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## Nutritional status and serum vitamin A, protein and albumin levels in children six to fifty-nine months in Ahmadu Bello University Teaching Hospital Zaria

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**Abstract:** *Background:* Protein energy malnutrition (PEM) is a major public health problem in the tropical and subtropical regions of the world and often arises during protein and/or energy deficits due to nutritional inadequacy, poor socio-economic and environmental conditions and infections. Vitamin A deficiency (VAD) is an important health concern in severe malnutrition and has been found to be associated with significant morbidity and mortality. Children with PEM have greater deficiency of total protein and albumin and in severe cases the total protein may be reduced to about 50 per-cent.

The objective of this study was to determine the serum vitamin A, total protein and albumin in malnourished children aged 6-59 months at Institute of Child Health Zaria.

*Methods:* This study was a case control health- based descriptive study to determine the relationship between serum vitamin A, protein and albumin levels among children with Protein Energy Malnutrition and their controls at Institute of Child Health, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. Using systematic sampling method, a total of 132 children (cases and controls) between 6 and 59 months of age were selected for assessment of their serum vitamin A, protein and albumin. Serum vitamin A level was analyzed by high performance liquid chromatography while the total serum protein and albumin levels were analyzed on

the Boehringer Mannheim Automated Hitachi system 704 using the Biuret and colour change methods respectively.

*Results:* The highest mean serum vitamin A ( $60.28 \pm 11.03 \mu\text{g/dl}$ ) and mean protein ( $61.24 \pm 10.12 \text{g/dl}$ ) among malnourished group were seen in marasmic -kwashiokor. For the controls, the mean serum protein is  $62.96 \pm 5.99 \text{g/dl}$  while the mean serum vitamin A is  $59.44 \pm 13.90 \mu\text{g/dl}$ . The overall mean serum protein for study group and controls were  $50.24 \pm 12.33 \mu\text{g/dl}$  and  $62.96 \pm 5.99 \text{g/dl}$  respectively and the difference between them was statistically significant ( $p < 0.01$ )

The highest mean retinol ( $60.28 \pm 11.03$ ) and albumin ( $38.43 \pm 30.14 \text{g/dl}$ ) were recorded among the marasmic-kwashiokor malnutrition, while for the controls, the mean retinol was  $50.44 \pm 13.90 \mu\text{g/dl}$  and the mean serum albumin was  $37.62 \pm 40.98 \text{g/dl}$ . The overall mean serum albumin for both study group and controls were  $37.17 \pm 12.22 \text{g/dl}$  and  $37.62 \pm 40.98 \text{g/dl}$  respectively and the difference between them was statistically significant ( $p < 0.04$ ).

*Conclusion:* The serum protein and albumin showed positive correlation with serum vitamin A levels. The highest mean serum vitamin A, protein and albumin was seen in marasmic-kwashiokor among under-nourished children

**Keywords:** Serum vitamin A; protein; Albumin; protein energy malnutrition; children

## Introduction

Protein energy malnutrition describes a spectrum of severe forms of malnutrition seen in childhood classified as kwashiorkor, marasmic - kwashiorkor, marasmus and underweight.<sup>1,2</sup>

The two severe forms of PEM are kwashiorkor and marasmus. Kwashiorkor is due to insufficiency of good quality proteins to meet the demand of growth and tissue repairs, while marasmus is due to chronic starvation and deficiency of total calories, including proteins.<sup>3</sup>

The World Health Organization (WHO) Global data base on child growth and Malnutrition uses a Z- score cut- off point of <- 2SD to classify low weight- for- age and low weight- for- height as moderate and severe under-nutrition, and <- 3SD to define severe under- nutrition and also called severe acute malnutrition.<sup>3,4</sup>

Protein energy malnutrition is a major public health problem in the tropical and subtropical regions of the world and often arises during protein and/or energy deficits due to nutritional inadequacy, poor socio-economic and environmental conditions and infections.

There is a wide variation in the pattern of incidence of PEM in all countries, including Nigeria.<sup>4</sup> The prevalence of PEM varies from one place to another and from time to time within the year.<sup>5,6,7</sup> It has been estimated that more than 20 million children of the world mostly developing nations suffer from severe malnutrition and 150 million children are underweight.<sup>8,9</sup>

It has been suggested that PEM has severe and lasting effects on immune functions, resulting in increased susceptibility to infections,<sup>10,11</sup> impact on growth and development of children,<sup>10</sup> learning ability,<sup>11</sup> socialadjustment,<sup>11</sup> work efficiency<sup>11</sup> and productivity of labour.<sup>11-</sup>

<sup>13</sup>Malnutrition is associated with vitamin A deficiency (VAD) and is one of the risk factors of VAD. Nutritional deficiencies measured by anthropometric measurements indicate some relationship with serum vitamin A levels even in the absence of eye signs of this disorder.<sup>13</sup>

Children with PEM are known to have low serum protein and albumin. The degree of severity of serum vitamin A, protein and albumin have not been widely determined. It is hoped that this study will help provide some insight into this so as to guide therapeutic intervention. The objective of this study was to determine the serum vitamin A, total protein and albumin in malnourished children aged 6-59 months at Institute of Child Health Zaria.

