ejpmr, 2017,4(1), 162-164

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF SERUM COBALAMIN LEVELS IN MALNOURISHED INFANTS IN UMUAHIA

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Article Received on 12/11/2016 **Article Revised on 02/12/2016** **Article Accepted on 22/12/2016**

ABSTRACT

The level of cobalmin was evaluated in the malnourished infants in Federal Medical Centre, Umuahia. Serum cobalamin levels were assayed in 30 healthy infants and 20 malnourished infants aged 1-12 months. Serum blood sample was used for the estimation of cobalamin using competitive ELIZA method. The result was analysed using t-test and level of significance set at $p<0.05$. The result showed significant increase ($p<0.05$) in serum cobalamin of malnourished infants (1490.2±170.2pg/ml) compared to the control subjects (345.6±237.4pg/ml). There was no significant difference ($p>0.05$) between the male and female malnourished infants(1495.6±540.3pg/ml) and (1475.2±173.4pg/ml) respectively. There was a significant increase ($p<0.05$) in level of cobalamin between malnourished infants aged (1483.0±172.3 pg/ml) 1-6 months compared to the control subjects (439.3±305.6 pg/ml) of the same age range and in malnourished infants with age range 7-12 months (1492.3±142.6 pg/ml) and control subjects within the same age bracket (352.1±240.5 pg/ml). The level of involvement of cobalamin in megaloblastic anaemia cannot be ascertained in this study because cobalamin was significantly high in the malnourished infants as they have started receiving treatment in the hospital including vitamin B complex.

KEYWORDS: Cobalamin (Vitamin B12), malnourished infants, megalobalstic anaemia, Umuahia.

INTRODUCTION

Cobalamin (vitamin B12) is a water soluble vitamin which plays 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]

Human source of cobalamin is of animal origin. Unfortified plant based foods do not contain cobalamin.[2]

Vitamin B12 consists of corrin ring and cobalt ion; and this cobalt-corrin ring complex gives vitamin B12 its red colouration.[3,4]

Vitamin B12 is particularly important for infants, and a lack of vitamin 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 haematologic, neurologic and gastrointestinal symptoms.[5]

VitaminB12 has profound effects on human health. Adequate body stores are essential for several crucial neurologic and haematologic functions.[6] I ts deficiency may cause megalobalstic anaemia.[7]

VitaminB12 deficiency is simple to prevent and simple to treat, but the diagnosis is easy to miss and is often overlooked in the outpatient setting. Malnourished infants might be at a great risk for developing serious sequelae if not detected early and followed with reassessment prophylaxis or treatment as needed. Cobalamin deficiency has been described in infants born to severely cobalamin-deficient mothers. These infants develop megalobalstic anaemia about 3-6 months of age, presumably because they are born with low stores of cobalamin and because they are fed with breast milk of low cobalamin content.

Malnutrition affects every system in the body and always results in increased vulnerability to illness, increased complications and in very extreme cases death .[8,9] Malnutrition has become a public health issue, killing or disabling millions of children each year. The World

Health Organisation estimated that malnutrition accounts for 54% of child mortality worldwide.[10]

AIM

To ascertain cobalamin level in malnourished infants in Umuahia.

MATERIALS AND MOETHOD

Study area: The study was done in Federal Medical Centre, Umuahia.

Subjects: A total of fifty (50) subjects were selected for this study. The test subjects included twenty (20) malnourished infants comprising 10 females and 10 males aged 1-12 months while thirty (30) apparently healthy aged matched infants were recruited as the control subjects.

Ethical Consideration: The consents of the parents were obtained and the procedure for the study explained to them and confidentiality assured to them.

Collection of blood: The subject and his/her attendants were given a detailed briefing about the purpose of the study. With all aseptic precautions 2.5ml of venous blood was drawn randomly. The serum was used for the estimation of cobalamin and was estimated using competitive ELIZA method.

Statistical Analysis: The results were presented as mean ± standard deviation (SD) and analysed using t-test and level of significance set at P<0.05.

