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Relationship Between Serum Vitamin A Levels and Maternal Education and Social Status Among Undernourished Children in Zaria

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Abstract
Background: Vitamin A deficiency (VAD) affects an estimated 6 million preschool children in Nigeria and 20 million in Africa. The deficiency significantly increases childhood morbidity and mortality when associated with undernutrition.
Objective: To determine the relationship between serum vitamin A levels, maternal education, and social status among undernourished Nigerian children.
Methodology: This was a case-control, hospital-based, descriptive study of children aged 6-59 months carried out at the Institute of Child Health (ICH) Banzazzau, Zaria. The serum vitamin A level was assayed by high-performance liquid chromatography. Values below a cut-off, usually taken as 0.70 µmol/L (20 µg/ dl) and 0.35 µmol/L (10 µg/dl), defined as low serum vitamin A and vitamin A deficiency, respectively.
Results: Sixty-six children were recruited as the cases and age-matched controls. Eleven children in each group had low serum vitamin A levels. The three children with vitamin A deficiency belonged to the cases.
The overall mean serum vitamin A for the cases was 59.44±12.93µg/dl, while it was 59.90±14.06µg/dl for the controls. The overall mean serum vitamin A levels based on maternal education were 57.26±16.2µg/dl for the cases and 61.02±5.36µg/dl for the controls (p = 0.01). The overall mean serum vitamin A levels based on social class were 59.36±7.38µg/dl and 61.67±6.74µg/dl for the cases and controls, respectively (p = 0.01).
Conclusion: The lowest mean serum vitamin A levels for the cases were recorded in the younger age groups compared to the controls. Maternal educational levels and social class greatly influenced their children's serum vitamin A levels.
Keywords: Childhood, Educational level, Retinol, Social class, Under-nutrition, Vitamin A.

Introduction

Vitamin A is a generic term referring to all compounds, other than carotenoids, that exhibit retinol activity.[1] Vitamin A deficiency refers to serum vitamin A level <0.7µmol/l or 20µg/l, and it is a public health problem when more than five per cent of the population is affected in developing countries of the world.[1]
Vitamin A deficiency (VAD) is a significant public health problem in many developing countries of the world, including Nigeria.[1-3] It is not only a cause of blindness but also a cause of childhood morbidity and mortality.[2, 3] It is well established that malnourished children have low serum vitamin A levels mainly due to inadequate intake of dietary vitamin A. It is estimated that over 124 million children are vitamin A deficient worldwide. In Africa, approximately 20 million preschool children are at risk of VAD, while 6 million preschool children may be at risk of VAD in Nigeria.[4]

A study carried out in Nigeria on a total of 6480 households with a mother and an under-five child randomly sampled nationwide reported that 24.8% of under-five children had marginal deficiency (serum vitamin A concentration < 20µg/dl) and, therefore, were vitamin A deficient, 4.7% had serum vitamin A concentration < 10 µg/dl and hence, were suffering from severe vitamin A deficiency (clinical deficiency), and 71.5 % of children were normal.[4] The prevalence of marginal deficiencies was 28.2% in the dry savannah and 22.8% in the humid forest. The clinically deficient cases were more common in the humid forest (7.1%) than in the dry savannah (3.1%) and moist savannah (2.4%). The distribution of marginal deficiency was 23.4% in rural areas and 25.1% in urban areas. Clinical deficiencies occurred in 7.5% of the medium areas (as defined in the Word VAD Map), while the urban (3.4%) and rural (2.2%) areas had much lower prevalences.[4] Low social status and low maternal education are risk factors for vitamin A deficiency. Malnutrition and vitamin A deficiency commonly affect children in developing countries.[5, 6] Both conditions have common predisposing factors. Vitamin A deficiency is a significant health concern in severe malnutrition and is associated with substantial morbidity and mortality.

