and fruit consumption ($\beta = -0.145$, $p = 0.046$) independently predicted haemoglobin concentration. In contrast, other variables were not significant independent predictors. Haemoglobin concentration among schoolchildren in Delta State was influenced primarily by age and dietary practices rather than by antioxidant vitamin concentrations. Comprehensive school nutrition programmes should integrate anaemia screening with interventions promoting healthy dietary practices to improve haematological health.

Keywords: Anaemia, Body Mass Index, Haemoglobin concentration, Vitamin A, Vitamin C, Vitamin E

INTRODUCTION
Anaemia remains one of the most important nutritional and population health challenges affecting children worldwide. World Health Organization (WHO) states that over 30% of the global population is affected by anaemia, with school-aged children constituting one of the most vulnerable groups (WHO, 2024). Childhood anaemia is linked with impaired physical maturation, restricted cognitive growth, reduced learning capacity, poor academic performance, decreased physical fitness, and increased susceptibility to infectious diseases (Pasricha *et al.,* 2021). Because these adverse consequences may persist into adulthood, prevention and early identification of anaemia remain important priorities for child health programmes, particularly in developing nations.

Haemoglobin concentration is the principal laboratory indicator used to assess anaemia and reflects the oxygen-carrying capacity of circulating erythrocytes. Adequate haemoglobin synthesis requires sufficient iron availability, as well as coordinated interactions among vitamins, minerals, erythropoietin, optimal bone marrow function, and normal red blood cell survival (Pasricha *et al*., 2021). Although iron deficiency remains the primary determinant of anaemia globally, growing evidence indicates that deficiencies in vitamins A, C, folate, vitamin B12, and other micronutrients also substantially contribute to impaired erythropoiesis (Stevens *et al*., 2022).

Vitamin A contributes to important functions in iron mobilization from body stores, erythroid differentiation, and immune regulation. Vitamin A deficiency is associated with impaired iron utilization and an increased risk of anaemia, even when iron stores appear adequate (Semba & Bloem, 2002). Experimental and epidemiological studies have demonstrated that vitamin A supplementation improves haemoglobin concentration in areas where vitamin A deficiency is prevalent, suggesting important physiological interactions between vitamin A metabolism and erythropoiesis (Sommer & West, 1996).

Vitamin C contributes to normal haematological function primarily by enhancing intestinal absorption of non-haem iron. By reducing ferric iron (Fe^{3+}) to the highly bioavailable ferrous iron (Fe^{2+}), vitamin C substantially improves iron bioavailability from plant-based diets, which predominate in many developing countries (Carr & Maggini, 2017). In addition, vitamin C functions as a potent water-soluble antioxidant, protecting erythrocytes from oxidative damage and supporting immune function. Consequently, inadequate vitamin C intake may indirectly contribute to iron deficiency and anaemia, particularly among children consuming diets low in bioavailable iron (Jang *et al*., 2018; McCall *et al*., 2019).

Vitamin E, the body's primary fat-soluble shield against oxidative stress, protects erythrocyte plasma membranes against oxidative injury as well as helps maintain normal red blood cell integrity.

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Although overt vitamin E deficiency is uncommon among healthy children, inadequate antioxidant protection may increase erythrocyte fragility and shorten red blood cell survival under conditions of increased oxidative stress (Jilani *et al*., 2015; Traber & Manor, 2012). The combined physiological functions of vitamins A, C and E therefore suggest that adequate antioxidant status may contribute to optimal haemoglobin synthesis and maintenance.

Despite improvements in global nutritional programmes, anaemia remains highly prevalent in sub-Saharan Africa. Multiple interacting factors, including inadequate dietary iron intake, recurrent malaria, intestinal helminth infections, chronic inflammation, haemoglobinopathies, limited access to adequate nutrition, and socio-economic deprivation, sustain elevated childhood anaemia prevalence (Odoko *et al*., 2020; Pasricha *et al*., 2021; Randrianarisoa *et al*., 2022). In Nigeria, the 2018 Nigeria Demographic and Health Survey demonstrated that anaemia remains a major public health concern among children, although substantial regional variations exist (National Population Commission (NPC) & ICF, 2019). Several Nigerian studies have investigated the prevalence of anaemia among preschool- and school-aged children; however, relatively few have simultaneously examined haemoglobin concentration alongside serum antioxidant vitamins in the same population. Consequently, evidence on the independent determinants of haemoglobin concentration among Nigerian schoolchildren remains limited.

