Studies on Manganese and Nickel in Hair of Malnourished Children.

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Introduction

Deficiencies of manganese (1) and nickel (2) in man have not been yet documented but manganese deficiency (3) and nickel deficiency (2) have been reported in several animal species.

There are numerous enzymes that can be activated by manganese which include hydrolases, kinaes, decarboxylases, and transferase (4). It is known to activate glycosyl transferases (5, 6), which are important in polysaccharide and glycoprotein synthesis. Leach et al (7) have identified the critical role of manganese in chondroitin sulfate synthesis.

There are significant concentrations of nickel present in DNA and RNA (8-11) and may contribute to the stabilization of the structure of nucleic acids. It may be an important cofactor in some enzyme system, since nickel can activate numerous enzymes in-vitro, including deoxy-ribonuclease (12) acetyl-CoA synthetase (13), and phosphoglucomutase (2, 14).

Protein-energy malnutrition is a deficiency disorder mainly of protein and energy but also frequently associated with deficiencies of various vitamins and trace metals.

Though there are some problems often encountered in the interpretation of hair analysis for trace elements, but there are some potential advantages of hair analysis for trace elements. Sample collection is easy and physically not traumatic. Of all human tissues, the head hair has been found to be most suitable for the analysis of many trace elements in human subjects (15) ; because during the period of growth, the hair being exposed to circulating blood, lymph, and extra-cellular fluid, keep a continuous records of the changes of concentrations of these elements in the body.

Due to widespread prevalence of varying degrees of malnutrition among preschool children of Bangladesh (16), this study was undertaken as preliminary study towards understanding the status of manganese and nickel in hair of malnourished children and healthy control children.

Materials and Methods

Selection of subjects

Different clinical types of 53 malnourished children of 1 to 5 years of age, were selected for this study and forty seven apparently healthy children of same age group selected randomly from the same socio-economic background served as control. Diagnosis was made from history, anthropometry, clinical examination, and supported

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by serum protein and serum albumin estimation. NCHS (17) standard was used to assess the nutritional status. Welcome classification was used to assess the clinical spectrum of malnutrition (18). Patients with severe systemic disorders were excluded from the study.

Sample collection and preparation

Hair sample, approximately 5g, cut close to the occipital portion of the scalp with stainless steel scissors, were collected from each of the subjects. Blood samples were collected from each of the subjects for the determination of serum total protein and serum total albumin. In case of malnourished children, the hair and serum samples were collected immediately after admission to hospital. Hair samples were kept in clean paper envelops until preparation for analysis.

The hair samples were washed according to the procedure described by International Atomic Energy (IAEA), (19) and the pellets were made from the washed samples according to the prescribed procedure (20). The pellets were mounted in 35 mm slide frame with adhesive tapes and preserved in a desiccator until irradiated for the determination of manganese and nickel levels.

Analytical methods

Manganese and nickel levels in hair matrix were determined by Proton Induced X-Ray Emission, external beam method (21). Serum total protein and serum total albumin were measured by Biuret method (22).

Statistical Analysis

Concentrations of manganese and nickel in hair of control and malnourished children were compared by Two sample Students t-test and also compared by Cochran's modified t-test to allow for inequality of variance. The t-test was also made following logarithmic transformation to ensure stability of variance and normality of sample (23). Analysis of variance by one way classification was done to find out the effect of age on hair manganese and nickels (23). In all analysis, results were considered statistically significant if $P \leq 0.05$.

Dietary Data

For the control children, dietary data were collected from their parents using dietary records for 3 consecutive weekdays combined with frequency of intakes of nutrients. For the malnourished children, dietary data were collected from their parents using 24 hour dietary recall method combined with frequency of intake of nutrients. The records were checked by an expert nutritionist. Energy, protein, carbohydrate and other nutrients values were obtained from food composition table (24).

Results

The levels of manganese and nickel in hair were calculated on the basis of only those values which were above the minimum detection limit of these elements. The concentrations of these elements are expressed in unit of parts per million (ppm) which is also equivalent to mcg/g dry hair.

One value, 33.24 ppm in case of control children and two values, 16.3 ppm and 27.7 ppm in case of malnourished children were shown to be much higher than the rest of the value of manganese and thereby excluded from the calculation from the mean.

With regards to nickel concentrations, five values, 33.2 ppm, 33.2 ppm, 38.1 ppm, and 40.2 ppm and 91.4 ppm in case of control children and one value, 73.7 ppm in case of malnourished children were found to be much higher than the rest of the values and therefore excluded from the calculation of mean.