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## Subjects And Methods

### Study settings

The study was carried out at the Institute of Child Health (ICH) Banzazzau, Zaria. The Institute serves the community/ children mainly from Zaria and its environs and is the Primary Health Care outlet of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. The ICH

offers out-patient service and receives an average of 400 patients in a day.

### Study design

This was a case-control hospital-based descriptive study, designed to evaluate the serum vitamin A, total protein and albumin in malnourished children aged 6-59 months at Institute of Child Health Zaria. The study was carried out between January to October 2017.

### Study population

The study population consisted of consecutive malnourished children between the ages of 6-59 months who presented to ICH. A simple classification of PEM is that proposed by the Well come working party in 1969 as cited by Wali and Sign.<sup>12</sup> This is based on the principle that a reduction in body weight below 80% of the Harvard standard (50<sup>th</sup>centile) is considered as malnutrition. This corresponds approximately to the Harvard third centile.

In this classification, only two criteria were used to classify the malnourished children, the presence or absence of oedema and the deficit in body weight. This gives rise to four groups as marasmus, kwashiorkor, marasmic-kwashiorkor and under-weight. Age and gender matched well malnourished children who presented to the ICH with clinical features of malaria, ARIs, acute diarrhoeal diseases among others were enrolled as controls. Informed consent was duly obtained from each child's parents or care givers before recruitment in the study.

### Study instrument

Relevant data using a proforma which included patient's name, age, sex, tribes and dietary history with particular emphasis on frequency of ingestion of vitamin A – rich foods were collected from all children enrolled for the study. Anthropometric measurements were conducted on each participant and documented. A detailed physical examination was conducted to check for features of under-nutrition such as oedema, skin and hair changes.<sup>4,5</sup>

### Sampling technique

The respondents were selected consecutively using convenient sampling technique

### Sample size determination

The minimum sample size was determined using the formular for two groups. The prevalence of serum vitamin A in malnourished under-five children from previous study

The sample size was determined using the following formular:<sup>13</sup>

Where

S= sample size

a= Level of significance = 0.05

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

1 -  $\alpha$  = power of the study 80%

$Z_1 - \alpha$  = SD for required CI = 1.96

$P_1$  = Proportion or prevalence of cases of vit

A deficiency in malnourished patients 52%<sup>95</sup> = 0.52

$P_2$  = Proportion of control 48% = 0.48

$Z_1 - \beta$  = SD for required power = 0.28

62.50 + 3.15 = 65.65 = 66 cases

Controls were also 66

Therefore, the overall sample size = 132

#### Ethical approval

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

#### Data collection

Each questionnaire was administered by an interviewer trained for the purpose. Ethical conduct guiding a research such as respecting the autonomy of the participants and confidentiality were upheld.

#### Biochemical tests

##### Blood collection and processing

Four milliliters of venous blood were collected into a plain bottle from selected patients and was wrapped in a black nylon so that retinol will not be denatured by light and the sera were separated by centrifugation at 1000 revolutions for 10minutes at the institute of child health laboratory by the investigator. Serum was taken with Pasteur pipette into 2ml tube, re- labelled and were wrapped in a black nylon. The samples were immediately placed in a cooler containing ice cubes by the investigator and transported by road to the Chemical Pathology Laboratory, ABUTH Zaria where they were frozen at -20°C until analysis. The samples were analyzed for serum vitamin A levels at Chemical Pathology Laboratory, University College Hospital, Ibadan, while total serum protein and albumin were analyzed in Chemical Pathology Laboratory ABUTH, Zaria. All these were done by the investigator except sample analysis which were done by the laboratory scientists.

##### Serum vitamin A estimation

Serum vitamin A levels were determined by High Performance Liquid Chromatography (HPLC Shimadzu prominence, made in Japan and functional) using Bieri method.<sup>14</sup>The principle was that a given volume of serum or plasma was diluted with methanol, which denatures plasma proteins, and retinol was extracted with a

suitable organic solvent which is hexane.

