



Effects of Antioxidant Vitamins ACE and Iron Supplements on Visual Impairment among Primary and Secondary School Children in Delta State, Nigeria

John Esimaje Moyegbone^{1*}, Ezekiel Uba Nwose^{2,3}, Charles Chukwuemeka Ofili², Eghonghon Ehianata Onoikhua¹, Emmanuel Agbonomhen Agege², Joseph Onyedenyifa Odoko², Omatseye Alero Akuirene² and Favour Alero Fregene²

¹Department of Optometry, Bingham University, Karu, Nasarawa State, Nigeria

²Department of Public and Community Health, Novena University, Ogume, Delta State, Nigeria

³School of Health and Medical Sciences, University of Southern Queensland, Toowoomba, Australia

*Corresponding Author: John Esimaje Moyegbone, Department of Optometry, Bingham University, Karu, Nasarawa State, Nigeria.

DOI: 10.31080/ASOP.2022.06.0662

Received: July 24, 2023

Published: July 28, 2023

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Abstract

The eyes like every other organ of the body, are biochemical elements that respond to both helpful or harmful biochemical stimuli leading to either a healthy or pathological state. Anaemia, antioxidant vitamin deficiencies and visual impairment (VI) are public health problems resulting in poor eye health globally. This study aimed to determine the relationship among anaemia, antioxidant vitamins and VI as well as the effect of antioxidant vitamins and iron supplements on visual acuity and anaemia. A pretest-posttest study of 201 respondents randomly selected from primary and secondary schools in three senatorial districts of Delta State, Nigeria. Sociodemographic data and visual acuity tests were done before and after intervention. Preventive intervention with a single dose of vitamin A, daily Vitamin C, weekly vitamin E, and twice weekly ferrous sulphate supplements was administered for five weeks. Spectacle prescriptions were administered to correct refractive errors (RE). Pretest-posttest haemoglobin and serum vitamins ACE were done using JENWAY 6320D Spectrophotometer. Data were analysed using SPSS version 28.0. Statistical significance was defined as $P < 0.05$. Findings showed that 83 (41.3%) were males and 118 (58.7%) were females with a mean age of 12.30 ± 3.14 years. Prevalence of anaemia and VI at baseline was 38.3% and 28.9% respectively, and 0% and 5.5% post-intervention respectively. Prevalence of vitamins A and C at baseline was 12.4% and 12.9% respectively, and post-intervention was 6.0% and 15.9% respectively. Prevalence of vitamin E at baseline and post-intervention was 0.0% respectively. RE was the major cause of VI with a prevalence of 23.4%. The association between visual impairment at baseline and impaired vision with spectacle prescription intervention was statistically significant ($P=0.001$). The correlation of anaemia, vitamins ACE and VI were not statistically significant ($P>0.05$). Odd ratios of vitamins ACE and iron supplements were predicted to decrease the risk of having anaemia and VI, though not statistically significant ($P>0.05$). This study showed that antioxidant vitamins and iron supplementation as well as spectacle prescription were effective in reducing the prevalence of anaemia, antioxidant vitamin deficiencies and visual impairment among primary and secondary school children in Delta State.

Keywords: Anaemia; Antioxidant Vitamins; Deficiency; Haemoglobin; Visual Impairment; Refractive Error

Abbreviations

EDTA: Ethylene Diamine Tetraacetic Acid; LGA: Local Government Area; PVA: Presenting Visual Acuity; RE: Refractive Error; VAD: Vitamin A Deficiency; VI: Visual Impairment; WHO: World Health Organization

Introduction

The eyes like every other organ of the body, are biochemical elements that respond to both biological and chemical stimuli. These stimuli can either be helpful, harmful or harmless to the eyes. While several chemical elements are helpful to the eyes and their deficiency result in lack of optimal function, others are harmful resulting in pathological state. Anaemia has been considered as a public health problem worldwide from time immemorial [1]. World Health Organization (WHO) estimates that approximately 1.3 to 2.2 billion people (more than 30% of the world's population) are suffering from anaemia, and approximately 50% of women and children in developing countries are anaemic, [2] and it affects 305 million (25.4%) school-age children [3]. Anaemia is a condition in which the blood contains low levels of haemoglobin as evidenced by low quality or quantity of Red Blood Cells [2,4]. Haemoglobin is an iron-rich protein that gives a red colour to blood. It carries oxygen from the lungs to the rest of the body. In people with anaemia, the blood does not carry enough oxygen to the rest of the body because the body does not have enough iron to form haemoglobin [2,4]. Anaemia causes fatigue, reduces work capacity, and makes people especially children more susceptible to infection [2,4]. An increased demand for iron as occurred in the early years of life and during pregnancy can result in iron deficiency anaemia [5]. According to Dreyfuss, *et al.* the direct causes of anaemia in the population are; inadequate dietary iron intake, hookworm infestation and presence of malaria [4,6]. Similarly, Mikki and Stigum stated that the main cause of anaemia is iron deficiency due to inadequate intake of bioavailable iron from the diet, and other causes include infectious diseases, deficiencies of micronutrients such as folate, vitamin B12, inherited conditions such as thalassaemia and environmental pollutants such as lead [7].