Therefore, this research evaluated the relationships between haemoglobin concentration and serum vitamins A, C and E and identified independent predictors of haemoglobin concentration among schoolchildren in Delta State, Nigeria. The outcomes are expected to yield data that support the enhancement of school health initiatives and nutritional policies aimed at reducing the burden of childhood anaemia in Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out in Delta State, situated in the South-South region of Nigeria, which spans between latitudes 5°00'N and 6°30'N and longitudes 5°00'E and 6°45'E, and has a population of over 6,037,667. (Delta State Government, 2025). The state comprises 25 Local Government Areas, which are grouped into three senatorial zones: Delta North, Delta Central, and Delta South. Delta State features a rich cultural landscape defined by numerous indigenous groups. The region is home to the Urhobo, Isoko, Ijaw, Itsekiri, and Anioma populations. Agriculture, fishing, trading, civil service, and petroleum-related occupations constitute the major means of livelihood (Moyegbone *et al*., 2023). The socioeconomic and dietary diversity within the State provides an appropriate setting for evaluating childhood nutritional status.

Study Population

The study population comprised school-aged children (6 – 17 years) in government-owned primary and secondary schools across Delta State, Nigeria. Children with known chronic illnesses, inherited haematological disorders, acute febrile illnesses, or those receiving medications that are likely to affect haemoglobin concentration or micronutrient metabolism were excluded from the study.

Sample Size Determination

We applied the sample size formula to establish the appropriate number of subjects required for the study (Charan & Biswas, 2013):

$$n = \frac{Z^2pq}{d^2} = \frac{1.96^2 x\ 0.871\ x\ 0.129}{0.05^2} = 172$$

Where,
Z = 1.96 at the 95% CI, P represented the estimated proportion obtained from the previous study (12.9%) (Hoang et al., 2019), and d was the desired precision of 5%.
After adjustment for multistage sampling and a 10% non-response rate, 201 eligible schoolchildren participated in our research.

Sampling Technique

We employed a multistage sampling method in our research to sample subjects from three senatorial districts. In stage 1, one each of public primary and secondary schools was randomly sampled from each senatorial district, making a total of three primary and three secondary schools, respectively. In stage 2, an equal allocation of 34 respondents was enrolled from each public primary school in the three senatorial districts, making a total of 102 primary school students. In stage 3, an equal allocation of 33 respondents was enrolled from each of the three public secondary schools in the three senatorial districts, for a total of 99 secondary school students. Overall, 201 primary and secondary school students participated in the study. Respondents received consent forms a day in advance to indicate their willingness to participate. The research team then collected the contact details of their parents or guardians and reached out via phone. During these calls, the researchers explained the study's objectives and formally requested parental permission.

Data Collection

A structured survey questionnaire was utilized to obtain comprehensive information from respondents between July 2024 and June 2025. The questionnaire consisted of closed-ended, pre-designed, and pre-tested questions. The questionnaire consisted of three sections:
Section A: Socio-demographic characteristics of respondents.
Section B: Assessment of nutritional status by anthropometry
Section C: Assessment of biochemical parameters
In Section A, the information obtained included age, gender, parental education, parental status, employment status, household income, and dietary practices, such as fruit and vegetable consumption. In section B, respondents' body mass indices were determined using weight (kg) and height (m). Hemoglobin, serum vitamins A, C and E were assessed and documented in section C.
A two-day training workshop was organized to ensure the investigators were proficient with the data collection instruments and methodological protocols.

Nutritional Status Anthropometry

Anthropometric data were collected in accordance with WHO guidelines. Weight was recorded to the nearest 0.1 kg using a calibrated digital scale. Height was measured to the nearest 0.1 cm using a stadiometer. BMI was determined using the following formula:

$$BMI = \frac{\text{weight (kg)}}{\text{H(m)}^2}$$

Measurements were taken in triplicate for each sample, with the averaged results serving as the basis for the analysis. Nutritional state categories were determined by applying the World Health Organization's BMI-for-age standards for individuals aged 5 to 19

(WHO, 2007). BMI-for-age Z-scores (BAZ) were generated and classified as follows: thinness (≤ - 3 SD to < - 2 SD); normal nutritional status (≥ - 2 SD to ≤ +1 SD); overweight (> +1 SD to ≤ +2 SD); and obesity (> +2 SD) (WHO, 2007).

