Original Article

The Use of Nuclear Medicine Invitro Technology to Study the Effect of Malnutrition on children's Thyroid Function

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Abstract

In this study, forty-nine – under five years, male & female Sudanese children, presented with protein energy malnutrition (PEM, Kwash) according to the WHO criteria have been studied for their thyroid function and other biochemical Parameters. Triiodothyronine (T3), Thyroxine (T4) and Thyroid Stimulating Hormone (TSH), as a thyroid function indicators, the study-included determination of Thyroglobulin (TG), Ferritin, Protein Moreover, Hemoglobin levels in serum. The study was done in different hospitals in Khartoum state. Patients being assessed & examined by pediatricians using a unified clinical protocol and a questionnaire for patient's characteristics. Thyroid hormones and Ferritin were measured by a sensitive radioimmunoassay (RIA) Technique in 49 children with malnutrition as well as a control group of 20 healthy children. When the results of malnourished children compared to normal children showed that; 22% of malnourished children have sever hypothyroidism (T4 < 30 ηmol/l) and (T3 < 0.5 ηmol/l) as the reference range (50 – 150ηmol/l) and (0.8 – 3 ηmol/l) respectively. The mean level of TSH for patients was within the normal range but

الملخص

تضمنت هذه الدراسة عدد تسعة وأربعين عينة مصل من الذكور والإناث الأطفال السودانيين (أقل من خمس سنوات) المصابين بسوء تغذية الخاص بنقص البروتين (كواش) وفقا لمعايير منظمة الصحة العالمية، تم دراسة العينات لمعرفة وظيفة الغدة الدرقية والمؤشرات الكيميائية الحيوية الأخرى مثل هرمونات الغدة الدرقية ثلاثي اليود (T3)، هرمونات الغدة الدرقية رباعي اليود (T4) والهرمون المحفز للغدة الدرقية (TSH)، هرمون الثيروجلوبولين (TG)، فيريتين (Ferritin)، ونسب الهيموغلوبين في المصل. أجريت الدراسة في مستشفيات ولاية الخرطوم المختلفة. تم تقييم وفحص المرضى من قبل أطباء أطفال متخصصين باستخدام البروتوكول السريري الموحد واستبيان خصائص المرضى. تم قياس هرمونات الغدة الدرقية والفيريتين بواسطة تقنية المناعة الاشعاعية (RIA) في ال ٤٩ طفلا المصابين بسوء التغذية فضلا عن مجموعة الأصحاء (٢٠ طفلا). كانت نتائج الأطفال الذين يعانون من سوء التغذية مقارنة مع الأطفال الأصحاء كالاتي: ٢٢٪ من الأطفال الذين يعانون من سوء التغذية لديهم قصور في الغدة الدرقية (T4 <30 نانو مول / لتر) و (T3 <0.5 نانومول / لتر) والمجموعة مرجعية (٥٠ -١٥٠ نانومول / لتر) و (٠,٨ -٣ نانومول / لتر) لل T3,T4على التوالي. كان مستوى الهرمون المحفز للغدة الدرقية (TSH) للمرضى في حدود المستوى الطبيعي ولكن كان أقل من متوسط مستوياته لدى مجموعة الاصحاء. وكانت القيم المتوسطة لهرمون الثيروجلوبولين (TG) وهرمون الفيريتين (Ferritin) للمرضى أعلى من تلك التي لدى مجموعة الأصحاء. أظهرت قيم البروتين والهيموغلوبين مستويات منخفضة لدى المرضى وذات ارتباط سريري سلبي بهرمون الفيريتين. استنتجت هذه

it was lower than the mean of TSH levels for controls. The mean values of TG and Ferritin for patients was higher than that of controls. Protein and Hemoglobin values showed low levels in Patients and negative clinical correlation between Ferritin and Hemoglobin has been found. Our conclusion of this study states that, malnutrition has severe effects on thyroid function.

الدراسة أن سوء التغذية له آثار سلبية على وظيفة الغدة الدرقية.

Introduction:

Malnutrition is one of the principal and extreme pathological disorders in comprehensive region globally. (1) In a survey done by Food and Agriculture Organization (FAO) in 1996, (1) several districts including Sudan presented high risk of insufficient nutrition. The incidence of protein energy malnutrition PEM in children under five of age in Khartoum state was likely 18.3% (4.5% severe, 13.8 moderate) (2). Malnutrition results mostly from relative or absolute deficiency of nutrients, and the most usually line of population exaggerated are children. (1.3)

The vital role of the thyroid hormones to children in stimulating growth and other functions is well-known, thyroid stimulating hormone (TSH), which secreted by the pituitary gland, stimulates the flow of T_3 and T_4. Thyroglobulin is a large iodinated glycosylated protein; it is the storage form of T_3 and T_4 in the colloid of thyroid gland (3.4)

Total plasma protein test is used to diagnose impaired nutrition. Normal range of total protein in blood is between 6.4 – 8.3g/dl. Hemoglobin is the main carrier of oxygen and carbon dioxide in the blood as with hematocrit, it is an important determinant of anemia. The typical child with protein energy malnutrition (PEM) has a moderate anemia with hemoglobin concentration of about 8-10g/dl (4)