Antioxidant vitamins such as vitamin A is necessary for the normal production of red blood cells, while others such as vitamins C and E protect mature red blood cells from premature destruction by free radical oxidation [4,8]. VAD is an important public health

problem. VAD is also a major cause of preventable childhood blindness [9]. VAD is the direct cause of xerophthalmia [10]. Primary vitamin A deficiency could be attributed to prolonged deprivation of vitamin A-rich foods and is further depleted by diarrhoea, measles, and respiratory infections [9]. In children, VAD is the leading cause of preventable visual impairment and blindness. VAD was estimated to affect between 75 and 254 million preschool children each year [11]. Study showed that 26% of vitamin A-deficient children live in Africa, with the largest number in Ethiopia (6.7 million). An estimated 250,000–500,000 vitamin A-deficient children become blind every year, and about half of them die within a year of becoming blind [11]. Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that cannot be produced by humans but is readily available in many types of food. Citrus fruits and vegetables are the best known sources of Vitamin C [12]. The reference range of vitamin C level in serum is 0.6-2 mg/dl (Vitamin C) below this range vitamin C level is considered as insufficiency [13]. A recent review of global vitamin C status has indicated a high prevalence of deficiency, particularly in low- and middle-income countries, as well as in specific subgroups within high-income countries [14]. Vitamin E is an essential vitamin for maintaining the metabolic function of the body and possesses antioxidant and scavenging free radical activities [15]. Recent research has suggested that increased intake can help to preserve brain function and protect against nerve cell degeneration [16]. Depletion of vitamin E among children, adolescence, and older populations in developing countries has been related to limited sources of food containing vitamin E and high prevalence of malaria [17] and human immunodeficiency virus in the region [18]. Deficiency of vitamin E has also been associated with low levels of vitamin C, β -carotene and other antioxidants in blood circulation, which supports the theory that deficiency of vitamin E is associated with poor intake and greater oxidative stress [18].

Materials and Methods

Materials

The study was a pretest-posttest (Quasi-experiment) study of 201 primary school (age 6-12 years) and secondary school (age 13-19 years) respondents randomly selected using multistage sampling from three primary and three secondary schools in the three senatorial districts of Delta State- Warri South LGA (Delta

South), Ethiope East LGA (Delta Central) and Ukwuani LGA (Delta North). Baseline and post-intervention sociodemographic information were collected using self-administered questionnaires. one public primary school and one public secondary school was selected by balloting from each of the three local government areas making a total of three (3) secondary schools and three (3) primary schools in the three senatorial districts of Delta State. Furthermore, 33 respondents of primary school age and secondary school age were enumerated from each of the enumerated schools. Consent forms were given to respondents who gave assent to participate in the study a day prior to the survey for their parents or guardian to give their consent. Phone numbers of their parents or guardians were collected by the researchers and calls were made through to the available phone numbers to explain the aim of the study and the need for their consents. The first 33 respondents from each of the six (6) schools that came with their consent forms signed were randomly recruited into the study making a total of 198 respondents. Three more respondents had consent forms signed and were included in the study making a total of 201 respondents.

Methodology

Hemoglobin (Hb) Concentration for Assessment of Anaemia

Haemoglobin (Hb) concentration was determined by cyanmethemoglobin [19] using JENWAY 6320D Spectrophotometer from venous blood sample. 2ml of venous blood was collected from each respondent into an EDTA container with disposable sterile syringe after cleaning the skin surface using sterile cotton wool (immersed in 70% alcohol). Two test tubes were labelled 'blank' and 'standard'. 2.0 ml of Hb reagent was dispensed into the test tube labelled blank. 0.01 ml of sample blood was added and mixed. The test tube was allowed to stand for 3 minutes at room temperature. 2.0 ml of standard solution (contained 60 mg/dl Methemoglobin dissolved in cyanmethemoglobin reagent) was placed in the test tube labelled standard. The JENWAY 6320D Spectrophotometer was set to 540 nm and zero with the reagent blank. Absorbance value of the standard was read and recorded. Thereafter, 2.0 ml of Hb reagent was mixed with 0.01 ml of blood sample of each respondent and read at 540 nm of wavelength. Haemoglobin concentration of each sample was calculated using the formula; [19,20].

Haemoglobin conc. of sample = $\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard}$

World Health Organization defined anaemia as haemoglobin concentration < 110 g/L.

Mild, moderate and severe anaemia was defined as haemoglobin concentration of 100–109 g/L, 70–99 g/L and lower than 70 g/L, respectively [3,21].

Laboratory assessment of antioxidant vitamins

A sample of 3.0 ml of free-flow venous blood was withdrawn from each of the intervention group and control group respondents using a 5ml disposable syringe and transferred to a plane microtube. The tubes were wrapped with black tape and placed in an ice-bag. The blood samples were allowed to stand for 1 hour and then centrifuged at room temperature for 15 minutes at 2500 rpm. The sera were separated into another plane tube labelled, and frozen at -20°C within 2-8 hours of being collected. Thereafter, it was transported using ice pack to department of Biochemistry, Novena University, Ogume, Delta State where absorbance of each sample was read using JENWAY 6320D spectrophotometer. Concentration of vitamins A, C and E were calculated from their various standard curves.

Vitamin A (Retinol)

Vitamin A content was determined using the method of Neeld and Pearson [22]. After separation, an aliquot (1.0 ml) of the solvent extract was carefully pipette with an auto pipette into a 1-cm quartz cell. To this was added 1 ml of TCA in chloroform from a fast delivery pipette with maximum absorbance recorded (usually within 5-7 seconds) at 620 nm. Cut-off for serum retinol was a prevalence of ≥15% with a concentration of 20 µg/dL (0.70 µmol/L) [23]. Serum retinol concentrations of <20 µg/dL was classified as: mild if prevalence is 2 to <10%; moderate if prevalence is 10 to <20%; and severe if prevalence is ≥20% [24].