Vitamins A, C and E Assessment

Researchers collected 3.0 mL of participants' venous blood using a 5 mL syringe and placed the blood into plain microtubes. The light-sensitive samples were wrapped in black tape, stored on ice, allowed to clot for 1 hour, and centrifuged (2500 revolutions per minute for 15 minutes). The serum was frozen at -20 ^{0}C and transported on ice to the Biochemistry Department at Novena University, Ogume, Delta State. Sample absorbance was measured with a JENWAY 6320D spectrophotometer and vitamin A, C and E concentrations were determined via standard procedures.

Vitamin A (Retinol)

Vitamin A levels were measured utilizing the Neeld and Pearson (1963) technique. A 1.0 mL extract was placed in a 1-cm quartz cell, combined with 1 mL of TCA in chloroform, and its maximum absorbance was read at 620 nm within 5-7 seconds. Serum retinol deficiency is defined as a prevalence of ≥15% at a concentration of 20 µg/dL (0.70 µmol/L) (Mejia & Arroyave, 1982). Levels below 20 µg/dL are classified by the World Health Organization (1997) as mild (2 to <10%); moderate (10 to <20%); and severe (≥20%).

Vitamin C (Ascorbic acid)

Vitamin C levels were measured using the 2,4-dinitrophenylhydrazine (DNPH) method described by Sadasivam and Manickam (2008). Standard ascorbic acid (20-100 µL) and the sample (40 µl) were dispensed into different test tubes. One drop of thiourea mixed with 1 mL of DNPH reagent was added to the tubes, and the volume was adjusted to 100 µL with 5% oxalic acid. Tubes were incubated for 3 hours at 37 $^{\circ}$C, then cooled on ice. After cooling, 5 mL of 85% sulphuric acid was thoroughly mixed into each tube. The resulting orange color was quantified at 540 nm against a reagent blank. The standard cut-off for serum vitamin C was 0.6–2 mg/dL. Levels falling below the threshold were classified as abnormal.

Vitamin E (α-Tocopherol)

α-Tocopherol concentrations were measured using the Kivcak and Mert (2001) spectrophotometric assay. 40 µL of the sample was combined with 20-100 µL of standard α-tocopherol solution. **Reagent volume was adjusted to 3 mL with chloroform, then 1 mL of 2,2-dipyridyl and 1 mL of FeCl3 were added, and the mixture was incubated for** 15 minutes at 37^{0}C. Reaction absorbance was evaluated at 520 nm. Clinically, Vitamin E levels in individuals aged 0–19 years should range from 3.8 to 18.4 mg/L. Concentrations below 3 mg/L indicate deficiency (Traber & Manor, 2012; Bates *et al.,* 2019).

Hemoglobin (Hb) Concentration Assessment

Haemoglobin (Hb) level was assessed with the reagent cyanmethemoglobin (Eiler, 1967), a JENWAY 6320D Spectrophotometer, and a venous blood sample. Each respondent's skin was cleaned with sterile cotton wool (immersed in 70% alcohol) before 2 mL of venous blood was drawn into an EDTA container using a disposable sterile syringe. "Blank" and "Standard" were the labels on two test tubes. The "Blank" tube was filled with 2.0 mL of Hb reagent. After adding 0.01 mL of whole blood to the reagent, the mixture was incubated at room temperature for 3 minutes. Two milliliters (2.0 mL) of standard solution (containing 0.6 g/L of Methemoglobin) was placed in the 'Standard' tube. The reagent blank was used to zero the JENWAY 6320D Spectrophotometer device at a wavelength of 540 nm. Absorbance of both the standard and the blood samples was measured and recorded.

Each respondent's blood haemoglobin level was evaluated utilizing the following standard equation (Eiler, 1967; Henry, 1974):

absorbance of Test Sample (g/L) = $\frac{\text{Absorbance of Test Sample}}{\text{Absorbance of Standard}}$ x Concentration of Standard

WHO defines an abnormal haemoglobin level as one below 115 g/L (WHO, 2011).

