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Estimation of Serum Cobalamin Level in Malnourished Infants in Imo State University Teaching Hospital Orlu, Imo State, Nigeria

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Abstract **Original Research Article**

Estimation of serum cobalamin level in malnourished infants was investigated at the Imo state university teaching hospital Orlu, Nigeria between January and November 2015. Serum vitamin B12 levels were assayed in 20 healthy and 20 malnourished aged 1-12 months using Competitive ELIZA Method. Serum proteins, albumin, globulin, iron, Total Iron Binding Capacity (TIBC), full blood count and anthropometric parameters (weight and height) of the infants were also measured. Stool analysis was also carried out. Serum vitamin B12, Anthropometric measurement, Full blood count, serum iron, TIBC, serum protein, albumin, globulin and stool analysis of malnourished infants were compared with that of normal healthy infants. The mean serum vitamin B12 level, was (1527.0±165. 1 pg/ml) significantly higher ($p<0.05$) in malnourished infants than in normal infants, (349.3±278.8pg/ml). Mean haemoglobin and PCV level in normal infants were (13.1 ± 1.5g/dl, 39.2 ±4.4%) respectively which was significantly higher ($p<0.05$) than that of malnourished infants (7.5 ± 1.8g/dl, 22.6±5.3%). The mean value of WBC in the infants with malnutrition was 12.9±2.7 x10^9/1 while it was 6.81±1.2x10^9/1 among the normal infants ($p<0.05$). The mean value of platelet counts were 282.8 ±72.2 x 10^9/1 and 326.0±54.4x 10^9/1 for the subjects and controls respectively ($p<0.05$). Anthropometric measurement of malnourished infants (Height 59.7 ± 8.8, weight 5.7 ± 1.7) decreased significantly ($p<0.05$) when compared with those of control (Height 69.5 ±8.7, weight 8.0± 2.5). All infants with malnutrition had an infection while normal infants had no infection. Mean serum total protein and albumin level in normal infants were 6.4 ± 0.4, 3.9 ± 0.4 g/dl respectively which was significantly higher ($p<0.05$) than that of malnourished infants (5.4 ± 0.8, 2.6 ± 0.4 g/dl). But mean-serum globulin level (2.8 ± 0.9 g/dl) was significantly higher ($p<0.05$) in malnourished infants than that of normal infants (2.5 ± 0.6 g/dl). The mean serum iron level was (43.7 ± 3.4μg/dl,) significantly higher ($p<0.05$) in malnourished infants than in normal infants, (79.0±1 10.0μg/dl) whereas serum TIBC had no significant difference between these two groups. This study has clearly shown that serum vitamin B12 and serum iron were significantly higher in malnourished infants than non malnourished infants. The most common haematologic change in the infants with malnutrition was anaemia and major cause of anaemia was malnutrition associated infection in this study. The level of involvement of vitamin B12 in megaloblastic anaemia cannot be ascertained in this study because vitamin B12 was significantly high in malnourished infants when compared to normal infants. Again macrocytes were not seen in the blood film.

Keywords: serum cobalamin, , malnourished infants, proteins, globulin, albumin.

INTRODUCTION

Vitamin B 12 is a water-soluble vitamin. It is one of the "B complex vitamins," which play roles in red blood cell formation, nerve cell maintenance, and methyl donation in DNA synthesis. Deficiency of vitamin B12 affects immunologic and haematologic parameters in the body [1].

It was in 1948 that vitamin B12 was first isolated from liver juice and it was used in treating pernicious anemia [2]. Vitamin B12 consists of corrin ring (synthesized by bacteria) and cobalt ion; and this cobalt-corrin ring complex gives vitamin B 12 its red colouration. Different forms of vitamin B 12 are similar in the cobalt central ion, the four parts of the corrin ring and a dimethylbenzimidazole group, but differ in the sixth site which may contain cyano group (CN), hydroxyl group (OH), methyl group (CH3) and/or 5'-deoxyadenosyl group (C-CO) [3, 4].

B12 is particularly important for infants, and a lack of B12 may ultimately slowdown baby's physical

and mental development and may cause irreversible nerve damage. Clinical manifestations among infants and young children are widely varied, encompassing hematologic, neurologic and astrointestinal symptoms [5].