Vitamin C (Ascorbic acid)

Ascorbic acid content was determined by 2,4 dinitrophenyl hydrazine method described by Sadasivam and Manickam [25]. About 40 µl sample and standard ascorbic acid (20-100 µl) was place in test tubes. A drop of thiourea solution and 2,4 dinitrophenyl

hydrazine reagent (1ml) was placed in each tube, the volume was made up to 100µl with 5% oxalic acid. The mixture was incubated at 37°C for 3 hours. The tubes were allowed to cool on ice water and about 5ml of 85% sulphuric acid was added to the tubes. The reaction mixture was mixed thoroughly. The orange colour of the reaction mixture was read at 540nm against a reagent blank. The reference range of vitamin C level in serum is 0.6-2 mg/dl. Below this range vitamin C level was considered as deficient.

Vitamin E (α-Tocopherol)

α-Tocopherol content was estimated using the method described by Kivcak and Mert [26]. About 20 µl-100 µl of standard α-tocopherol solution and 40 µl of the sample were used for the estimation. Volume was made up to 3ml using chloroform, 1 ml of 2, 2-dipyridyl, and 1 ml of FeCl₃ solution were added and then incubated at 37°C for 15 minutes. The absorbance of the reaction mixture was read at 520nm. Reference value of Vitamin E for 0 – 19 years is 3.8–18.4mg/L. Value less than 3 mg/L of vitamin E indicate significant deficiency [27,28].

Determination of visual impairment using presenting visual acuity

Presenting visual acuity (PVA) was assessed monocularly using the Snellen's chart, illiterate E, or picture chart for each eye depending on the level of literacy of the respondent. This was placed six metres (20 feet) away in an open space in daylight [29]. The smallest line read on the chart is written as a fraction, where the numerator refers to the distance at which the chart is viewed, and the denominator is the distance at which a "healthy" eye is able to read that line of the vision chart. The last completed line on the chart was recorded as the visual acuity for that eye. For example, a visual acuity of 6/18 means that, at 6 metres from the vision chart, a person can read a letter that someone with normal vision would be able to see at 18 metres. "Normal" vision is taken to be 6/6. Visual acuity assessment was the criteria used to determine visual impairment in the better eye. Normal/near normal VA is presenting vision ≥ 6/12 in the better eye. Visual impairment was defined as a visual acuity of <6/18 and categorized as follows [30].

- Mild visual impairment (Mild VI): presenting VA <6/12 to 6/18 in the better eye.
- Moderate visual impairment (Mod VI): presenting VA of <6/18 to 6/60 in the better eye.

- Severe visual impairment (SVI): presenting VA of <6/60 to 3/60 in the better eye.
- Blindness: presenting VA (with glasses for distance if normally worn or unaided if glasses for distance not worn) of <3/60 in the better eye.

Intervention therapy

Antioxidants vitamins A, C, E and iron supplements as well as spectacle prescription were administered to respondents as intervention therapy following pretest data collection. Single dose of 200,000 IU [31] vitamin A was administered. Daily 100mg [31] of Vitamin C, weekly 1000 IU [28] of vitamin E and twice weekly 200 mg [32] of ferrous sulphate (equivalent to 65mg ferrous iron) supplements were administered for five (5) weeks [33].

Ethical consideration

Ethical clearance was obtained from the Ethics Committee of Central Hospital, Warri and Eku Government Hospita, Eku, Delta State. Permission to gain access to primary and secondary schools in the various LGA were obtained from the Local Education Authority (LEA) and Chief Inspector Education (CIE) respectively. Informed consent was obtained from parents or caregivers of the respondents and assent from each respondent was sought as well.

Method of data analysis

The statistical data was analysed using the Statistical Package for Social Sciences (IBM SPSS for Window 10 Version 28.0; SPSS Inc., Chicago, USA). Frequency distribution and descriptive statistics presented in tables and charts were used to determine the prevalence of anaemia, antioxidant vitamins deficiencies, and visual impairment. Cross tabulation, Chi square test and Fisher's exact test were used to compare the differences between the prevalence of visual impairment at baseline and spectacle prescription at post intervention. Spearman's rho bivariate Correlation was used to determine the relationship between visual impairment, anaemia, and antioxidant vitamins among the respondents. Paired t-test was used to compare the effect of antioxidant vitamins and iron supplements before and after intervention. Multinomial logistic regression was used to determine the effects of antioxidant vitamins and iron supplements on visual acuity and anaemia. All p-values reported were two tailed and statistical significances was defined as $P < 0.05$ and power of 80%.

Results

In Table 1, 83 (41.3%) of the respondents were male and 118 (58.7%) were female with mean age of 12.30± 3.14 years. 72 (35.8%) were of primary school age (6-12 years), while 129 (64.2%) were of secondary school age (13-19 years). Majority of parent educational and employment status were secondary schooling 87 (43.3%) and self-employed 139 (69.2%) respectively. Majority of respondents parents 81 (40.3%) received income of less than ₦ 30, 000 per month.

Variable	Frequency (n = 201)	Percent (%)
Gender		
Male	83	41.3
Female	118	58.7
School Age Group*		
Primary School age (6-12 Years)	72	35.8
Secondary School age (13-19 Years)	129	64.2
Parent Educational Status		
No Schooling	11	5.5
Primary Schooling	29	19.9
Secondary Schooling	87	43.3
Tertiary Schooling	72	35.8
Employment Status of Parent		
Unemployed	16	8.0
Employed	29	14.4
Self Employed	139	69.2
No Response	17	8.4
Income Per Month of Parent		
< 30,000	81	40.3
30,000 - 50,000	72	35.8
51,000 - 100,000	26	12.9
> 100,000	20	10.0
No Response	2	1.0

Table 1: Socio-demographic characteristics of the respondents.