Ferritin is found mainly in the cytoplasm of reticulo-endothelial cell, liver cell, and too lesser extend in developing red blood cells precursors in bone marrow. It has normally been considered as storage compound from which iron is readily mobilized either into the transferring-bound plasma pool or for intracellular heme synthesis. In simple iron deficiency, Ferritin concentration of <15mg/l indicate an absence of storage iron. In patients with iron overload high concentration of circulating Ferritin are found. In patient's infection, inflammatory disease, tissue damage or malignancy, the serum Ferritin concentration is still related to the level of iron store. (5)

MATERIALS AND METHODS:

Study subject and selection criteria:

Forty-nine under five malnourished children were randomly selected for this study. The selected patients comprised males and females. Other 20 well-nourished children were selected to serve as control subjects. All patients participated in this study were seen at different hospital in Khartoum state, in which all clinical examination and assessment of disease were conducted by medical pediatricians. The subjects were also selected to be free of systemic or endocrine disease, and they were not under effect of any drug known to affect thyroid function.

Sample collection:

After the use of local antiseptic for skin

(70%ethanol), 3 ml of femoral blood sample were obtained by puncturing using disposable syringes. The serum was separated after centrifugation at room temperature and separated sera were kept frozen at -20C^{o} until used.

Radioimmunoassay (RIA) method to measure serum T_3 and T_4:

- T_4 (or T_3) radioimmunoassay method depend on the competition between I^{125} labeled T_4 (or T_3) and T_4 (or T_3) contained in standard or in specimens to be assayed for a fixed and limit number of T_4 (or T_3) antibody binding sites.
- After incubation, the amount of I^{125} labeled T_4 (or T_3) bound to the antibody is inversely related to the amount of T_4 (or T_3) present in the sample.
- In the antibody suspension of this kit, the antibody is covalently bound to the magnetic particle. Separation of the antibody bound fraction is achieved by magnetic separator and decanting the supernatant.
- By measuring the proportion of the I^{125} labeled T_4 (or T_3) bound in the present of references standards containing various known amount of T_4 (or T_3) the concentration of T_4 (or T_3) present in unknown sample can be interpolated.

Immunoradiometric assay method to measure TSH:

- Immunoradiometric assay for measuring TSH in human serum involves the reaction of TSH present in serum with monoclonal and polyclonal antibody.
- The monoclonal antibody is labeled with I^{125} (I^{125}-Mcab) and the polyclonal antibody is coupled to magnetic iron oxide particles (PcAb<M>).
- The formed I- Mc Ab-TSH-PcAb< M >complex (sandwich) is separated from unbound tracer by placing the assay tube in the magnetic separator decanting supernatant.
- The radioactivity of tracer in the tubes is directly proportional to the concentration of TSH in the specimen.

Immunoradiometric assay to measure Thyroglobulin (TG):

- The tubes were labeled for T (total account), standards, samples and control serum. Systematically the coated tubes allowed reaching room temperature before opening the bag.
- 100µl of each standard were added to corresponding tubes.
- 100µlof each sample or control serum were added into corresponding tubes.
- Incubation for an hour at room temperature on horizontal shaker.
- The incubation mixture of all tubes was decanted.
- 2ml of washing solution was added to each tube. Washed for one minute and decanted carefully.
- The washing procedure was repeated.
- 200µl I^{125} mAb tracer (red) was added to each tube.
- Incubated 2 hours at room temperature on horizontal shaker.
- The incubation mixture of all tubes carefully decanted.
- 2ml of washing solution was added to each tube, after one minute decanted carefully.

- The washing procedure was repeated.
- The radioactivity fixed in each tube was counted for one minute in Gamma counter.

Ferritin Measurement (Unit: µl)

Table (1) Radioimmunoassay to measure Ferritin levels in serum:

	T	NSB	S_0	Standard	Sample
Incubation solution		200	100		100
Ferritin standard				100	
Sample					100
^{125}I Ferittin	100	100	100	100	100
Ferritin Ab			100	100	100

Principles of total protein measurement:

Under alkaline conditions, substances containing two or more peptide bonds form a purple complex with copper salts in the reagent.

Table (2) Estimation of total serum protein methodology:

	Test	Standard	Blank
Sample	0.02 ml		
Standard		0.02 ml	
Distilled water			0.02 ml
Biuret reagent	1.0ml	1.0ml	1.0ml

The mixture incubated for 15min at 37C, and then absorption was read at 540 nm using spectrophotometer. Then the results were calculated according to the equation:

$$\text{Serum total protein con. (g/dl)} = \frac{\text{Abs of sample} \times \text{conc. STD}}{\text{Abs of STD}}$$

Estimation of plasma hemoglobin using Cynomet Hemoglobin Method:

All form of hemoglobin reacts with drabkin solution to form stable color solution (cynomet hemoglobin).

Table (3) hemoglobin concentration methodology

	Test	Standard	Blank
Blood	0.02		
Standard		0.02	
Drabkin reagent	5ml	5ml	5ml

All samples incubated for 5min.