Statistical Analysis

Researchers utilized SPSS (version 23) to process and analyze the dataset. Socio-demographic traits, anthropometric parameters, and biochemical parameters were presented in frequency tables. Pearson's correlation analysis was used to determine relationships among haemoglobin concentration, serum vitamins A, C, E and BMI. Multiple linear regression analysis was conducted to point out independent determinants of haemoglobin concentration. The statistical threshold was set at p-value < 0.05.

Ethical Approval

Ethical approval was obtained from Warri Central Hospital (Ref. No: CHW/ECC VOL 1/243) and Eku Government Hospital (Ref. No: EBGH/AD/112/REM/V/101). Institutional approval for school access was granted by the Chief Inspector of Education (secondary schools) and the Chairman of the Local Education Area (primary schools). Additionally, written informed consent was secured from the child's parent or guardian, and assent by the children themselves.

RESULTS

Overall, 201 school children participated in our study. Females constituted 58.7% (n = 118), while males accounted for 41.3% (n = 83). The participants comprised pupils from primary schools and students from secondary schools, aged 6 to 17 years. Most participants were from households with low monthly income (≤ ₦50,000), and the majority of parents had at least secondary school education. Although most parents were employed, regular fruit and vegetable consumption remained suboptimal across the participants. Approximately three-quarters reported inadequate fruit consumption (less than three times weekly), while almost half reported inadequate vegetable intake (less than three times weekly) (Table 1).

Table 1: Socio-demographic and Dietary Lifestyle Characteristics of Respondents

Variables	Options	Frequency (n)	Percentage (%)
Gender	Male	83	41.3
	Female	118	58.7
Age Group (in years)	Primary (6 – 11)	72	35.8
	Secondary (12 - 17)	129	64.2
Educational Status	Primary School	41	20.4

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of Parent	Secondary School	88	43.8
	Tertiary School	72	35.8
Employment Status of Parent	Unemployed	22	10.9
	Employed	179	89.1
Income of Parents per Month (Naira)	≤ 50,000	155	77.1
	> 50,000	46	22.9
Fruit consumption	Low fruit consumption	145	72.1
	Adequate fruit consumption	56	27.9
Vegetable consumption	Low Vegetable Consumption	95	47.3
	Adequate Vegetable Consumption	106	52.7

In Table 2, mean haemoglobin concentration was 115.32 ± 41.15 g/L, indicating considerable variation in haematological status among the study population. While mean BMI was 17.43 ± 3.46 kg/m², mean serum concentrations of vitamins A, C and E were 25.85 ± 6.48 µg/dL, 11.00 ± 6.27 mg/L and 16.58 ± 6.89 mg/L, respectively.

Table 2: Anthropometric and Biochemical Characteristics of Respondents

Variable	Mean	Std. Deviation
Haemoglobin	115.32	41.15
BMI	17.43	3.46
Vitamin A	25.85	6.48
Vitamin C	11.0	6.27
Vitamin E	16.58	6.89

According to the WHO haemoglobin classification, 38.3% of participants were anaemic, while 61.7% had normal haemoglobin concentrations. Biochemical assessment further showed that 12.4% of participants had vitamin A deficiency and 12.9% had vitamin C deficiency. No participant had a serum vitamin E concentration below the recommended deficiency threshold (Table 3)

Table 3: Prevalence of Anaemia and Micronutrient Deficiencies

Variable	Options	Frequency (n)	Percentage (%)
Haemoglobin	Normal	124	61.7
	Anaemia	77	38.3
Vitamin A	Normal	176	87.6
	Deficient	25	12.4
Vitamin C	Normal	175	87.1
	Deficient	26	12.9
Vitamin E	Normal	201	100.0

Table 4 showed the relationships between haemoglobin concentration, BMI, and serum vitamins A, C, and E. Although, not statistically significant, haemoglobin concentration showed a negative correlation with BMI (r = –0.048, p = 0.503), serum vitamin A (r = –0.062, p = 0.381), serum vitamin C (r = –0.047, p = 0.509), or serum vitamin E (r = –0.058, p = 0.415). Similarly, none of the antioxidant vitamins demonstrated a significant relationship with haemoglobin concentration. These findings suggest that variations in haemoglobin concentration among the participants were not directly associated with BMI or serum antioxidant vitamin concentrations at the bivariate level.