*Mean age ± standard deviation = 12.30 ± 3.14 years.

In figure 1, the prevalence of anaemia in baseline was 38.3% (77) and 0.0% at post intervention. The prevalent of vitamin A deficiency at baseline and post intervention was 12.4% (25) and 6.0% (12) respectively. The prevalence of vitamin C deficiency at baseline and post intervention was 12.9% (26) and 15.9 (32) respectively. There was no vitamin E deficiency at baseline and post intervention. The prevalence of visual impairment at baseline and post spectacle prescription intervention was 28.9% and 5.0% respectively.

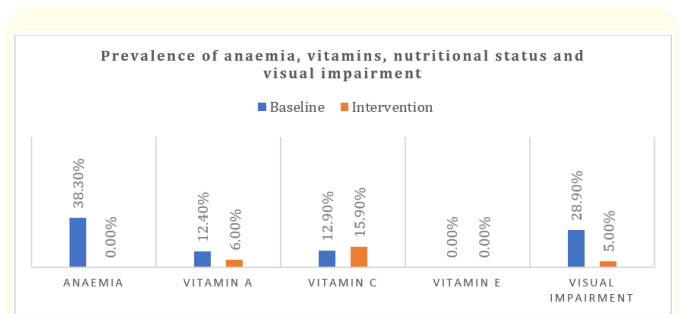


Figure 1: Prevalence of Anaemia, Antioxidant Vitamins ACE, and Visual Impairment among Baseline and Intervention Respondents.

In Table 2, Out of the 38.3% prevalence of anaemia, 18.4% (37) were severely anaemic and 15.9% had moderate anaemia. Out of the 28.9% with VI, majority (19.9%) had mild VI. Visual impairment was categorised into mild, moderate and severe with percentages of 19.9%, 6.5% and severe 2.5% respectively.

In Table 3, 23.4% (47) out of 28.9% (58) of respondents who were visually impaired in baseline have normal vision after spectacle prescription intervention. While 5.5% (11) of respondents who were visually impaired in baseline still had impaired vision after spectacle prescription intervention. The association between visual impairment in baseline and with spectacle prescription intervention was statistically significant ($\chi^2= 28.691, df= 1, P=0.001$).

Variable	Baseline		Intervention	
	Frequency (n = 201)	Percent (%)	Frequency (n = 201)	Percent (%)
Anaemia Category				
Normal	124	61.7	201	100
Mild Anaemia	8	4.0		
Moderate Anaemia	32	15.9		
Severe Anaemia	37	18.4		
Visual Impairment Category				
Normal (VA > or = 6/12)	143	71.1	190	94.5
Mild (< 6/12 - 6/18)	40	19.9	8	4.0
Moderate (< 6/18 - 6/60)	13	6.5	3	1.5
Severe (< 6/60 - 3/60)	5	2.5	0.0	0.0

Table 2: Prevalence of Anaemia and Visual Impairment by Categories among Baseline and Intervention Respondents.

Variables	Visual Impairment with Spectacle (%)		P-Value (P < 0.05)
	Normal Vision	Impaired Vision	
Visual Impairment Baseline	Normal Vision	Impaired Vision	
Normal Vision	143 (71.1)	0	
Impaired Vision	47 (23.4)	11 (5.5)	0.001

Table 3: Comparison of Prevalence of Visual Impairment of Baseline and Spectacle Prescription of Intervention.

In Figure 2, refractive error with prevalence of 23.4% (47) was the major cause of VI among the respondents. Glaucoma and Cornea opacity constituted a prevalence of 4.5% (9) and 1.0% (2) respectively.

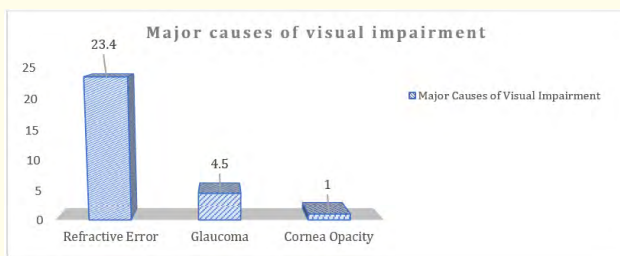


Figure 2: Major Causes of Visual Impairment among Respondents.

In Table 4, Spearman’s rho Bivariate Correlation was used to ascertain the relationship between visual impairment, anaemia

and antioxidant vitamins ACE among the respondents. The correlation of visual impairment was not significant with anaemia ($r = -0.064, p = 0.364$), vitamin E deficiency ($r = 0, p = 0$), vitamin C deficiency ($r = 0.078, p = 0.271$) and vitamin A deficiency ($r = -0.131, p = 0.064$).

In Table 5, the mean values of vitamin A and haemoglobin was higher among respondents in post intervention than that of baseline. While the mean values of vitamin C and vitamin E was higher in baseline than that of post intervention. The mean serum antioxidant vitamins and haemoglobin between baseline and intervention were statistically significant at $P < 0.05$.