#### Procedure

One hundred microlitre (100  $\mu$ l) of serum and 100 $\mu$ l of the internal standard 0.6 $\mu$ g retinyl acetate/ml (used as internal standards to correct for losses during extraction or during analysis) were transferred in a test tube. 100 $\mu$ l of methanol was added to denature and precipitate proteins (essential for release of retinol from retinol - binding protein), and the sample mixed. Two hundred microlitre (200 $\mu$ l) of spectograde hexane, was added and the contents mixed vigorously but intermittently for 45 seconds on a vortex mixer and then centrifuged at 50,000 rpm for 3 minutes to ensure phase separation using a fixed head centrifuge, made from Japan and of bucket size. The upper hexane layer was transferred to another tube using a Pasteur pipette. The combined hexane extracts was then evaporated under a gentle stream of argon and the residue was re-dissolved in 50 $\mu$ l of propan-2-ol. This was injected into the HPLC column specification with a 100  $\mu$ l 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 by use of peak height ratios relative to an internal standard (retinyl acetate).<sup>14</sup>

High performance liquid chromatography method was considered in this study because of its high specificity and sensitivity.<sup>14</sup>HPLC is available to measure individually both retinol and retinyl esters. HPLC provides precise and accurate means of analyzing serum vitamin A. It offers a quick, automated and highly reproducible methods compared to other methods. Thus, this method was used to determine the serum vitamin A levels among malnourished children as seen in Institute of Child Health {ICH}, Ahmadu Bello University Teaching Hospital {ABUTH} Zaria. Other biochemical methods for vitamin A assay are ultraviolet absorption spectrophotometry methods are the least expensive to use but also least sensitive. Fluorimetric methods are sensitive but the laboratory should be able to maintain conditions that avoid contamination from interfering with fluorescing substances. HPLC is available to measure individually both retinol and retinyl esters. In contrast, spectrophotometric and fluorimetric techniques generally measure total vitamin A. Calorimetric methods is not recommended because it is not as sensitive as the other methods.<sup>10</sup>

The limitation of measuring serum vitamin A is that the profile obtained reflects a static situation and fails to capture the dynamics that may be occurring because of seasonal, ecologic, economic or other biologic factors.<sup>10</sup> Another biochemical method is the plasma retinol binding protein (RBP) response test. Plasma RBP is determined by quantitative radial immunodiffusion or by enzyme linked immunosorbent assay.<sup>11</sup>The total body pool of vitamin A can be determined by a new method called isotope dilution analysis.

Total serum protein estimation

Total serum protein was analyzed on the Boehringer Mannheim Automated Hitachi System704 using Biuret method. The principle was that the peptide linkages in the amino acids which make up a protein were capable of reacting with copper in alkaline solution to produce a violet colour (Biuret). The coloured complex is measured at a wavelength of 546nm against a reagent blank. Biuret reagent consists of 0.2N sodium hydroxide 8g/l, sodium potassium tartrate 45g/l, potassium iodide 5g/l and copper sulphate 15g/l.<sup>15</sup>

Serum albumin estimation

Serum albumin was analyzed on the Boehringer Mannheim Automated Hitachi System 704. The principle was the formation of an albumin / bromocresol green complex at pH 4.2. This caused a change in colour which is proportional to the amount of albumin present. This colour change was measured at a wavelength of 600nm against a reagent blank. The reagents were stock succinate buffer pH 4.2, 0.5mol /l, stock bromocresol green solution 10mmol/l, stock sodium azide 40g/l and stock brij (35) 250g/l.<sup>15</sup>

Statistical analysis

Obtained data was compiled and analyzed using statistical package for social sciences (SPSS) version 20.0. Comparison of mean values was done using Student t-test and level of significance was set at p<0.05.

Results

The Demographic variables for both groups showed 26 (39.4%) were males and 40 (60.6%) were females with a male: female ratio of 1:1.5 among cases while for the controls, 30 (45.5%) were males and 36 (54.5%) were females. Table 1 shows the nutritional status of the study group and controls. Among the study group, under-weight malnutrition had the highest score of 30 (45.5%) followed by marasmus comprising 25 (37.8%), kwashiorkor 6 (9%) and marasmic-kwashiorkor 5 (7.5%). This was calculated based on percentage of reference weight for age, that is patients weight divided by weight of normal child of the same age, multiplied by one hundred, in which 80% weight-for-age is the level below which wasting malnutrition is defined.<sup>12,13</sup>

Table 2 shows the mean serum levels of retinol and protein. The highest mean serum vitamin A (60.28±11.03µg/dl) and mean protein (61.24±10.12g/dl) among malnourished group were seen in marasmic – kwashiorkor. For the controls, the mean serum protein is 62.96±5.99g/dl while the mean serum vitamin A is 59.44±13.90µg/dl. The overall mean serum protein for study group and controls were 50.24±12.33µg/dl and

62.96±5.99g/dl respectively and the difference between them was statistically significant (p<0.01)

Table 3 shows the relationship between serum vitamin A and albumin. The highest mean retinol (60.28±11.03) and albumin (38.43±30.14g/dl) were recorded among the marasmic-kwashiorkor malnutrition, while for the controls, the mean retinol was 50.44±13.90µg/dl and the mean serum albumin was 37.62±40.98g/dl. The overall mean serum albumin for both study group and controls were 37.17±12.22g/dl and 37.62±40.98g/dl respectively and the difference between them was statistically significant (p<0.04).