Dietary intake

Mean daily intakes of energy, protein, carbohydrate, calcium and iron by Control and Malnourished children are presented in Table-I. Energy intakes by malnourished children could meet only 30% to 60% of their requirements according to US Recommended Dietary Allowance (RDA) (25) and protein intakes by them supply only 3.3 ± 0.3 % of their total energy need but carbohydrate supplies about 84.5 ± 5.0% of their total energy requirements. The daily intakes of calcium and iron by both control and malnourished children are beyond their RDA.

Levels of manganese and nickel in hair of Control and Malnourished children

Even with higher values excluded, there was considerable variation among individual subjects and the range of values for hair manganese and nickel concentrations are relatively large.

Table I. *Mean daily intakes of energy, protein, carbohydrate, calcium and iron by Control and Malnourished children.*

	Control	Malnourished
Energy (Kcal/day)	973 ± 428	609.5 ± 252
(Kcal/kg/day)	87.4 ± 38.5	54.8 ± 252
Protein (g/day)	12.2 ± 5.6	10.0 ± 22.7
(g/kg/day)	1.6 ± 0.4	0.88 ± 0.2
(% of total energy)	6.6 ± 0.6	3.3 ± 0.3
Carbohydrate (g/day)	182.9 ± 47.1	119.2 ± 54.2
(g/kg/day)	15.3 ± 6.7	10.5 ± 4.8
Calcium (mg/day)	184 ± 32.5	132.0 ± 50.9
Iron (mg/day)	6.2 ± 4.2	4.4 ± 1.6

Table II. *Mean Manganese and Nickel levels in hair of Control and Malnourished children.*

	Hair manganese (ppm or mcg/g dry hair)		Nickel (ppm or mcg/g dry hair)	
Sex	Control	Malnourished	Control	Malnourished
Male	5.92 ± 3.4 [a]	6.2 ± 3.2	11.45 ± 6.0 [a]	12.1 ± 8.1
	(No = 28)	(No = 28)	(No = 29)	(No = 30)
Female	6.9 ± 2.9	7.9 ± 2.9	14.5 ± 7.8	9.4 ± 7.1
	(No = 14)	(No = 21)	(No = 15)	(No = 22)
P--Value	P > 0.05 (NS) [b]	P > 0.05 (NS)	P > 0.05 (NS)	P > 0.05 (NS)

a. Mean ± S. D. b. NS means not significant

Table III. *Analysis of variance table for the effect of age on hair manganese level of control children.*

	Degree of freedom	Sum of squares	Mean squares	F--ratio	P--value
Ages	3	61.91	20.64	2.13	P > 0.05 (NS) [a]
Errors	39	378.82	9.7	---	---
Total	42	440.23			

a. NS means not significant.

Table--IV *Analysis of variance table for the effect of age on hair nickel levl of control children*

	Degree of freedom	Sum of squares	Mean squares	F--ratio	P--value
Ages	3	225.3	75.1	2.1	P > 0.05 (NS) [a]
Errors	38	1354.9	35.7	---	---
Total	41	1580.2			

a. NS means not significant.

Table V. *Concentrations of manganese in hair of Control and Malnourished children.*

Nutritional status	No. of children	Hair manganese (ppm or mcg/g dry hair)	Comparison with control group	Range
Control	43	6.2 ± 3.2 [b]	-	1.7 -- 33.2
Kwashiorkor	9	7.6 ± 3.2	P>0.05 (NS) [c]	4.3 -- 14.3
Marasmic-Kwashiorkor	16	8.0 ± 4.2	P>0.05 (NS)	3.2 -- 19.2
Marasmus	25	6.9 ± 3.7	P>0.05 (NS)	2.0 -- 27.7

a. Two sample student t-test. b. Mean $\pm$ SD. c. NS means not significant

Table VI. *Concentrations of nickel in hair of Control and Malnourished children.*

Nutritional status	No. of children	Hair nicke (ppm or mcg/g dry hair)	Comparison with control group	Range
Control	48[a]	14.4 ± 9.9 [b]	-	3.7 ± 91.2
Kwashiorkor	10	12.8 ± 9.7	P>0.05 (NS) [c]	4.2 -- 31.6
Marasmic-Kwashiorkor	18	12.4 ± 9.7	P>0.05 (NS)	2.0 -- 34.4
Marasmus	25	10.5 ± 7.0	P>0.05 (NS)	2.6 -- 39.0

a. Two sample student t-test. b. Mean ± SD c. NS means not significant

Analysis of variance by one-way classification to find out the effect of age on hair manganese and nickel are presented in Table-III and Table-IV. respectively. The results show no significant effects of age on hair manganese and nickel concentrations of control children aged I to 3 years (P) 0.05).