In Table 6, using a multinomial logistic regression, vitamin E and iron supplements intake were predicted to decrease the risk of having mild VI with odd ratios of 0.961 times and 0.993 times (relative to normal vision) respectively compare to when the supplement was not taken. The association between mild VI

Variables	Baseline Mean ± S.D	Intervention Mean ± S.D	T- Value	Degree of Freedom	P-Value
Vitamin A Baseline and Intervention	25.8468 ± 6.47586	27.5502 ± 5.82645	-2.672	200	0.008
Vitamin C Baseline and Intervention	11.0045 ± 6.27169	9.5831 ± 6.65954	2.219	200	0.028
Vitamin E Baseline and Intervention	16.5841 ± 6.89161	14.4975 ± 3.16679	4.292	200	< 0.001
Haemoglobin Baseline and Intervention	135.5975 ± 70.88628	239.2527 ± 47.87183	-17.328	200	< 0.001

Table 5: Intervention Vitamins and Iron Supplements on Baseline Vitamins and Haemoglobin.

and vitamin E supplement (B= - 0.039; P = 0.552; 95% CI: 0.844 – 1.095) and iron supplement (B= - 0.007; P = 0.107; 95% CI: 0.984 – 1.002) was not statistically significant (P > 0.05). Vitamin C and iron supplements were predicted to decrease the risk of moderate VI with odd ratios of 0.996 times and 0.997 times respectively,

though their associations were not statistically significant. Likewise, vitamins A, C and iron supplements were predicted to decrease the risk of severe VI with odd ratios of 0.964 times, 0.873 times and 0.984 times respectively, though their associations were not statistically significant (P > 0.05).

Visual Acuity ^a	Variables	B	P-Value (P < 0.05)	Exp(B)	Lower Bound	Upper Bound
Mild (< 6/12 - 6/18)	Intercept	1.647	0.455			
	Vitamin A	0.014	0.668	1.014	0.952	1.080
	Vitamin C	0.053	0.036	1.055	1.004	1.108
	Vitamin E	-0.039	0.552	0.961	0.844	1.095
	Haemoglobin	-0.007	0.107	0.993	0.984	1.002
Moderate (< 6/18 - 6/60)	Intercept	-1.328	0.691			
	Vitamin A	0.020	0.713	1.020	0.916	1.136
	Vitamin C	-0.004	0.935	0.996	0.895	1.107
	Vitamin E	0.137	0.172	1.146	0.942	1.395
	Haemoglobin	-0.003	0.649	0.997	0.983	1.011
Severe (< 6/60 - 3/60)	Intercept	13.617	0.291			
	Vitamin A	-0.036	0.814	0.964	0.712	1.305
	Vitamin C	-0.136	0.434	0.873	0.622	1.227
	Vitamin E	0.154	0.434	1.167	0.793	1.718
	Haemoglobin	-0.016	0.516	0.984	0.937	1.033

Table 6: Effects of Antioxidant Vitamins and Iron Supplements on Visual Acuity of Respondents.

a. The reference category is: Normal (VA ≥ 6/12).

In Table 7, vitamin A was predicted to decrease the risk of mild and severe anaemia with odd ratios of 0.627 times and 0.491 times respectively compare to when the supplement was not taken, though their associations were not statistically significant (P > 0.05). Vitamin C was predicted to decrease the risk of moderate

and severe anaemia with odd ratios of 0.160 times and 0.037 times respectively compare to when the supplement was not taken, though their associations were not statistically significant (P > 0.05). Iron supplement was predicted to decrease the risk of mild, moderate and severe anaemia with odd ratios of 0.745

times, 0.658 times and 0.829 times respectively compare to when the supplement was not taken, though their associations were not statistically significant ($P > 0.05$).

Anaemia Category ^a		B	P-Value	Exp(B)	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
Mild Anaemia	Intercept	207.260	0.937			
	Vitamin A	-0.467	0.977	0.627	1.027E-14	3.829E+13
	Vitamin C	0.054	0.998	1.055	3.872E-23	2.875+22
	Vitamin E	0.693	0.992	2.001	5.249E-58	7.626E+57
	Haemoglobin	-0.295	0.964	0.745	2.206E-6	251374.200
Moderate Anaemia	Intercept	963.550	0.876			
	Vitamin A	0.341	0.999	1.407	2.720E-303	7.276E+302
	Vitamin C	-1.833	0.968	0.160	5.086E-40	5.026E+37
	Vitamin E	4.061	0.995	58.014	0.000	. ^b
	Haemoglobin	-0.419	0.995	0.658	1.362E-59	3.178E+58
Severe Anaemia	Intercept	1093.477	0.965			
	Vitamin A	-0.712	0.999	0.491	0.000	. ^b
	Vitamin C	-3.285	0.990	0.037	2.759E-232	5.084E+228
	Vitamin E	1.255	0.999	3.506	0.000	. ^b
	Haemoglobin	-0.188	0.998	0.829	9.291E-69	7.389E+67

Table 7: Effects of Antioxidant Vitamins and Iron Supplements on Anaemia.

a. The reference category is: Normal.

b. Floating point overflow occurred while computing this statistic. Its value is therefore set to system missing.

Discussion

Anaemia

In this study, the prevalence of anaemia at baseline was 38.3% (of which 4.0% mild, 15.9% moderate and 18.4% severe) and 0.0% at post intervention [8]. The prevalence of anaemia in this study is classified as moderate public health problem [3,34]. The result of this study is similar to the prevalence of anaemia among primary school children in Lagos, Nigeria which was reported to be 38.1% [35], and that of the study done by Assefa, *et al.* [34] among school children in Ethiopia whose prevalence of anaemia was 37.6%. The result of this study was higher than the prevalence of anaemia in school children of which 3% was reported in Canada [36], 23.1% in Somaliland [37], 15.5% in Ethiopia [38] and 14% in Indonesia [39]. On the contrary, it was lower than the 43% worldwide prevalence among children [40], 40.7% in Gaza Strip, Palestine [41] and

88.3% in Sudan due to the report of high prevalence of infectious diseases [42]. This study also showed that iron supplementation reduced anaemia from 38.3% to 0%. Similarly, in another study, iron supplementation was shown to reduce the risk of anaemia by 50% and the risk of iron deficiency by 79% [36]. With the 100% reversal of anaemia in this study, the cause of anaemia among the study population may probably be due to low bioavailability of heme iron in their diet.