In Africa, it is estimated that approximately 20 million preschool children are at risk of VAD[6,] while in Nigeria, 6 million preschool children may be at risk of VAD.[4, 5] The blinding form of severe VAD afflicts 350,000 – 500,000 young children annually; most of them residing among people with low incomes in the developing countries.[5] Improved vitamin A nutriture would be expected to prevent approximately 1-2 million deaths annually among children aged 1 – 5 years. An additional 0.25 – 0.5 million deaths may be averted if improved vitamin A nutriture can be achieved during the latter half of infancy.[5,6] This study was carried out to determine the relationship between vitamin A status and maternal education and social class among undernourished Nigerian children.

Methodology

Study settings

The study was conducted at the Institute of Child Health (ICH) Banzazzau, Zaria. This institute, located in the Banzazzau area within the walled city of Zaria, serves the community and children population mainly from Zaria and its environs. It is also the primary healthcare outlet of the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, which offers outpatient services to an average of 200 patients daily.

Study design

This was a case-control, hospital-based descriptive study conducted over ten months.

Study population

The study population consisted of consecutive malnourished children aged 6-59 months who presented to ICH. The WHO Z-score for anthropometric parameters[7] were used to classify malnutrition into mild, moderate and severe using the weight-for-age, height/length-for-age, mid-arm circumference (MAC) and, presence or absence of oedema, socioeconomic and educational status of the mothers were recorded. Age-matched normal children who presented to the ICH with clinical features of malaria, ARIs, and acute diarrhoeal diseases were recruited as controls. Informed consent was duly obtained from each child's parents or caregivers before recruitment into the study.

Sample size determination and sampling

The minimum sample size was determined using formula [8] to compare the proportions in two groups based on the vitamin A status in malnourished under-five children from a previous study.[9]

$$S = \frac{[Z1 - \frac{\alpha}{2}\sqrt{2P_2(1 - P_2)} + Z_1 - \beta\sqrt{P_1(1 - P_1) + P_2(1 - P_2)}]^2}{(P_1 - P_2)}$$

The calculated sample size was 132.

The children were selected consecutively using a convenient sampling technique.

Study instruments

Relevant data such as name, age, sex, parental occupation and educational status, and dietary history with particular emphasis on the frequency of ingestion of vitamin A–-rich foods were obtained using a proforma designed from the study from all the children enrolled on the study. History of illnesses such as measles, diarrhoeal diseases and acute respiratory infections within the preceding two weeks were also recorded.

Physical examination was performed, emphasising anthropometry, skin changes, hair changes and oedema.[4 - 6]

Data collection

The questionnaire was administered by an interviewer who was trained for the purpose. Ethical principles guiding research, such as respecting the participants' autonomy and confidentiality, were upheld. Socioeconomic classification of the family was done by scoring the highest educational qualification and present occupation of each parent using the model recommended by Ogunlesi *et al.* [10] Each child was assigned to a socioeconomic class (I-V) based on the score.[10] The data recorded included the details of the highest educational qualification and the present occupation of both parents of each child if the child lives with biological parents. Otherwise, the data on foster parents or guardians was obtained. A score was awarded for the education and occupation of each parent or guardian. The mean of the four scores (from the father's education and occupation and the mother's education and occupation) was determined. This mean score, to the nearest whole number, was the socioeconomic status (I, II, III, IV and V respectively) assigned to the child.[10]

Biochemical Procedures

Blood collection and processing

Four millilitres of venous blood were drawn into a plain bottle from the selected patients, and the sample bottle was wrapped in black nylon to prevent light from denaturing the retinol content. The sera were separated by centrifugation at 1000g for 10 minutes at the ICH laboratory. The serum was taken with a Pasteur pipette into a 2ml tube, re-labelled and wrapped in black nylon. The samples were immediately kept in a cooler containing ice cubes and transported to the Chemical Pathology Laboratory, ABUTH Zaria, where they were frozen at $-20^{0}C$ until analysis. The samples were analysed for serum vitamin A levels at the Chemical Pathology Laboratory, University College Hospital, Ibadan.