Values for hair manganese concentrations of control, kwashiorkor, marasmic-kwashiorkor and marasmic children are presented in Table-V. No significant differences are found between the hair manganese levels of children with either kwashiorkor, or marasmic-kwashiorkor, or marasmus and control children (P> 0.05.).

Values for hair nickel concentrations of control, kwashiorkor, marasmic-kwashiorkor and marasmic children are presented in Table-VI. Mean levels of hair nickel of children with either kwashiorkor or marasmic-kwashiorkor or marasmus show no significant differences (P> 0.05) with that of control children.

Discussion

Value of analysis of hair for manganese as an index of nutritional status of manganese is not yet established in human subjects. From various studies with animal models (26), it is found that manganese level in hair reflects the manganese dietary supply better than any other part of the body studied but no such relationship was observed in two other studies with cattle (26).

The results of this study show that sex and age have no significant effects on hair manganese and nickel levels of both control and malnourished children. Gibson, R.S. et al (27) also reported that sex has no significant influence on hair manganese levels of healthy children aged 4.5 to 5.5 years. But no study has yet been reported showing the effects of sex and age on hair manganese and nickel levels of control and malnourished children.

The results of the present study show no significant differences in the hair manganese and nickel levels between control children and children with either kwashiorkor or marasmic-kwashiorkor or marasmus. This may be partly explained by results from dietary data.

Analysis of dietary data shows that about 80% to 90% of energy consumed by these malnourished children come from cereal based carbohydrate diets and protein supplies only 3% of their energy. Schroeder et al (2,28) reported that highest manganese levels occur normally in whole cereals, variable amounts in vegetables and low concentrations in meat, fish and dairy products. They also reported that the foods of plant origin are rich in nickel content and that animal foods are very low in nickel (29). So, it is apparent that though the diets based predominantly on cereals, consumed by these malnourished children are grossly deficient in energy and protein but probably rich in manganese and nickel. Thus, intakes of dietary manganese and nickel are probably quite adequate for their requirements. Many components of the diets affect the bioavailability of manganese and nickel and hence may be considered as contributing factors in causing deficiency or excess state of these elements.

With regards to manganese Thomson et al (30) showed that iron competitively inhibits the absorption of manganese in iron-deficient rats. Analysis of dietary data shows that intake of dietary iron by malnourished children is very much low according to their RDA, so the interaction of dietary iron with manganese probably accelerate the absorption of dietary manganese from the gut. Similarly, excess intake of dietary calcium also affect the bioavailability of manganese (31, 32) in birds. Here also, analysis of dietary data show that the intake of dietary calcium by malnourished children is markedly low. Therefore, less intake of dietary calcium by these children probably facilitates the increased absorption of manganese in these children.

With regards to nickel, very little is known about the factors regulating the bioavailability of dietary nickel. It has been shown that nickel translocates in plants as a stable anionic amino acid complex (33), whether organic nickel complexes are the usual compounds of nickel in the plant tissues, and whether they influence the bioavailability of nickel in any way, remains to be determined. Most probably high intakes of cereal foods rich in nickel may be responsible for increased availability of nickel for absorption. However, we need more data for a valid comparison of the results from this study.

The results from this study support evidences that age and sex have no significant effects on manganese and nickel levels in hair of control children aged 1 to 5 years. This study also provides evidences that there are no significant differences in hair manganese and nickel levels between control and malnourished children. Thus it may be concluded that most probably our malnourished children are not deficient in manganese and nickel. But it needs extensive research works on this field for a valid conclusion and should include a) manganese and nickel contents of the foods consumed by our children and their chemical nature; b) the relations of different chemical forms to their bioavailability from the gut, c) correlations of their levels in hair with those in various organs, tissues, metabolic pools and subcellular fractions.

Summary

Hair manganese and nickel concentrations were determined by proton Induced X-Ray Emission Spectroscopic, external beam, method in Bangladeshi malnourished and control children aged I to 5 years. No significant influences (P> 0.05) of sex and age have found on hair manganese and nickel levels. This study also shows no significant differences in hair manganese and nickel levels between control children and children with either kwashiorkor or marasmic-kwashiorkor or marasmus (P> 0.05). Mean daily intakes of energy, protein, carbohydrate were significantly different in the two groups. Malnourished children are markedly deficient in energy and protein intakes and carbohydrate supplies 80% to 90% of their total energy while protein supplies only about 3% of their total energy. High intakes of manganese and nickel rich cereal based diets and low intakes of dietary calcium and iron may increase the absorption of manganose and nickel by these malnourished children.

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