The aim of this work was to estimate serum Cobalamin level in malnourished infants in Imo State University Teaching Hospital, Orlu, Imo State, Nigeria

MATERIALS AND METHOD

STUDY AREA

This study was carried out at the children emergency unit of the Imo state university Teaching Hospital (lMSUTH).

STUDY POPULATION AND ENROLMENT

Subjects for this research work were forty infants. The test subjects included 20 malnourised infants comprising 10 female and 10 male between the age of 1-12months of age while 20 apparently healthy age matched infants were used as control comprising ;12 males and 8 females.

The latter group constituted the control group was subjects of the same age range. All consecutive admissions into the Emergency Pediatric Unit with a diagnosis of malnutrition based on the standard deviation below the growth standards of the United States National Center for Health Statistics (NCHS) as published by WHO that fulfilled the inclusion criteria were enrolled over a period of one year (January - November 2015). Controls were well infants attending the routine clinic without haematologic or infectious condition. Infants were broadly divided into two groups.

Group I: Consisted of 20 apparently healthy infants whose weight for height Z-score (WHZ) were >-2.

Group II: Consisted of 20 infants whose WHZ were <-3.

Detailed history including birth history, feeding history, present infection, and socio-economic status of both the groups and anthropometric measurements were taken. For the purpose of taking history and other laboratory findings, pre-tested questionnaire was used by the investigator herself. Laboratory investigations were performed including complete blood count, total proteins, albumin, iron profile and Vitamin B12

SELECTION CRITERIA FOR SUBJECTS INCLUSION CRITERIA

The apparently healthy male and female infants aged 1-12 months of age with weight-for-height (WHZ) >-2 of the median NCHS/WHO reference values without any systemic disease and whose consent was obtained through the parents were selected as control. Then malnourished male and female infants between 1-12 months of age with weight-for-height (WHZ) <-3 of the median NCHS/WHO reference values whose consent was obtained were selected as the case.

EXCLUSION CRITERIA

Infants above 12 months, those who refused consent, and immune compromised subjects (HIV subjects)

ETHICAL CONSIDERATION

Approval was obtained from the Ethical Committee of Imo State University Teaching Hospital Orlu (IMSUTH).

STUDY DESIGN

The study was a case control study carried out at the Imo State University Teaching Hospital among infants (1-12 months old) with Malnutrition whose WHZ were <-3 and controls were children with normal nutritional status without haematological or infectious conditions. Whose weight for height Z-score (WHZ) >-2 Controls were well infants attending the routine clinic without haematologic or infectious condition. A semi-structured questionnaire (proforma) was used to obtain information from the subjects using interview method. Relevant information on the child's sociodemographic characteristics, nutritional indices and laboratory findings were documented. Informed consent was obtained from the parents of the infants before commencement of the study. Blood specimen of both malnourished and non malnourished infants were collected and analysed.

COLLECTION OF SPECIMEN

The patient and his/her attendants were given a detailed briefing about the purpose of the study. With all aseptic precautions 5 ml of venous blood was drawn randomly on day of admission before the treatment was started by the physician. 2.5ml was dispensed into dipotassium EDTA anticoagulant tube containing 1.5mg/ml of blood of the anhydrous salt mixed. This was used for haemoglobin (HB) estimation, red blood cell count, platelet count, total white cell count and differential count, using manual method. The remaining 2.5ml was dispensed into a plastic tube and centrifuged at 3,000 rpm for 5 minutes on the same day. The sera obtained from the samples were used for Serum total protein, serum albumin, serum globulin, serurn iron and TIBC by spectrophotometric technique then cobalamin by competitive ELIZA method. After processing, specimens for cobalamin were kept frozen until analyzed. Then stool sample was collected for stool examination. Naked eye examination of stool was made, and the stool was then examined for ova under the microscope (macroscopic and microscopic) using

visual and wet mount method respectively.