Calculation:

$$\text{H b con. (g/dl)} = \frac{\text{Absof sample x conc. of STD}}{\text{Abs of STD}}$$

Results and discussion:

Means of studied parameters level have been taken for patients group and compared with the means of control group, table (4) showed the comparison.

Table (4): means of the biochemical parameters for patients of control groups.

Subjects	T4 mmol/l	T3 mmol/l	TSH mIU/l	TG ng/ml	Ferr mg/l	Protein g/dl	HB% g/dl
Patient	84.3	1.09	2.47	84.86	129.70	4.86	60.4
Control	98.0	1.62	2.80	34.04	65.88	6.73	85

Referring to the normal range of thyroid hormones among Sudanese children, T_3: 0.8-3/ mmol/l, T_4: 50-150 mmol/las recorded by Nagi *et- al,* 2000, [6] the means of thyroid

hormones (T_3& T_4) for the Two-study groups were in the normal range. Nevertheless, when we look to the patients individual results, 40% (11 out of 49) showed low levels of T4 thyroid hormone– i.e. below the lower limit of the normal range. 37% (18 out of 49) of T_3, while 10% exhibit low levels of both T_3 and T_4.

6.25% (3 out of 49) of the patients were found to be complained of secondary hypothyroidism-low levels of T_3, T_4 and TSH, which may indicates that the pituitary gland was also affected by malnutrition. Low TSH levels (figure 3) may be due to hypo-secretion of thyrotrophic releasing hormone (TRH) from the hypothalamus as mentioned in [7.8]

Upon the comparison of the means of protein and hemoglobin between patients and controls groups, patient group levels were below the normal range for the two parameters, which indicates the protein energy malnutrition (PEM) of the patients. This also told by Getahun et-al in .2017.

From the results, the patients group shows high levels of thyroglobulin TG (>60ng/ml). These high levels of TG among malnourished children may be due to the dysfunction of thyroid hormones. [8, 9]

On the other hand, malnourished children showed high levels of Ferritin compared with the controls group, nearly two time, and table (4). This elevation of serum Ferritin among patient may be due to increase hemolysis of red blood cells, which caused by anemia in malnourished children.(This is not known in pure PEM) in addition, maybe because ferritin is acute phase reactant, & in PEM there are inflammatory changes . This finding is in agreement with Jain et-al in [10, 11]

Conclusion:

- Form this study; the clear conclusion is that, there is significant ratio of severe hypothyroidism among the society of malnourished children on Khartoum state.
- So that it will be strongly recommended the routine investigation of thyroid function for the malnourished children immediately upon diagnosis by the pediatrician.
- The immediate treatment of hypothyroidism and follow up will be of a great benefit for children.
- It will be recommended to prevent protein energy malnutrition by availability of adequate nutrition, education of individuals of the importance of good nutrition and immunization, good surveillance is necessary to avoid periods of famine and follow the WHO priority program.

References:

1. Food and Agriculture Organization of the United Nations, FAO. The Sixth World Food Survey (FAO, Rome, 1996), pp. v-vi.
2. Alaverdashvili M[1], Li X[2], Paterson PG[2]. Protein-Energy Malnutrition Causes Deficits in Motor Function in Adult Male Rats.J Nutr. 2015 Nov; 145(11):2503-11. Doi: 10.3945/jn.115.216382. Epub 2015 Sep 30.
3. Sawsan M. Abdullah nutritional assessment of children under 5 in Khartoum state,2000,MD,in community medicine
4. Clark clinical medicine, second edition, edited by Parveen J. Kumar and Michael L.
5. Correia, Maria Isabel TD, Mario Ignacio Perman, and Dan LinetzkyWaitzberg.

"Hospital malnutrition in Latin America: A systematic review." Clinical Nutrition (2016).

6. N.I.Ali, Determination of the normal range of thyroid hormones in Sudanese by locally produced reagents, Fifth Arab conference on the peaceful uses of atomic energy, Beirut 13-17/11/2000.

7. Poulia, Kalliopi-Anna, et al. "The two most popular malnutrition screening tools in the light of the new ESPEN consensus definition of the diagnostic criteria for malnutrition." Clinical Nutrition (2016).

8. Molin, M., Ulven, S. M., Dahl, L., Lundebye, A. K., Holck, M., Alexander, J. &Ydersbond, T. A. (2017). Arsenic in seafood is associated with increased thyroid-stimulating hormone (TSH) in healthy volunteers–a randomized controlled trial. Journal of Trace Elements in Medicine and Biology.

9. Jain, R. B. (2017). Associations between the levels of thyroid hormones and lipid/lipoprotein levels: Data from National Health and Nutrition Examination Survey 2007–2012. Environmental Toxicology and Pharmacology, 53, 133-144.

10. Pollit, Ernesto, and Dan Granoff. "Mental and motor development of Peruvian children treated for severe malnutrition." RevistaInteramericana de Psicologia/Interamerican Journal of Psychology 1.2 (2017).

11. Getahun, Z., Urga, K., Ganebo, T., &Nigatu, A. (2017). Review of the status of malnutrition and trends in Ethiopia. The Ethiopian Journal of Health Development (EJHD), 15(2).