Serum vitamin A estimation

The Bieri method determined serum vitamin A levels using high-performance liquid chromatography (HPLC Shimadzu prominence, Japan).[11] The principle is that a given volume of serum or plasma is diluted with methanol, which denatures plasma proteins, and retinol is extracted with hexane, a suitable organic solvent. One hundred microlitres (100µl) of serum and 100 of the internal standard 0.6µg retinyl acetate/ml (used as internal standards to correct for losses during extraction or analysis) were transferred in a test tube. A hundred microlitres of methanol was added to denature, and precipitate proteins (essential for the release of retinol from retinol–binding protein), and the sample was mixed. Two hundred microlitres (200µl) of spectograde hexane was added, and the contents were mixed vigorously but intermittently for 45 seconds on a vortex mixer and then centrifuged at 50,000 rpm for three minutes to ensure phase separation using a fixed head, bucket-sized centrifuge, made from Japan. The upper hexane layer was transferred to another tube using a Pasteur pipette. The combined hexane extracts were then evaporated under a gentle stream of argon, and the residue was re-dissolved in 50µl of propane-2-ol. This was injected into the HPLC column specification with a 100 µl syringe for High-Performance Liquid Chromatography. Elution was carried out with methanol: water (95:5 v/v at a flow rate of 1.5ml/min, monitored at 5 minutes. Retinol was quantitated using peak height ratios relative to an internal standard (retinyl acetate).[11]

The serum vitamin A levels were classified as deficient (<10µg/dl), low (10 - <20µg/dl) and normal (≥20µg/dl).

Data management and analysis

The questionnaires were examined for completeness and accuracy. The data were subsequently entered into an electronic database using the Statistical Package for Social Sciences version 15.4. The mean values were compared using the Student's t-test, and the significance level was set at *a P* value of less than 0.05.

Ethical approval

Approval for the study was obtained from the ethical committee of Ahmadu Bello University Teaching Hospital Zaria.

Results

A total of 66 children in each of the cases and the age-matched controls were sampled for the study. There were 26 (39.4%) males and 40 (60.6%) females with a male-to-female ratio of 1:1.5 among cases, while for the controls, there were 30 (45.5%) males and 36 (54.5%) females.

The distribution of serum vitamin A levels is shown in Table I. Three (4.5%) and 11 (16.7%) of the cases with wasting had deficient and low serum vitamin A levels, respectively, compared to 11 (16.7%) of controls with low serum vitamin A levels, while none had vitamin A deficiency. Similarly, 3 (4.5%) and 11 (16.7%) of the cases with stunting had deficient and low serum vitamin A levels, respectively, compared to 11 (16.7%) of controls with low serum vitamin A levels. The differences between wasting and the controls lacked statistical significance (p = 0.492) between wasting and controls and stunting and controls.

Table I: Distribution of vitamin A status among the cases and controls

	Serum vitamin A status					
	Deficient*	**Low#**	**Normal^**	**Total**		
Nutritional status	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**X^2**	**p-value**
Wasting	3 (4.5)	11 (16.7)	52 (78.8)	66 (100.0)	1.416	0.492
Stunting	3 (4.5)	11 (16.7)	52 (78.8)	66 (100.0)	1.416	0.492
Controls	0 (0.0)	11 (16.7)	55 (83.3)	66 (100.0)		

***Deficient vitamin A = < 10µg/dl** **#Low vitamin A= 10 - < 20µg/dl** **^Normal vitamin A = ≥20µg/dl**

Table II compares serum vitamin A levels between the two groups, distributed by age group and sex. Eleven cases had low serum vitamin A levels. Seven (77.8%) females had the highest prevalence of low serum vitamin A levels compared to 2 (22.2%) males aged 13-24 months. Similarly, 11 controls had low serum vitamin A levels, of which 4 (66.7%) males had the highest prevalence of low serum vitamin A levels compared to 2 (33.3) females within the age range of 6-12 months. Only 3 cases of vitamin A deficiency were seen among the cases in the 13-24 months age group; these comprised 2 (66.7%) males and 1 (33.3) female.