METHOD OF MEASURING/ASSESSING MALNUTRITION ANTHROPOMETRIC PARAMETERS

The basic information and measurements that constitute anthropometric measurements in children are Age, Sex, Length, Height, Weight and Oedema. These measurements are the key building blocks of anthropometries and are essential for measuring and classifying nutritional status in children under 5 years. Anthropometric indices used in this study were Standard deviations, or Z-scores. To define nutritional status based on anthropometric indices, cutoff values were used. This is the preferred expression for anthropometric indicators in surveys. It is the difference between the value for an individual and the median value of the reference population for the same age or height, divided by the standard deviation of the reference population. In other words, by using the Z-score, you will be able to describe how far a child's weight is from the median weight of a child at the same height in the reference value.

$$\text{Z-SCORE} = \frac{\text{measured value} - \text{median of reference population}}{\text{standard deviation of the reference population}}$$

WEIGHT-FOR-HEIGHT MEASUREMENT

Each child who is weighed and measured was examined for the presence of bilateral pitting oedema.

WEIGHT

The weight of the infants was obtained using a weighing balance. The weighing balance was adjusted to zero, the infants were weighed and the weight was recorded in kilogram (kg).

1. **HEIGHT:** Their lengths were measured lying down (supine) with measuring tape. The height was recorded in cm and
2. **CLINICAL SIGNS OF MALNUTRITION** (e.g. oedema, hair and skin changes). Identifying the presence or absence of oedema was done by applying moderate pressure to the back of the foot or to the shin just above the ankle for a full 3 seconds (1 ... 2 ... 3, was counted slowly while applying pressure). Oedema is present if, when the pressure is removed, an indentation remains in the skin. This aspect was done by the attending clinicians.

LABORATORY METHODS AND PROCEDURES

All reagents were commercially purchased and the manufacture's standard operating procedures were strictly followed.

Determination of serum cobalamin level by competitive eliza

Cobalamin was estimated quantitatively with AccuDiag™ Vitamin 812 ELISA kit by Diagnostic Automation Inc. Woodland Hills USA (catalog number 3125 - 15).

Procedure

Before proceding with the assay, all reagents, patient specimens and controls were brought to room temperature (20-27°C). The mircoplates were formatted for each control and patient specimen to be assayed. Enough test tubes were prepared for all patient samples, controls and serum references. The serum references and controls were treated as test. Dispensed 100μl (0.10ml) of each patient sample, control and serum references into individual test tubes according to the number. Pipetted 50μl (0.050ml) of the prepared extraction agent to each test tube, shaking after each addition. The reaction was allowed to proceed for 15minutes. At the end of the 15min, dispensed 50μl (0.050ml) of the neutralizing buffer. After the neutralizing buffer was added and mixed the reaction went to completion by waiting an additional 5min before dispensing into the microwells. 50μl (0.050ml) of extracted vitamin B12 control & specimen was pipetted into the assigned well. Added 50μl (0.050ml) of the vitamin B12 Biotin Reagent to all wells and mixed for 20-30 seconds. Covered and incubated for 45minutes at room temperature. 50μl (0.050ml) of vitamin B12 Enzyme Reagent was added to all wells: Mixed gently for 20-30 seconds. Covered and incubated for 30 minutes at room temperature. The contents of the microplate were decanted by decantation and the plate blotted dry with absorbent paper. 350ml (0.350ml) of wash buffer was added, decanted, taped and blotted two additional wash was done making it a total of three washes. 100ml (0.100ml) of substrate reagent was added to all wells. Incubated at room temperature for twenty (20) minutes. 50ml of stop solution was added to all wells and gently mixed for 15-20 seconds. Absorbance of each well was read at 450nm. The result was read within 15 minutes of adding the stop solution. A dose response curve was used to ascertain the concentration of vitamin B 12 in unknown specimens.
Full blood count [6].

Haemoglobin estimation (cyanmethaemoglobin method)

Procedure

With the test tubes properly arranged according to subject's number 4mls of drabkin solution was pipette into the tubes, following with the addition of 0.02mls of well mixed venous blood. Mixed and was allowed to stand for 4minutes at room temperature. The samples absorbance was read spectrophotometrically against reagent blank (drabkin solution) at 540nm wavelength. Using the table prepared from the calibration graph, the samples haemoglobin value was taken.