Table 4: Relationship between Haemoglobin, BMI and Serum Vitamins among Respondents

		BMI	Vitamin A	Vitamin C	Vitamin E	Haemoglobin
BMI	Pearson Correlation	1	.119	.193**	-.111	-.048
	Sig. (2-tailed)		.093	.006	.118	.503
Vitamin A	Pearson Correlation	.119	1	.017	-.108	-.090
	Sig. (2-tailed)	.093		.815	.126	.203
Vitamin C	Pearson Correlation	.193**	.017	1	.039	.009
	Sig. (2-tailed)	.006	.815		.586	.895
Vitamin E	Pearson Correlation	-.111	-.108	.039	1	.029
	Sig. (2-tailed)	.118	.126	.586		.687
Haemoglobin	Pearson Correlation	-.048	-.090	.009	.029	1
	Sig. (2-tailed)	.503	.203	.895	.687	

**. Correlation is significant at the 0.01 level (2-tailed).

Table 5 presents the results of a multiple linear regression analysis to identify independent determinants of haemoglobin concentration. The regression model explained only 5.6% of the variation in haemoglobin concentration (R^2 = 0.056; adjusted R^2 = 0.017) and was not statistically significant overall (F = 1.434, p = 0.184), indicating that the variables included in the model accounted for only a small proportion of the observed variability in haemoglobin concentration.

Despite the model's modest explanatory power, age emerged as a statistically significant independent predictor of haemoglobin concentration (B = 2.785, β = 0.212, p = 0.022). After adjusting for other variables included in the model, each one-unit increase in age was linked with an average increase of 2.785 units in haemoglobin. This finding indicates that haemoglobin concentration increased significantly with increasing age.

Fruit consumption was also identified as an independent predictor of haemoglobin concentration (B = –14.019, β = –0.153, p = 0.036). Participants with adequate fruit consumption (greater than three times weekly) had, on average, haemoglobin levels that were 14.019 units lower than those with low fruit consumption (less than three times weekly), after controlling for other variables in the model. Conversely, BMI (p = 0.136), gender (p = 0.307), serum vitamin A (p = 0.513), serum vitamin C (p = 0.759), serum vitamin E (p = 0.921), and vegetable consumption (p = 0.854) were not significant independent predictors of haemoglobin concentration after adjustment for other variables. Overall, the findings indicate that age and dietary behaviour exerted greater influence on haemoglobin concentration than anthropometric status or serum antioxidant vitamin concentrations within the study population.

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Table 5: Determinants of Haemoglobin Concentration among Respondents

	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
	B	Std. Error	Beta	t	P-Value	Lower Bound	Upper Bound
(Constant)	133.626	25.804		5.178	<.001	82.728	184.525
BMI	-1.666	1.114	-.139	-1.496	.136	-3.863	.530
Vitamin A	-.306	.467	-.048	-.655	.513	-1.227	.615
Vitamin C	-.148	.482	-.023	-.307	.759	-1.098	.803
Vitamin E	-.042	.428	-.007	-.099	.921	-.887	.802
Age	2.785	1.207	.212	2.308	.022	.405	5.164
gender	6.655	6.498	.080	1.024	.307	-6.162	19.473
Fruit Consumption	-14.019	6.623	-.153	-2.117	.036	-27.083	-.955
Vegetable Consumption	1.104	5.990	.013	.184	.854	-10.741	12.948

Dependent Variable: Haemoglobin

DISCUSSION

This study investigated the relationships between haemoglobin concentration and serum vitamins A, C, and E among schoolchildren in Delta State, Nigeria, and identified the independent determinants of haemoglobin concentration. The findings demonstrated that anaemia remains an important public health burden, affecting over thirty percent of the study population. Although deficiencies of vitamins A and C were identified among a proportion of participants, neither antioxidant vitamin concentrations nor body mass index (BMI) showed significant correlations with haemoglobin concentration. Multiple linear regression analysis further demonstrated that age and fruit consumption independently predicted haemoglobin concentration, whereas BMI, serum vitamins A, C, and E, gender, and vegetable consumption did not. These findings underscore the multifactorial nature of childhood anaemia and suggest that haemoglobin concentration is influenced by a wider array of physiological and ecological factors than the biochemical indicators assessed in this study.