Table II: Distribution of low serum vitamin A levels by age and sex among the children in the cases and controls

	Cases			Controls		
Age (months)	**Males, n (%)**	**Females, n (%)**	**Total, n (%)**	**Males, n (%)**	**Females, n (%)**	**Total, n (%)**
6-12	0 (0.0)	1 (100.0)	1 (100.0)	4 (66.7)	2 (33.3)	6 (100.0)
13-24	2 (22.2)	7 (77.8)	9 (100.0)	2 (66.7)	1 (33.3)	3 (100.0)
25-36	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100)	1 (100.0)
37-48	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
49-59	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1(100.0)
Total	**2 (18.2)**	**9 (81.8)**	**11 (100.0)**	**6 (54.5)**	**5 (45.5)**	**11 (100.0)**

For the 6-12 months age group, the mean serum vitamin A levels of the 18 cases was 65.69±9.51µg/dl and 58.36±13.21µg/dl for the controls. For the 49-59 months age group, the mean serum vitamin A level of the two cases was 57.23±2.45µg/dl and 58.20±13.58µg/dl for the controls. The overall mean serum vitamin A was 59.44±12.93µg/dl for the cases and 59.90±14.06µg/dl for the controls. The observed differences between the cases and controls were statistically significant for the age groups of 6–12 months (t = 3.659; p = 0.0004) and 13–24 months (t = 2.163; p = 0.032), as shown in Table III.

Table III: Comparison of the mean serum vitamin A levels among the cases and controls

Mean serum vitamin A level (µg/dl)				
	Cases, n = 66	**Controls, n = 66**		
Age (months)	**Mean**	**Mean**	**t**	**p-value**
6 – 12	65.69±9.51	58.36±13.21	3.659	0.0004
13 – 24	56.08±13.78	62.11±17.98	2.163	0.032
25 – 36	59.96±1.31	59.80±11.67	0.111	0.912
37 – 48	59.40±8.94	63.15±14.09	1.826	0.070
49 – 59	57.23±2.45	58.20±13.58	0.571	0.569
Total	**59.44±12.93**	**59.90±14.06**	**0.196**	**0.845**

Table IV shows the distribution of vitamin A status in relation to maternal education. Two (8.0%) out of 25 cases whose mothers had secondary education were vitamin A deficient, while 1 (3.6%) case belonging to a mother with Islamic education had serum vitamin A deficiency. There were no children with serum retinol deficiency among the controls.

Table IV: Distribution of vitamin A status among cases and controls in relation to maternal education

Serum vitamin A levels (µg/dl)						
	Cases			**Controls**		
	<10	**10-19**	**>20**	**<10**	**10-19**	**>20**
Maternal education	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*
None	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Islamic	1 (3.6)	5 (17.9)	22 (78.6)	0 (0.0)	0 (0.0)	8 (100)
Primary	0 (0.0)	4 (40.0)	6 (60.0)	0 (0.0)	6 (46.1)	7 (53.8)
Secondary	2 (8.0)	2 (8.0)	21 (84.0)	0 (0.0)	3 (8.8)	31 (91.1)
Tertiary	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (18.2)	10 (81.8)
Total	**3 (4.5)**	**11 (16.6)**	**52 (78.8)**	**0 (0.0)**	**11 (16.7)**	**55 (83.3)**

Table V shows the levels of serum vitamin A among children distributed according to maternal levels of education. Apart from the high mean serum vitamin A of 60.92±12.40µg/dl among the cases whose mothers had secondary education, there was no gradual increase in mean serum vitamin A levels as maternal education improved. However, for the controls, there was a progressive increase in mean serum vitamin A levels as maternal education improved. There was no child whose mother had no formal education. Statistically significant differences were observed in the mean serum vitamin A levels of the cases and controls whose mothers had Islamic education ($t = 4.161$; $p = 0.0001$) and tertiary education ($t = 4.841$; $p = 0.0001$).