Packed cell volume (pcv)

Procedure

- The capillary tubes were filled two-thirds full with well mixed venous blood each subject. Sealed the unfilled end, using a sealant material.
- The filled tubes were then placed in the microhaematocrit centrifuge and spun at 12,000g for 5 minutes. Immediately after centrifuging, the PCV was read.

TOTAL WHITE CELL COUNT

Platelet count

Procedure

With the test tubes properly arranged according to subject's number, 0.38ml of filtered ammonium oxalate diluting fluid was dispensed into the tubes, following with the addition of 0.02mls of well mixed venous blood and mixed. The counting chamber was assembled and filled with each of the well-mixed sample one after the other and left undisturbed for 20 minutes on blotting paper and covered with a lid. The chamber was placed on the microscope using 10x focused the rulings of the grid and brought the central square of the chamber into view. Then changed to the 40x objective and focused the small platelets.

Blood film/differential white cell count making, fixing and staining blood films

Procedure

Thin blood films were made from well mixed EDTA anticoagulated blood. Immediately air dried. The blood film was covered with Leishman stain for 2 minutes. Double diluted with buffered water of pH 6.8 and allowed to stain for 10 minutes. The stain was washed of with tap water. The back of the slide was wiped clean and stood it in a draining rack for the smear to dry. A drop of immersion oil was placed on the lower third of the blood film and covered with a clean cover glass.

The film was examined microscopically using the 10x objective with condenser iris closed sufficiently to see the cells clearly. Then changed to 100x for differential count.

Determination of total serum protein by biuret method of doumas [7]

Total protein was determined by using the Randox kit according to the manufacturer's instructions. Cat No TP245

Procedure

Four tubes were labeled as test sample, blank, control sample and standard respectively. 5mls of protein reagent (Biuret solution) were pipetted into each tubes respectively. Into the tubes, 0.1 ml of serum was pipetted into the tube labeled test. 0.1 ml of protein standard (standard solution 60g/L) was pipetted into the tube labeled standard 0.1 ml of distilled water was pipetted into the tube labeled blanks; 0.1 ml of control serum into the tube labeled control. The four tubes were mixed thoroughly by avoid frothing and incubate at 37°C for 10 minutes and at 540nm wavelength using the blank to zero the instrument.

The readings were calculated using the formular

$$\text{Total protein g/l} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \frac{\text{Concen std (10g/l}}{1}$$

Determination of serum albumin by modification of bromocresol green method of doumas [7]

The quantitative determination of serum albumin concentration was carried spectrophotometrically with RANDOX kit by Randox laboratories limited crumlin, county Antrim, United Kingdom catalog Number (AB 362)

Procedure

Four tubes were labeled as test, blank, control, standard respectively. 4mls of (BCG reagent) albumin reagent were pipetted into each tube respectively. 0.02mls of distilled water was added into the tube labeled blank, 0.02mls of standard concentration, (30g/L) was pipetted into the tube labeled standard; 0.02mls of control serum was pipetted into the tube labeled blank. 0.02mls of patient's serum into the tube labeled test. The tubes were mixed thoroughly and incubate at 37°C for 10minutes. The intensity of the test, standard and control were read at 632nm wavelength using blank to zero the instrument.

The reading was calculated using the formula:

$$\text{Serum albumin level giL} = \frac{\text{AT x Concentration of std } \textit{(g/dl)}}{\text{As}} \times \frac{}{1}$$

Key:

At = Absorbance of test(s) or control

As = Absorbance of standard (30g/L)

Determination of serum iron concentration by ferozine method of young [8]

The quantitative determination of serum iron concentration was carried spectrophotometrically with TECO Diagnostic IRON/TIBC Reagent, set, Anaheim, USA (CA 92807).

Procedure

Iron free clean tubes were labeled as "test", "blank" and "standard".

The 2.5mls of iron buffer reagent was added to all the labeled tubes. Again, 0.5ml of the subject's samples was added to the respective tubes and was mixed. The samples added to the blank and were also mixed as well. The reagent blank was used to zero the spectrophotometer at 560nm. The absorbance of all tube was read and value was recorded (A 1 reading).