Determination of serum cobalamin levels by competitive ELIZA method

Cobalamin was estimated with ACCUDiag™ Vitamin B12 Hills USA (Catalog Number: 3125-15)

Principle

A competitive binding assay is used. The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. This depends on competition between VitaminB12 present in the sample with a horseradish peroxidase (HRP)-labeled vitaminB12 for limited number of binding sites on a biotinylated antibody present in the liquid phase. Vitamin B12 present in the patient sample is released from its endogenous binding proteins by alkaline denaturation. Biotinylated antibody is added and incubated with the treated neutralized sample. An aliquot of the treated sample is transferred into a streptavidin coated well and vitamin B12-HRP conjugate added. Following a competitive binding reaction, the vitamin B12 intrinsic factor complexes are captured by streptavidin on the wells. Unbound conjugate is removed by washing. The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration.

Procedure

Before proceeding with the assay, all reagents, patients' samples and controls samples were brought to room temperature. The micro plates were formatted for each control and patient sample assayed. Enough tubes were prepared for all patient samples, controls and serum references. The serum references and control were treated as test.100µl of patient sample, control and serum reference were added into individual tubes according to their number.50 µl of prepared extraction agent was added to each tube, mixed and allowed for 15 minutes. After that 50 µl of the neutralizing buffer was added, mixed and allowed for 5 minutes and was dispensed into the microwells.50 µl of extracted vitamin B12 control and patient sample was added into the assigned well.50 µl of vitamin B12 Biotin reagent was added to the wells, mixed and allowed for 25 seconds, covered and incubated for 45 minutes at room temperature and 50 µl of vitamin B12 Enzyme Reagent was added to all well, mixed gently for 25 seconds, covered and incubated for 30 minutes at room temperature. The contents of the micro plates were decanted, blotted dry with absorbent paper.350 µl of wash buffer was added, decanted, taped, blotted and 2 additional wash were done.100 µl of substrate reagent was added to all wells, incubated at room temperature for 20 minutes.50 µl of stop solution was added to all wells and gently mixed for 15 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 B12 in unknown specimens.

RESULTS

Table 1: Comparism of cobalamin levels between control subjects and malnourished infants

Parameter	control (30)	malnourished infants (20)	p-level
Vitamin B12(pg/ml)	345.6±237.4	1490.2±170.2	P<0.05

Table 2: comparism of cobalamin levels between control and malnourished infants based on age range

Parameter	Age (months)	control (30)	malnourished infants (20)	p-level
Vitamin B12 (pg/ml)	1-6	439.3±305.6	1483.0±172.3	P<0.05
	7-12	352.1±240.5	1492.3±142.6	P<0.05

Table 3: comparism of cobalamin levels between control and malnourished infants based on sex

Parameter	Sex	control	malnourished infants	p-level
Vitamin B12 (pg/ml)	Male	283.4±120.9	1495.6±540.3	P<0.05
	Female	564.6±336.3	1475.2±173.4	P<0.05

DISCUSSION

The study showed significant increase ($p<0.05$) in cobalamin in the malnourished infants (1490.2±170.2pg/ml) compared to the control subjects (345.6±23.7 pg/ml) respectively. The study followed the same trend when cobalamin was compared according to age range and sex between malnourished infants and the control subjects (1483.0±172.3pg/ml, 439.3±305.6pg/ml) and (1492.3±142.6pg/ml, 352.1±240.5 pg/ml) respectively. The result is contrary to the findings of Turkan *et al.*[11] and Koc *et al.*[12], who observed a reduced cobalamin in the malnourished infants. The significant increased level of cobalamin observed among the malnourished infants could be as a result of vitamins and other supplements used to treat these infants by their attending physicians in the hospital.

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. Macrocytosis and anaemia may be absent despite neuropathy. Hypersegmented neutrophils were not invariably present, they may occur during cytotoxic therapy.

CONCLUSION

The study showed significant increase in the levels of cobalamin in the malnourished infants which could be as a result of multivitamins the affected infants was placed by their physicians. This does not show that malnourished infants may not have cobalamin deficiency and because of that, it is necessary that all malnourished infants with or without unexplained haematologic or neurologic symptoms should be evaluated for a vitaminB12 deficiency. If such a deficiency is found, the cause should be determined. Irreversible neurologic damage can occur if diagnosis and treatment are delayed. It is important to be alert especially with the malnourished infants who are at risk for vitamin B12 deficiency; remedial measures should be adopted such as dietary modifications, supplementation and fortification of specific foods, mainly those that are commercially processed

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