Antioxidant Vitamins ACE

The prevalent of vitamin A deficiency (VAD) in our study was 12.4%. This was lower than the nationwide prevalence of 29.5% and 17% in the northwest part of Nigeria [11] as well as 29.6% in Benin city, Nigeria [43] and 44.0% in Nsukka, Nigeria [44]. The result of this study was also lower than the prevalence of VAD

in Uganda (27.9%) and Tanzania (24%) [11]. It is similar to the prevalence of 12.0% in northeast among children < 6 years old [11]. The lower prevalence of VAD in this study may be due to high consumption of red palm oil rich in beta-carotene which is a staple diet in Delta State, and the educational level of the respondent's parent which was majorly secondary and tertiary may influence the choice of vitamin A rich food being given to the children.

The 12.9% prevalence of vitamin C deficiency in this study may be due to the lower socioeconomic status of the respondent's parent due to higher deprivation of good quality, nutrient-dense food [12,27,45].

There was no vitamin E deficiency in this study. It was in consonance with Rizvi, *et al.* reported that vitamin E deficiency is quite rare in humans, that it happens almost exclusively in people with an inherited or acquired condition such as cystic fibrosis, short bowel syndrome, bile duct obstruction or disorders of fat metabolism that impairs their ability to absorb the vitamin [46].

Visual impairment

The 28.9% prevalence of visual impairment (VI) at baseline in this study was similar to the study conducted in India with a prevalence of VI of 26.68% [47] and higher than the prevalence of 6.7% among school children aged 5 to < 16 years in Ogun State, Nigeria [48] and 8.0% among school children 8-18 years in North West Ethiopia [49]. This higher prevalence of VI is probably due to variation in the measurement of visual impairment. In addition, the discrepancy could also be attributed to the difference in age of children, this study included children up to age of 19 years since increasing age is an important predictor of visual impairment. However, the VI prevalence of 28.9% in this study was lower than 37.58% in North-East Ethiopia [50]. Refractive error (RE) was the major cause of VI with prevalence of 23.4% [8,30]. This was similar to the systematic review carried out among children and adolescence in Ethiopia, in which RE was the most common cause of visual impairment [51].

Relationship between anaemia, antioxidant vitamins and visual impairment

In this study, VI negatively correlate with anaemia, though, their association was not significant ($r = - 0.064$, $p = 0.364$). The prevalence of VI and anaemia in this study was 28.9% and 38.3%

(13.4% of male and 24.9% of female) respectively. Study done by Nayak, *et al.* revealed that 67.0% girls were suffering from anaemia compare to 58.7% of boys, while 13.9% adolescents had visual impairment [52]. Thakor, *et al.* study among adolescents aged 5-19 years revealed that 46.7% girls were suffering from anaemia compare to 37.3% of boys and 12.9% adolescents had visual impairment [53]. Another study conducted by Srinivasan and Prabhu 61.4% among adolescents aged 10-14 years, reported the prevalence of anaemia in adolescents to be 79.6% and 4.4% adolescents had defective vision [54]. Panda, *et al.* reported that the prevalence of anaemia in boys was 22.9% and in girls was 30.5%, and 5.6% adolescents had refractive errors [55]. The correlation between visual impairment and anaemia in this study was not statistically significant ($p = 0.364$). Meanwhile, the few studies on anaemia and visual impairment did not research on their relationship [52-55]. In this study, antioxidant vitamins were not correlated with anaemia ($P > 0.05$). Similarly, Hashizume, *et al.* in their findings reported that vitamin A (Serum retinol) concentrations were not associated with anaemic status [56].

Effect of antioxidant vitamins and iron supplements on visual acuity

There is little known research done on the effect of antioxidant vitamins on VI in children. This study showed that antioxidant vitamins ACE and iron supplements were predicted to decrease the risk of anaemia and VI compare with when they were not taken. Though, their associations were not statistically significant ($P > 0.05$), the importance of their intake cannot be over emphasized. An increasing amount of evidence suggests that deficiency of vitamin A not only causes night blindness but may also cause structural degeneration of the retina. Daily intake of antioxidant vitamin supplements may provide protection against possible transient decrease in serum retinol concentration which may adversely affect photoreceptor function as well as reduce risk of cataract development [57-59].

Conclusion

The burden of visual impairment, anaemia, vitamin A deficiency and vitamin C deficiency among school children in Delta State was high. Uncorrected refractive error was the commonest visual impairment among school children. This study showed that antioxidant vitamins supplementation, iron supplementation and

spectacle prescription was effective in reducing the prevalence of anaemia, antioxidant vitamins deficiencies and visual impairment among primary and secondary school children in Delta State.

Acknowledgements

We are thankful to the Head of Department of Public and Community Health - Prof Josiah O Adjene, Dr. Joel Okpoghono and all lecturers and staffs of Novena University, Ogume, Delta State, Nigeria who supported us in this study.