The prevalence of anaemia observed in our study indicates a moderate public health burden according to the World Health Organization (2024) classification of anaemia in children and adolescents. Anaemia during school age is associated with impaired cognitive function, reduced attention span, decreased school performance, poor physical endurance, impaired immunity, and reduced productivity later in life (Pasricha *et al.*, 2021). The present prevalence is comparable to reports from several Nigerian and other sub-Saharan African studies, in which childhood anaemia continues to affect between 30% and 50% of the school-aged population despite ongoing nutritional interventions (National Population Commission (NPC) & ICF, 2019; Stevens *et al.*, 2022). The persistence of anaemia reflects the continued influence of inadequate dietary intake, infectious diseases, poverty, and limited access to healthcare.

Although approximately one in eight participants had vitamin A deficiency and a similar proportion had vitamin C deficiency, no participant had vitamin E deficiency. These findings align with recent evidence showing a decline in vitamin A deficiency following supplementation programmes and the rarity of vitamin E deficiency among healthy children (Bates *et al.*, 2019; Stevens *et al.*, 2022). This pattern is consistent with reports indicating that vitamin A deficiency remains prevalent among kids in many developing nations, whereas clinically significant vitamin E deficiency is relatively uncommon outside conditions associated with severe fat malabsorption or chronic disease (Sommer & West, 1996; Traber & Manor, 2012). Although vitamins A and C are known to support iron metabolism and erythropoiesis, neither vitamin A, C, nor E showed significant associations with haemoglobin concentration. This contrasts with studies reporting positive relationships between vitamin A and haemoglobin (Semba & Bloem, 2002; Htet *et al.*, 2014), but agrees with evidence that childhood anaemia is multifactorial and influenced by iron deficiency, infections, inflammation, and socioeconomic factors rather than antioxidant vitamin status alone (Pasricha et al., 2021). The coexistence of anaemia with relatively low rates of antioxidant vitamin deficiencies suggests that factors other than antioxidant vitamin status contributed substantially to the burden of anaemia in the study population.
Since serum vitamins A, C, and E were not significant predictors of haemoglobin concentration, interventions should extend beyond vitamin supplementation to include iron-rich diets, malaria prevention, deworming, nutrition education, and improved food security. Future public health programmes should also incorporate assessment of iron status, inflammatory markers, and infectious diseases to strengthen anaemia prevention among school-aged children.

An important outcome of our study was the absence of statistically significant correlations between haemoglobin concentration and the serum levels of vitamins A, C, and E. Although these vitamins play important physiological roles in erythropoiesis, iron metabolism, and antioxidant defense, their circulating concentrations were not directly associated with haemoglobin concentration among the participants. This finding differs from studies that have demonstrated positive relationships between vitamin A status and haemoglobin concentration, particularly among populations with widespread vitamin A deficiency (Semba & Bloem, 2002; Jilani *et al.*, 2015). Vitamin A facilitates mobilization of iron from hepatic stores, enhances erythropoietin production, and supports differentiation of erythroid precursor cells. Consequently, vitamin A deficiency has frequently been linked to anaemia, even in the absence of overt iron deficiency (Htet *et al.*, 2014).

Similarly, vitamin C promotes intestinal absorption of non-heme (plant-based) iron by converting poorly absorbed ferric iron into a highly bioavailable ferrous form, thereby optimizing physiological iron utilization and haemoglobin synthesis (Carr & Maggini, 2017). Nevertheless, serum vitamin C was not significantly linked with haemoglobin concentration in our study. One possible explanation is that serum vitamin C primarily reflects recent dietary intake rather than long-term nutritional status. Furthermore, iron absorption depends not only on vitamin C availability but also on total dietary iron intake, the presence of mineral absorption inhibitors such as phytates and polyphenols, gastrointestinal health, and inflammatory status (Osunu et al., 2024). These interacting factors may have attenuated any measurable correlation between serum vitamin C and haemoglobin concentration in this cross-sectional study.