Table VI shows the pattern of vitamin A status among the cases and controls distributed according to the social classes. Among the cases, one (12.5%) of 8 children in social class II was vitamin A deficient, and 2 (7.1%) out of 28 in social class IV had vitamin A deficiency. There was no child with serum vitamin A deficiency among the controls in all the social classes.

Table VII shows the mean serum vitamin A levels for each social class category. There was only one child in social class I among the cases, while three children among the controls belonging to social class I had mean serum vitamin A of 66.86±1.70µg/dl. The observed differences in the mean serum vitamin A levels between the cases and controls were statistically significant for social classes II ($t = 14.045$; $p = 0.0001$), III ($t = 8.606$; $p = 0.0001$) and IV ($t = 20.707$; $p = 0.0001$).

Table V: Comparison of the mean serum vitamin A levels among the cases and controls distributed according to maternal education

	Mean serum vitamin A levels (μg/dl)			
	Cases n = 66	**Controls n = 66**		
Maternal education	*Mean*	*Mean*	*T*	*p-value*
No formal education	-	-	-	-
Islamic	59.76± 12.69	66.84 ±5.48	4.161	0.0001
Primary	57.04± 16.24	51.26± 18.91	1.884	0.062
Secondary	60.92± 12.40	61.42 ± 1.30	0.326	0.745
Tertiary	51.30 ± 9.19	62.80± 16.97	4.841	0.0001
Total	**57.26 ± 16.2**	**61.02 ± 5.36**	**1.790**	**0.076**

Table VI: Distribution of vitamin A status among children in the cases and controls in relation to maternal social classes

	Serum vitamin A levels (μg/dl)					
	Cases			***Control***		
	<10	***10-19***	***>20***	***<10***	***10-19***	***>20***
Social class	***n (%)***	***n (%)***	***n (%)***	***n (%)***	***n (%)***	***n (%)***
I	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	1 (33.3)	2 (66.7)
II	1 (12.5)	3 (37.5)	4 (50.0)	0 (0.0)	4 (21.0)	15 (78.9)
III	0 (0.0)	5 (26.3)	14 (73.7)	0 (0.0)	2 (16.7)	10 (83.3)
IV	2 (7.1)	3 (10.7)	23 (82.1)	0 (0.0)	4 (18.2)	18 (81.8)
V	0 (0.0)	0 (0.0)	10 (100)	0 (0.0)	0	10 (100)
Total	**3 (4.5)**	**11 (16.7)**	**52 (78.8)**	**0 (0.0)**	**11 (16.7)**	**55 (83.3)**

Table VII: Comparison of the mean serum vitamin A levels among the cases and controls distributed according to maternal social classes

	Mean serum vitamin A levels (μg/dl)			
	Cases	**Controls**		
Social class	**Mean n = 66**	**Mean n = 66**	**T**	**p-value**
I	-	66.86±1.70		
II	61.27±1.73	57.58±1.25	14.045	0.0001
III	58.46±1.37	60.52±1.38	8.606	0.0001
IV	60.28±1.04	65.08±1.57	20.707	0.0001
V	57.42±3.24	58.32±4.20	1.378	0.171
Total	**59.36±7.38**	**61.67±6.74**	**1.878**	**0.063**

Discussion

This study showed that VAD is a public health problem among undernourished children in Zaria, as 16.7% of children in both study groups had low serum vitamin A levels (10-19 μg/dl) during the rainy season when this study was carried out. In this study, the mean serum vitamin A levels among normal children was 59.90±14.06μg/dl, while that of undernourished children was 59.44±12.93μg/dl. The highest mean serum vitamin A was recorded in the fourth year of life, and the lowest level occurred in the fifth year of life among normal children. This is comparable to the study carried out in the United States of America (USA) by Szymanski and Longwell,[12] who reported the mean serum vitamin A of normal children reaching the highest level similar to the level recorded in the fourth year in the present study. In the setting of the present study, the children were fed both pro-vitamin A-containing foods and preformed vitamin A-rich foods. In contrast, in the USA, the children depended mainly on preformed vitamin A-rich foods.