**Table 1:** Nutritional status of study group and control

Nutritional status	Study group (%)	Controls n=66 (%)
Normal	0	66 (100)
Marasmus	25 (37.8)	0
Kwashiorkor	6 (9.0)	0
Marasmic-kwashiorkor	5 (7.7)	0
Under-weight	30 (45.5)	0

**Table 2:** Relationship between serum vitamin A and protein according to classes of malnutrition and controls

Malnutrition	Mean vitamin A (µg/dl)	Mean protein (g/dl)	P-value
Marasmus	58.82±13.78	59.12±11.12	0.03
Kwashiorkor	59.36±12.13	60.23±16.02	0.01
Marasmic-kwashiorkor	60.28±11.03	61.24±10.12	0.01
Under-weight	58.20±12.20	59.30±30.21	0.04
Controls	59.44±13.90	62.96±5.99	0.02

Overall mean serum protein: study group=59.24±12.33g/dl, controls=62.96±5.99g/dl  
 Overall mean serum vitamin A: study group= 59.44±12.93g/dl, control=59.90±14.06g/dl

**Table 3:** Relationship between serum vitamin A and albumin according to classes of malnutrition in study group and control

Malnutrition	Mean vitamin A (µg/dl)	Mean albumin (g/dl)	P-value
Marasmus	58.82±13.78	37.06±16.15	0.02
Kwashiorkor	59.36±12.13	37.83±40.14	0.01
Marasmic-kwashiorkor	60.28±11.03	38.43±30.14	0.02
Under-weight	58.20±12.20	37.17±12.22	0.01
Controls	59.44±13.90	37.62±40.98	0.03

Overall mean albumin: study group=37.17±12.22, control=37.62±40.98  
 Overall mean serum vitamin A: study group= 59.44±12.93g/dl, control=59.90±14.06g/dl

## Discussion

The present study demonstrated low serum vitamin A, protein and albumin among the study group. This study group showed majority of cases were underweight followed by marasmus comprising, kwashiorkor and marasmic-kwashiorkor. The highest mean serum vitamin A was seen in marasmic-kwashiorkor patients. There was significant association between the nutritional levels and mean serum vitamin A levels. This is not comparable to findings by West et al<sup>16</sup> in which they found no difference in the nutritional status of children with and without xerophthalmia. Molla et al in Karachi,<sup>17</sup> Pakistan also found no correlation between degree of malnutrition and serum vitamin A levels.

The present study showed protein and albumin (useful biochemical measures of nutrition status) showed positive correlation with serum vitamin A levels. There was also low mean serum protein and albumin children with serum vitamin A deficiency. Protein deficiency might enhance VAD in one of two ways or both (1) by impairing transport of vitamin A to the liver from the gut or (2) from liver to tissues. Protein deficiency is probably often primary and VAD secondary, but the reverse is also possible. This was comparable to the study done in Ijaiye- Orile<sup>18</sup> in which they found low mean serum protein and albumin in children with vitamin A deficiency.

The highest mean serum vitamin A and mean protein among malnourished group were seen in marasmic – kwashiorkor.. Statistical analysis showed significant difference among malnourished and controls. The overall mean serum protein for study group and controls were statistically significant.

The highest mean retinol and albumin were recorded among the marasmic-kwashiorkor malnutrition. Statistical analysis showed significant difference in both malnourished and controls. The overall mean serum albumin for both study group and controls were statistically significant.

## Conclusion

The present study showed protein and albumin (useful biochemical measures of nutrition status) showed positive correlation with serum vitamin A levels. The highest mean serum vitamin A, protein and albumin was seen in marasmic-kwashiorkor among under-nourished children studied in Zaria

## Recommendations

Children who are 6-59 months of age with Protein Energy Malnutrition should receive the daily recommended nutrient intake of vitamin A throughout the treatment period. Children with severe acute malnutrition should be provided with about 5,000 IU vitamin A daily, either as an integral part of therapeutic food or as part of a multi-micronutrient formulation.

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## Authors Contributions

Abdullahi S M: Planning, literature search, data collection, analysis and writing of manuscript

Mado S M: Revised the manuscript and supervised the conduct of the study

Akuyam S A: Revised the manuscript and supervised the conduct of the study

**Conflict of interest:** None

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