Following the stages, an addition of 0.05ml of iron color reagent was made all the tube, and was properly mixed. The tube were placed in a heating bath at 37°c or 10 minutes The reagent blank was also used to zero the spectrophotometer at 560nm and another absorbance of all tube were read d the value obtain were recorded (A_2 reading).

CACULATION

To obtain the final result, this formula was used:
A2 test-A 1 x Conc of =Total iron ug/dl
A2std – A1 std
A1 = Absorbance of the of first reading of the test
A2 = Absorbance of the second reading of the test
A1std = Absorbance of the reading of the test
A2std = absorbance the second reading of standard
Note ug/dl)x 0.179= umol/L

Detemination of total iron binding capacity using Rrozine method of young [9]

The total binding capacity (TIBC) was estimated quantitatively with TECO- Diagnostic Iron /TIBC Reagent set, U.S.A. using the standard iron ferrozine method. The method used was indirect method which estimate, the unsaturated iron binding capacity (UIBC) and the value were computed with that of serum iron to obtain the for total iron binding capacity. The kit was also supplied by TECO Diagnostic, Anaheim, U.S.A. TIBC/IRON (CA NO 92807)

Procedure

Iron free clean test tubes were labeled as "Test", "blank" and "Standard". Then 2.0ml of unsaturated iron binding capacity buffer reagent were added to all the tube according to the subject's number, while 1.0ml of iron free water was added to the blank tube. And 0.5ml of standard plus 0.5ml of iron free water were added to standard tube and was properly mixed. To the "test" 0.5ml of respective sample plus 0.5ml iron standard was added to the "test" tubes and were also properly mixed. The reagent blank was used to zero the spectrophotometer at 560nm wave length. The absorbance of the samples was read and was recorded as (A1 reading). Then 0.0ml of iron colour of the tube was mixed properly and was placed in a heating bath at 37°C for 10 minutes. The reagent blank was again used to zero the spectrophotometer at 560nm another reading was taken as the (A2 reading).

UIBC CALCULATONS

$$\text{Conc.of std} - \frac{(A_2\text{test} - A_1\text{test})}{(A_2\text{std} - A_1\text{std})} \times \text{Cone. of std} = \text{UIBC in ug/dl}$$

TlBC CALCULATION

TIBC in mg/dl = Iron level + UIBC
Note: ug/dl x 0.179 = umol/L

STOOL ANALYSIS. Adopted from [10]
Macroscopic examination

Procedure

The stool sample for each infant was visually examined for its colour, consistency and presence of blood, pus, mucus or parasites.

Microscopic examination:Wet mount

Procedure

A drop of saline was dropped in the centre of the left half of the slide and placed a drop of iodine solution in the centre of the right half of the slide. With an applicator stick, a small portion of the stool was picked from the specimen container and mixed it with saline on the slide to form a uniform suspension. In the same way, a suspension of stool in the iodine on the slide was prepared. The drop of saline suspension was covered with coverslip by holding it at an angle and lowering it gently on to the slide to reduce formation of air bubbles. The iodine suspension was covered as well with another coverslip. The wet mount was focused using a low power (10X) objective. When any-parasite or suspicious material was observed, the objective was changed to the high-dry objective (40X) and the light increased.

RETROVIRAL SCREENING

The Uni-Gold HIV Rapid Test Kit was used for the subjects. Lot number:
R1870988.

Procedure

The device was removed from the package and labeled with client identification number. Specimen was collected using the disposable pipette. Two drops (approx. 60ul) of specimen was added to the sample port in the device. Two drops (approx. 60ul) of the appropriate wash reagent was added to the [sample port. Result were read after 10 minutes (no longer than 20 minutes) and recorded in the worksheet. Two lines of intensity appear in both the control and test areas. Non-reactive: One line appears in the control area and no line in the test area.

STATISTICAL ANALYSIS

Data entry and analysis were carried out with a micro-computer using the Epi info version 3.5 (2008) software packages and p value of <0.05 was regarded as significant. Then non parameters were analysed using median percentage.