Likewise, serum vitamin E showed no significant relationship with haemoglobin concentration. Although vitamin E protects erythrocyte membranes against oxidative damage and contributes to red blood cell stability, deficiency is uncommon in healthy school-aged children (Traber & Manor, 2012; Jilani *et al.*, 2015). Since no participant in the present study had vitamin E deficiency, the relatively narrow range of serum vitamin E concentrations may have limited the study's power to detect statistically significant links with haemoglobin concentration.
BMI was also not linked with haemoglobin concentration. Our finding supports previous evidence indicating that anthropometric and haematological nutritional status represent distinct dimensions of child health (Black *et al.*, 2013). Children with normal BMI may still develop anaemia because haemoglobin synthesis depends on adequate iron stores, micronutrient availability, erythropoietic activity, and the absence of chronic infection or inflammation. Conversely, underweight children are not invariably anaemic if these physiological processes remain intact. Therefore, BMI alone should not be used as a haematological biomarker among the school-aged population.

Multiple linear regression analysis identified age as an independent predictor of haemoglobin concentration. Increasing age was associated with higher haemoglobin levels, a finding that is physiologically plausible because erythropoietic activity, blood volume, and lean body mass increase progressively throughout childhood and adolescence. Similar observations have been reported in previous studies, where haemoglobin concentrations increased significantly with age after adjustment for nutritional and socioeconomic variables (Mesfin *et al.*, 2015; Hoang *et al.*, 2019; Pasricha *et al.*, 2021).

Interestingly, serum vitamins A, C, and E were not independent predictors of haemoglobin concentration after adjustment for age, dietary variables, BMI, and gender. This finding suggests that haemoglobin concentration among schoolchildren is determined by multiple interacting factors rather than by isolated biochemical indicators. In addition to nutritional deficiencies, childhood anaemia may result from malaria, helminthic infections, chronic inflammation, inherited haemoglobin disorders, folate deficiency, vitamin B12 deficiency, and socioeconomic deprivation (Pasricha *et al.*, 2021; WHO, 2024). Since these factors were not measured in the present study, they may account for the relatively low explanatory power of the regression model.

The regression model explained only 5.6% of the variability in haemoglobin concentration and was not statistically significant overall. This finding indicates that most determinants of haemoglobin concentration were not captured by the variables included in the present analysis. Consequently, the regression outcomes should be interpreted with caution and regarded as exploratory rather than definitive. Future studies should incorporate additional variables such as dietary iron intake, inflammatory biomarkers, malaria parasitaemia, intestinal helminth infections, haemoglobinopathies, menstrual history among adolescent girls, and household food security to provide a broader understanding of childhood anaemia.

Strengths and Limitations of the Study
The major strength of this study is the simultaneous assessment of haemoglobin concentration together with serum antioxidant vitamins using standardized laboratory methods. The inclusion of children from both primary and secondary schools across Delta State also enhances the external validity of our results. Nevertheless, these findings should be interpreted with certain limitations in mind. Firstly, the cross-sectional design hinders conclusions regarding temporal or causal relationships between antioxidant vitamins and haemoglobin concentration. Secondly, the study's modest sample size may have limited the statistical power to detect subtle associations. Furthermore, important determinants of haemoglobin concentration, such as serum ferritin, transferrin saturation, C-reactive protein, malaria infection, intestinal helminthiasis, sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, dietary iron intake, folate status, and vitamin B12 status, were not evaluated. Their omission may partly explain the modest explanatory power of the regression model.

Conclusion
Anaemia remains a pressing medical challenge affecting the pediatric population across Delta State despite relatively low prevalence of vitamins A and C deficiencies and absence of vitamin E deficiency. Haemoglobin concentration showed no significant association with serum vitamins A, C and E or with BMI. Age and fruit intake were the principal independent determinants of haemoglobin concentration, highlighting the importance of physiological development and dietary behaviour in childhood haematological health. Future longitudinal studies incorporating comprehensive assessments of iron status, infectious diseases, inflammatory markers, and dietary intake are recommended better to elucidate the determinants of haemoglobin concentration among Nigerian schoolchildren.

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