Bibliography

1. Hung NV, et al. "Anemia, Malaria and Hookworm Asian, Infections in a Vietnamese Ethnic Minority". *Southeast Asian Journal of Tropical Medicine and Public Health* 36 (2005): 816-821.
2. Nelima D. "Prevalence and Determinants of Anaemia among Adolescent Girls in Secondary Schools in Yala Division Siaya District, Kenya". *Universal Journal of Food and Nutrition Science* 3 (2015): 1-9.
3. World Health Organization/UNICEF. Iron deficiency anaemia, assessment, prevention and control: a guide for programme managers. World Heal. Organ. Geneva. (2001).
4. Moyegbone JE., et al. "Epidemiology of visual impairment: focus on Delta State, Nigeria". *The International Journal of Community Medicine and Public Health* 7 (2020): 4171-4179.
5. Ifebajo A., et al. "Assessment of Anthropometric and Iron Status of School Children in Somolu Local Government Area of Lagos State, Nigeria". *International Journal of Advanced Academic Research* 6 (2020): 60-82.
6. Dreyfuss E., et al. "Hookworms, Malaria and Vitamin A Deficiency Contribute to Anemia And Iron Deficiency among Pregnant Women in the Plains Of Nepal". *Journal of Nutrition* 130 (2000): 2527-2536.
7. Mikki N and H Stigum. "Anaemia prevalence and associated sociodemographic and dietary factors among Palestinian adolescents in the West Bank". *Eastern Mediterranean Health* 17 (2011): 208-217.
8. Moyegbone J., et al. "Preventive programs in public health : seminar presentations on HIV, malaria and visual impairment". *International Journal of Advances in Medicine* 9 (2022): 856-859.
9. Wirth JP, et al., Vitamin A Supplementation Programs and Country-Level Evidence of Vitamin A Deficiency". *Nutrients* 9 (2017): 190-107.
10. Ross DA. "Recommendations for Vitamin A Supplementation". *Journal of Nutrition* 131 (2002): 2902S-2906S.
11. Maziya-Dixon BB., et al. "Vitamin A deficiency is prevalent in children less than 5 y of age in Nigeria". *Journal of Nutrition* 136 (2006): 2255-2261.
12. Hayder MA-T., et al. "Association between Low Serum Vitamin C Level and Cataract in Elderly Patients". *EC Ophthalmology* 10 (2019): 751-760.
13. Kamath YS., et al. "The association of age-related cataract and serum Vitamin C". *Indian Journal of Clinical and Experimental Ophthalmology* 4 (2017): 287-290.
14. Rowe S and AC Carr. "Factors Affecting Vitamin C Status and Prevalence of Deficiency : A Global Health Perspective". *Nutrients* 1 (2020): 1-19.
15. Chen H., et al. "Role of serum vitamin A and E in pregnancy". *Experimental and Therapeutic Medicine* 16 (2018): 5185-5189.
16. Morris M., et al. "Vitamin E and cognitive decline in older persons". *Archives of Neurology* 59 (2002): 1125-1132.
17. Odoko J., et al. "Factors Influencing Utilization of Insecticide-treated Nets among Pregnant Women and Children in Bayelsa and Rivers States, Nigeria". *International Journal of Tropical Diseases* 44 (2023): 55-69.
18. Alghadir AH., et al. "Association of physical activity, vitamin E levels, and total antioxidant capacity with academic performance and executive functions of adolescents". *BMC Pediatrics* 19 (2019): 156-163.
19. Eiler RJ. "Cyanmethemoglobin". *American Journal of Clinical Pathology* 47 (1967): 212.
20. Henry RF, et al. "Principles and Techniques in Clinical Chemistry". 2nd ed., MD, Hagerstown, (1974).
21. Chan M. "Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity". Geneva, Switz. World Heal. Organ. (2011): 1-6.