RESULTS

Table-1: Characteristics of Malnourished! and non-Malnourished Infants

Malnourished infants (n=20)	**Non malnourished infants (n=20)**
Age (months) Frequency (%)	Age (months) Frequency (%)
0-6 (n=9) 45	0-6 (n=8) 40
17-12 (n=11) 11	7-12 (n=12) 60
Sex	Sex
Male	Male
1-6 (n=5) 50	1-6 (n=4) 33
7-12 (n=5) 50	7-12 (n=8) 67
Female	Female
-6 (n=4) 40	1-6 (n=4) 50
7-12 (n=6) 60	7-12 (n=4) 50
Socioeconomic status(SES)* of the parents of the study patients (n=20)	
SOCIOECONOMIC STATUS	
FREQUENCY PERCENTAGE	
Poor 16 80%	
ower middle class 3 15%	
pper middle class 1 5%	
Affluent 0 0%	
* Status were evaluated by Rahaman *et al* (2009) (ICDDRB)	
Feeding habit of the patients (n=20)	
Finding yes no(%) no number(%)	
Prelacteal feeding: 2(10%) 18(90%)	
Breast Feeding 15(75%) 5(25%)	
Exclusive breast feeding: 12(60%) 8(40%)	
Complementary feeding: 17(85%) 3(15%)	

This prospective study included 40 infants, among them 20 were malnourished and 20 were non malnourished; (normal infants). All the subjects were between 1-12 months old. The characteristics of malnourished and non-malnourished infants are shown in Table 6.0. Among the malnourished, 10 (50%) of patients were female and 10(50%) of the patients were male. Most of the infants were from the families of low socioeconomic status 16(80%). 3(15%) infants were from upper middle class who had step mother. As nutrition is maintained in the children of affluent families. Malnourished children were not found among them. Among the 20 study subjects prelacteal feeding was given among 18(90%) children, breast feeding was given 15(75%), exclusive breast feeding was given to 12(60%) of infants and complementary feeding after 6 months was given to 29(58%) patients.

Table 1 also shows the characteristics of normal children enrolled for the study. When age range and sex of the normal children were considered, 8(40%) were females, 4(50%) were at the age range of (1-6) and 4(50%) were at the age range of (7-12). Then 12(60%) were males, 4(33%) were at the range of (1-6) and 8(67%) were at the age range of (7-12).

Table-2:Comparison of Mean ± standard deviation Anthropometric Levels between Control subjects and Test subjects

Parameters	**Control Samples**	**TEST Samples**	**P Value**	**Inference**
Weight(kg)	8.0 ± 2.5	5.7± 1.7	p<0.05	s
Height(cm)	69.5 ± 8.7	59.7 ± 8.8	p<0.05	s

Anthropometric measurement of malnourished infants decrease significantly (p<0.05) when compared with those of control.

Table-3: Comparison of Mean ± standard deviation Protein profile Levels between Control subjects and Test subjects

Parameters	**Samples Control**	**TEST Samples**	**P Value**	**Inference**
Total protein(g/dl)	6.4 ± 0.4	5.4± 0.8	<0.05	S
Albumin(g/dl)	3.9± 0.4	2.6 ±0.4	<0.05	S
Globulin(g/dl)	2.5 ± 0.6	2.8 ± 0.9	<0.05	S

The mean serum total protein and albumin level in non-malnourished infants were significantly higher ($p<0.05$) than malnourished infants. On the other hand, mean serum globulin level in malnourished infant was higher than in normal infants.

Table-4: Comparison of Mean ± standard deviation Iron profile Levels between Control subjects and Test subjects

Parameters	Control Samples	TEST Samples	P Value	Inference
Serum iron (g/dl)	43.7±3.4	79.0 ± 10.0	<0.05	S
TIBC (g/dl)	345.6±43.1	310.9 ± 43.9	>0.05	NS

The mean serum iron was significantly high ($p<0.05$) in malnourished infants than in non malnourished infants whereas serum TISe had no significant difference between these two groups.