Another finding in this study is the higher prevalence of VAD among males compared to females in the control group and the other way round for the cases. The higher prevalence in males among the controls needs to be clarified. No cultural factor could be offered to explain this. There was no obvious difference in prohibited foods fed to boys and girls. A similar observation was reported from Sri Lanka[13] and the Philippines.[14] In the Philippines, it was discovered that males consumed 36% less vitamin A foods than females. However, the figures in the present study were too insignificant to make a meaningful comparison.

The lowest mean serum vitamin A level of 58.20±13.58 µg/dl was found in the fifth year of life; the mean retinol was highest in the fourth year, followed by the second, third and first year of life, in that order, for the controls. In the study group, the lowest level was obtained in the second year of life, possibly due to reduced dietary intake and weaning practices during this period and the highest in the first year of life. The explanation for the low mean serum retinol levels in the fifth year of life might be due to increased demand for vitamin A as the child grows older, which may be met by dietary intake and drawing from hepatic stores. The trend in the distribution of serum vitamin A in the present study is not comparable to the findings in Ijaye-Orile in Ibadan.[9] Szymanski and Longwell [12] in the USA showed the lowest mean serum level in the first year and highest in the second year; after that, it decreased until the fifth year of life.

The present study demonstrated that 34.8% of the mothers had at least primary education. There were statistically significant differences in the mean serum vitamin A levels of children in the two study groups whose mothers had Islamic and tertiary educations. Serum retinol levels improve with maternal education among the controls. The findings in the present study were not comparable with that of Nepal[15] which showed that maternal literacy was not associated with a significant reduction in risk of xerophthalmia or malnutrition.

Improvement in socioeconomic status affects the vitamin A status of the study children, though most of the mothers were in social classes III and IV. There was a significant difference in the mean serum vitamin A levels between the cases and controls in relation to children in social class II. There is an increase in serum retinol levels as social class improves. It is acknowledged that families in the lower social classes have poor access to foods rich in preformed vitamin A owing to the high cost of such foods. On the other hand, the effect of income changes in the studied area is minimal.[15] This is because the parents' incomes are low; they depend mainly on agricultural products from their farms, from which their children are fed.

The findings in this study may not be comparable to the study in Ijaye – Orile,[9] which showed that improvement in socioeconomic status did not seem to affect the vitamin A status of the studied children. Wolde-Gabriel *et al*.[16] also found no association between occupation of the head of household, household size and low serum vitamin A levels.

Limitation

Serum carotene was not measured because of the limitation of obtaining the commercial standard preparations. Carotene estimation could have provided an adequate reflection of the carotenoid status, especially as the population derives most of their vitamin A from carotenoid sources. The controls had the same illnesses as the cases except for the better nutritional status. Those illnesses may also affect serum vitamin A levels. The controls should have been apparently healthy children.

Conclusion

As defined by WHO, low serum vitamin A is of public health significance in the population studied in Zaria. The lowest mean serum vitamin A levels were recorded in the age groups 13-24 months and 49-59 months for the children in the study group and control group, respectively, probably because of increased demand for vitamin A as the child ages. The male undernourished children had higher mean serum vitamin A than their female counterparts, while the reverse was the case among the controls. The reason for this needs to be clarified because there are no obvious differences in their dietary intake patterns. Maternal education and social class greatly influenced their children's serum vitamin A levels.

Mothers should be enlightened about the importance of vitamin A-rich diets in children aged 13-24 months and 49-59 months. They should also be encouraged to increase their consumption of vitamin A-rich foods, particularly preformed vitamin A sources such as liver, eggs, and milk. Mothers should be educated on the storage and cooking of vitamin A-containing foods. Such food should be stored for a short time, either in the sun or at home, and neither should they be cooked for too long. Red palm oil, for example, should not be bleached so as not to denature the carotenoids and vitamin A contents.

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