22. Neeld WN and JB Jr Pearson. "Macro and micro methods for the determination of serum vitamin A using trifluoroacetic acid". *Journal of Nutrition* 79 (1963): 454-462.
23. Mejia L and G Arroyave. "The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala". *American Journal of Clinical Nutrition* 36 (1982): 87-93.
24. World Health Organization., et al. "Vitamin A supplements : a guide to their use in the treatment of vitamin A deficiency and xerophthalmia / prepared by a WHO/UNICEF/IVACG task force". 2nd ed., World Heal. Organ. (1997).
25. Sadasivam A and S Manickam. "Biochemical methods". 3rd ed., New Age International Publishers, New Delhi, India, (2008).
26. Kivcak T and B Mert. "Quantitative determination of a-tocopherol in Arbutus Ledo densitometry and Colorimetry". *Fitotetrapia* 72 (2001): 656-661.
27. Bates B., et al. "National Diet and Nutrition Survey Years 1 to 9 of the Rolling Programme (2008/2009-2016/2017): Time Trend and Income Analyses". *Public Heal. Engl. London, UK.* (2019): 56.
28. Traber M and D Manor. "Vitamin E". *Advances in Nutrition* 3 (2012): 330-331.
29. Ramke J., et al. "Prevalence and causes of blindness and low vision in Timor-Leste". *British Journal of Ophthalmology* 91 (2007): 1117-1121.
30. Moyegbone J., et al. "Prevalence of Visual Impairment among Primary and Secondary School Children in Delta State, Nigeria". *Journal of the Nigerian Optometric Association* 25 (2023): 42-53.
31. World Health Organization. "Guideline: Vitamin A supplementation in infants and children 6-59 Months of Age". Geneva World Heal. Organ. (2011).
32. Stoltzfus R and M Dreyfuss. "Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia". ILSI Press, Washington DC, 1998.
33. Tanumihardjo S. "Biomarkers of vitamin A status: what do they mean?", in: World Heal. Organ. Rep. Priorities Assess. Vitam. A Iron Status Popul., Geneva, World Health Organization., Panama City, Panama, 15-17 September 2010, (2012).
34. Assefa S., et al. "Prevalence and severity of anemia among school children in Jimma Town, Southwest Ethiopia". *BMC Hematology* 14 (2014): 1-9.
35. Akeredolu IA., et al. "Iron, Zinc, and Copper Malnutrition among Primary School Children in Lagos, Niagara". *Food and Nutrition Sciences* 02 (2011): 1063-1070.
36. Low M., et al. "Effects of daily iron supplementation in primary-school- aged children: systematic review and meta-analysis of randomized controlled trials". *Cmaj* 185 (2013): 1477-1478.
37. Salim S., et al. "Prevalence and Factors Associated with Iron Deficiency Anemia among School-Age Children in Primary Schools in Burao City, Somaliland, 2020". *Journal of Blood Disease* 11 (2021): 67-80.
38. Getaneh Z., et al. "Prevalence of Anemia and Associated Factors among School Children in Gondar Town Public Primary Schools, Northwest Ethiopia: A School-Based Cross-Sectional Study". *PLoS One* 12 (2017): E0190151.
39. Andriastuti M., et al. "Prevalence of Anemia and Iron Profile among Children and Adolescents with Low Socio-Economic Status". *International Journal of Pediatrics and Adolescent Medicine* 7 (2020): 88-92.
40. Nkurunziza J., et al. "Prevalence and factors associated with anaemia in children aged 6 - 24 months living a high malaria transmission setting in Burundi". *PLoS One* 17 (2022): e0273651.
41. Jalambo M., et al. "Prevalence of Anemia, Stunting and Parasitic Infection Among 5-6 Years Prevalence of Anemia, Stunting and Parasitic Infection Among 5-6 Years Children in Gaza Strip, Palestine, Public Heal". *Preventive Medicine* 5 (2020): 5-15.
42. Eltayeb MS., et al. "Assayed, Prevalence of Anaemia among Quranic School (Khalawi): Students (Heiran): in Wad El Magboul Village, Rural Rufaa, Gezira State, Central Sudan: A Cross-Sectional Study". *Pan African Medical Journal* (2016): 24.
43. Atimati A., et al. "High prevalence of vitamin A deficiency in school-age children in Benin City". *Annals of Biomedical Sciences* 11 (2012): 106-116.
44. Ayogu RNB., et al. "Prevalence and factors associated with anthropometric failure, vitamin A and iron deficiency among adolescents in a Nigerian urban community". *African Health Sciences* 16 (2016): 389-398.
45. McCall S., et al. "Plasma vitamin C levels: Risk factors for deficiency and association with self-reported functional health in the European Prospective Investigation into Cancer-Norfolk". *Nutrients* 11 (2019): 1552.

46. Rizvi S., *et al.* "The Role of Vitamin E in Human Health and Some Diseases". *SQU Medical Journal* 14 (2014): 157-165.
47. Vashist P., *et al.* "Blindness and visual impairment and their causes in India: Results of a nationally representative survey". *PLoS One* 17 (2022): e0271736.
48. Alabi A., *et al.* "Visual Impairment and Ocular Morbidities among Schoolchildren in Southwest, Nigeria". *Nigerian Postgraduate Medical Journal* 25 (2018): 166-171.
49. Zelalem M., *et al.* "Prevalence of visual impairment among school children in three primary schools of Sekela Woreda, Amhara regional state". *SAGE Open Medicine* 7 (2019): 1-8.
50. Seid M., *et al.* "Visual impairment and its predictors among people living with type 2 diabetes mellitus at Dessie town hospitals , Northeast Ethiopia: institution-based cross-sectional study". *BMC Ophthalmology* 22 (2022): 52-64.
51. Atlaw D., *et al.* "Prevalence of visual impairment due to refractive error among children and adolescents in Ethiopia: A systematic review and meta-analysis". *PLoS One* 17 (2022): e0271313.
52. Nayak S., *et al.* "Prevalence of Anaemia and Visual Impairment among School Going Adolescents of Patan City, Gujarat: A Cross-Sectional Study". *National Journal of Community Medicine* 6 (2015): 449-451.
53. Thakor N., *et al.* "Health status of children of primary and secondary boarding schools of Gandhinagar district". *International Journal of Medical Science and Public Health* 3 (2014): 866-869.
54. Srinivasan K and G Prabhu. "A Study of The Morbidity Status of Children In Social Welfare Hostels In Tirupati Town". *Indian Journal of Community Medicine* 31 (2007): 51-56.
55. Panda P., *et al.* "Health Status of School Children in Ludhiana city". *Indian Journal of Community Medicine* 4 (2000): 150-155.
56. Hashizume M., *et al.* "Anaemia, iron deficiency and vitamin A status among school-aged children in rural Kazakhstan". *Public Health Nutrition* 8 (2005): 564-571.
57. Zhao Y., *et al.* "Vitamins and Mineral Supplements for Retinitis Pigmentosa". *Hindawi Journal of Ophthalmology* 2019 (2019): 1-11.
58. Klaver C and A Thiadens. "Vitamin A for children with retinitis pigmentosa: an unresolved mystery". *JAMA Ophthalmology* 136 (2018): 496-497.
59. Francisco SG., *et al.* "Dietary Patterns, Carbohydrates, and Age-Related Eye Diseases". *Nutrients* 12 (2020): 1-19.