Table-5: Comparison of Mean ± standard deviation Full Blood Count Levels between Control subjects and Test subjects

Parameters	Control Samples	TEST Samples	P Value	Inference
Haemoglobin (g/dl)	13.1± 1.5	7.5 ± 1.8	<0.05	S
Platelet count(x1 0^9/1)	39.2 ±*4.0*	22.6 ±5.3	<0.05	S
White cell count(x10^9/1)	326.0 ± *54.0*	282.8±72.2	<0.05	S
Neutrophils(%)	6.81 ±1.2	12.9 ± 2.7	<0.05	S
Lymphocyte (%)	44.2±4.7	51.8 ± 11.6	<0.05	S
	50.0 ±6.3			

The mean haematocrit value for the control subjects (non malnourished) subjects was significantly higher ($p<0.05$) than the test subjects (malnourished). There was a significant difference between the Mean haemoglobin values of malnourished and that of control (non malnourished). The difference between the mean platelets count of and controls was statistically significant ($p<0.05$) with infants having a lower platelet count compared to the controls. The mean value of WBC in the infants with Malnutrition was - significantly higher than the controls ($p<0.05$). The subjects had higher an values of total white cell count, neutrophil and lower lymphocytes counts compared with controls ($p<0.05$).

Table-6: Comparison of Mean ± standard deviation Vitamin B12 levels between Control subjects and Test subjects

Parameters	Control Samples	TEST Samples	P Value	Inference
Vitamin 812 (pg/ml)	349.3±278.8	1527.0±165.1	p<0.05	S

There was a significant difference between the mean vitamin b12 of test subjects (malnourished) and that of control subjects (non malnourished)

Table-7: Comparison of Mean ± standard deviation Vitamin B12 Levels between Control and Test subjects according to age range

Parameters	Samples Control	Samples TEST	P Value	Inference
Vitamin B12(pg/ml)				
1-6months	451.6±314.6	1502.0.0±182.5	p<0.05	S
7-12months	360.2±237.7	*1574.3.0±157A*	p<0.05	S

There was a significant difference between the mean vitamin b12 of test malnourished) subjects at 1-6 months and that of control subjects. There was a significant difference between the mean vitamin b12 of test malnourished) subjects at 7-12 months and that of control subjects. ($p<0.05$) but there was no significant difference when compared with their ($p<0.05$), mean vitamin B 12 in the control (non malnourished) subject according age range had no significant difference ($p<0.05$)

Table-8: Comparison of Mean ± standard deviation Vitamin 812 Levels between Control and Test subjects according to sex

Parameters	Control Samples	TEST Samples	P Value	Inference
Vitamin B12(pg/ml)				
Male	279.1±108.9	1586.0±559.4	p<0.05	S
Female	573.1 ±345.1	1468.0±162.1	p<0.05	S

Key: (NS) = Non significant,
(S) = Significant

There was a significant difference between the mean vitamin B 12 of test subjects (malnourished) and that of

control subjects (non malnourished) ($p<0.05$) in both sexes. There was no significant difference in the mean value of vitamin B 12 in both female and male control ($p>0.05$). Also vitamin B 12 of both sexes in the test had no significant increase ($p>0.05$).

Table-9: Stool examination of 40 subjects studied

FINDINGS	Control Samples	TEST Samples	P value	Inference
Parasite	-	-	-	-
Ancylostoma duodenale	0	80	p<0.05	s
Ascaris lumbricodes	0	20	p<0. 05	s

The current study found Ancylostoma duodanale in 80% of malnourished infants examined, the other infestation being ascariasis, 20%. While no worm infection was found among normal infants. This was analysed and found statistically different ($p<0.05$).

DISCUSSION

This study has clearly demonstrated the level of vitamin 812 in malnourished infants. Vitamin BI2 was significantly higher in cases than its level in the control group ($p<0.05$). Statistical analysis distinctly showed the lower 812 status in the control group ($p<0.05$). This is in agreement with works of Osifo and Adadevoh [11], but is contrary to the findings of Turkan, *et al.* [12] and Koc *et al*. [13] who observed a reduced vitamin B12 in malnourished infants. The increased level of Vitamin B12 observed in this study may be as a result of vitamins and supplement used to treat these malnourished infants by some of doctors in the tertiary level hospital which might have affected the B12 of the cases in the current study.

For haematological parameters, lower mean values were also observed in the haematocrit and haemoglobin values of infants with Malnutrition as compared to controls a finding similar to previous studies [14, 15]. These changes can be attributed to adaptation to lower metabolic oxygen requirements and decrease in lean body mass seen in malnutrition [16]. These changes have also been attributed to changes in the plasma volume as well as the intracellular body water in the body [17, 18]. An increase in plasma volume is seen and is said to be responsible for changes in haematocirt and haemoglobin levels [19]. This study also found a significant leucocytosis and neutrophilia among infants with Malnutrition as compared to controls; this is similar to a previous study where there was a significant rise in leukocyte count in the patients with malnutrition compared to the controls [18]. Leucocytosis in these infants can be a result of infection which is seen commonly in malnutrition: both malnutrition and infection, either clinical or subclinical have been reported to act synergistically. This has been an important factor in determining morbidity and mortality attributed to malnutrition [14]. However, several other studies revealed leucopenia as well as neuropenia as a common finding in malnutrition [18]. Furthermore, a lower lymphocyte count was observed in the malnourished children compared to controls. The lower lymphocyte count can be attributed to changes in the thymus which is greatly reduced in infant during severe malnutrition. The degree of thymic atrophy correlates closely with depletion of lymphocytes and a decrease in the thymic dependent lymphocyte is also associated with impaired immunity [20]. Infants with malnutrition had a significantly lower platelet count. This decrease in platelets seen in malnutrition can be attributed to a purported decrease in bone marrow activities which indirectly affect megakaryocyte functions. A similar finding has been reported by a previous study [17].

Macrocytes and hypersegmented neutrophils were not seen in the blood film of both control and test subjects. Therefore the level of involvement of cobalamin deficiency cannot be ascertained in this study and again cobalamin level was statistically high in this study. Macrocytosis and hypersegmented neutrophils in the blood film are two major diagnostic features suggestive of cobalamin deficiency. Macrocytosis and anaemia, may be absent despite neuropathy. Hypersegmented neutrophils are not invariably present they may occur during cytotoxic therapy [6].

Serum total proteins and albumin levels were lower in the cases than that in the control group. This difference was proved statistically significant ($p<0.05$). This well expected finding was very much similar to the observations of the previous study [21]. The globulin level was higher in the cases than that of the control and this was statistically significant ($p<0.05$). The result is consistent with the findings of the previous study [15]. This could be due to the increased incidences of malnutrition associated infection.

Iron status was significantly higher in cases than its level in the control group ($p<0.05$). Accordingly total iron binding capacity (TIBC) level also supported better iron status in the cases. This did not show any statistical significance ($p>0.05$). Statistical analysis distinctly showed the lower iron status in the control group ($p<0.05$). This observation and statistically acceptable inferences are explainable as iron deficiency is very common in our community regardless of the status of PEM (HKI, Nutritional Surveillance Project, Bull 10, 2002). Malnu-trition associated with iron deficiency is more common in kwashiorkor but not in marasmic children [22-24]. Also the severely malnourished infants admitted in the tertiary level hospitals are usually seen by some of the doctors and treated by certain vitamins and supplements which might have affected the iron profile of the cases in the

current study.

The current study found Ancylostoma duodanale in 80% of malnourished infants examined, the other infestation being ascariasis, 20%. While no worm infection was found among normal infants. Musaiger *et al.* [25] observed that Malnutrition is one of the predisposing factors for intestinal worm infection. Others are lack of sanitation and hygiene.

CONCLUSION

This study has clearly shown that serum vitamin B12 was significantly higher in malnourished infants than non-malnourished infants. The same was observed in serum iron levels of these malnourished infants. The most common haematologic change in the infants with malnutrition was anaemia and major cause of anaemia was malnutrition associated infection in this study. Nutritional anaemias (vitamin B12 and Iron) were ruled out because they were high in malnourished infants in this study. Anaemia is common in children suffering from severe malnutrition, even where there is no evidence of a concurrent deficiency of vitamin B12 and iron.

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