ESTIMATION OF SERUM COPPER AND ZINC IN ANAEMIC PATIENTS

AISHWARYA S[1]*, SOHARA PARVEEN[1], VISHNUPRIYA V[2], GAYATHRI R[2]

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[1]???, Saveetha Dental College, Chennai, Tamil Nadu, India. [2]Department of Biochemistry, Saveetha Dental College, Chennai, Tamil Nadu, India. Email: aishuswami27@gmail.com

ABSTRACT

Objective: Estimation of serum zinc and copper levels in anemic patients.

Methods: The study design included 30 subjects presenting to clinical laboratory, global hospitals. The subjects were divided into 2 groups. The Group 1 consists of 15 healthy individuals and Group 2 consists of 15 anemic patients.

Results: The serum copper and zinc levels were evaluated in anemic patients. The serum copper and zinc were found to be significantly lower in anemic patients when compared with normal individuals.

Conclusion: Thus, the study concludes that the serum zinc and copper in anemic patients is significantly less when compared to the control individuals. Hence, it is important to add adequate amount of zinc and copper rich food in their diet to control the prevalence of anemia.

Keywords: Anemia, Zinc, Copper, Hemoglobin.

INTRODUCTION

Iron deficiency (ID) and ID anemia (IDA) are common worldwide especially among young women and have been shown to decrease general health and well-being [1-3]. IDA constitutes an important public health problem worldwide. It is a hypochromic microcytic anemia representing the most severe degree of ID due to insufficient iron stores to maintain red blood cell synthesis [4]. Copper levels in tissues and body fluids depend on diet, state of health, sex, and age. It is a component of at least 16 mammalian metalloprotein, many of which are central to hematopoiesis, bone and connective tissue physiology, and parts of nervous system [5,6]. Copper toxicity is rare, resulting in nausea, vomiting, hemodialysis, hepatic necrosis, oliguria, azotemia, convulsions, and coma [7]. Copper plays a critical role in human metabolism. It acts as a cofactor of key metabolic enzymes, which are involved in respiration, neurotransmitter biosynthesis, radical detoxification and iron metabolism. The average daily intake of copper is between 1 mg and 3 mg, and this amount is adequate for body needs. However, in lower socio-economic groups chances of copper deficiency exist [8,9]. Copper is an essential trace element, playing a critical role in multiple functions in the body. Copper has an important role in maintaining good health. When paired with iron, it helps create red blood cells. It also helps keep blood vessels, bones, nerves, and the immune system healthy. Humans typically ingest copper by drinking water from copper pipes and eating foods such as nuts, some fruits and vegetables, shellfish, and red meat. One unique thing about copper is that women need more of it than men. This seems to be primary because copper is required for the production of the enzymes which convert progesterone into estrogen. It is the metal's deficiency that is manifested in many diseases. The measurement of this metal in additional biological samples such as hair, erythrocytes, leukocytes as well as some enzymes such as superoxide dismutase, relative exchangeable copper, and tissue copper will be a useful approach for the differential diagnosis of various diseases pertaining to this metal. As a reliable colorimetric method using zinc, dibenzyldithiocarbamate is available and hence measurement of this metal could be carried out in any clinical laboratory. Assayed controls with reference values are readily available with reputed commercial organizations and hence the reliability of results could be easily established. Supplementation studies such as prescribing copper along with iron tablets for better

pregnancy outcome could also help the laboratory diagnosis of anemia related gestational problem [10].

Zinc is an antioxidant, micronutrient that has been playing a very significant role in maintaining immune function and neutralizing the reactive oxygen intermediates produced by activated macrophages and neutrophils in their response to micro-organisms. The deficiency of antioxidant trace elements in the people living with HIV and AIDS is due to excessive release of pro-oxidants and cytokines leading to increased

Table 1: Group statistics

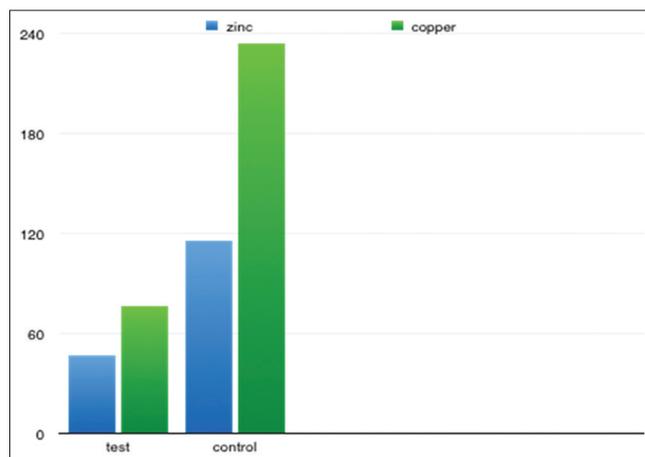| Group	n	Mean	SD	Standard error mean
Zinc test	15	47.04	9.740	2.515
control	15	115.87	42.572	10.992
Copper test	15	76.327	24.6008	6.3519
Control	15	233.600	229.4617	59.2468

Fig 1: ???

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Table 2: Independent samples test

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???	Levene's test for equality of variances		t-test for equality of means						95% confidence interval of the difference	
	F	Sig	t	Df	Significant (two tailed)	Mean difference	Standard error difference	Lower	Upper	
Zinc										
Equal variances assumed	12.183	0.002	−6.104	28	0.000	−68.827	11.276	−91.925	−45.729	
Equal variances not assumed			−6.104	15.461	0.000	−68.827	11.276	−92.799	−44.854	
Copper										
Equal variances assumed	17.205	0.000	−2.639	28	0.013	−157.2733	59.5863	−279.3303	−35.2164	
Equal variances not assumed			−2.639	14.322	0.019	−157.2733	59.5863	−284.8043	−29.7423	

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Table 3: Ranks

Group	n	Mean rank	Sum of ranks
Copper			
Test	15	8.93	134.00
Control	15	22.07	331.00
Total	30		

Table 4: Test statistics

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???	Copper
Mann-Whitney U	14.000
Wilcoxon W	134.000
Z	−4.086
Asymp. Sig. (two tailed)	0.000
Exact sig. (2* [one tailed sig.])	0.000[a]

[a]Not corrected for ties, [b]Grouping variable: Group

utilization of antioxidant like zinc [11-15]. Zinc is a part of over 80 metalloenzymes found in all metabolic pathways. It is required for DNA, RNA and protein synthesis, cell-mediated immunity, reproduction and development of the epidermis and Central nervous system [16,17]. Zinc deficiency has been associated with dementia, depression, thought disorders and schizophrenia. It has also been linked with senile dementia but it has been disputed. In senile dementia [18], there is evidence for a primary degeneration of the noradrenergic system and loss of nerve cells in the locus coeruleus. Clinical symptoms of zinc deficiency are similar to those of acrodermatitis enteropathica, itself due to a congenital often fatal inability to absorb zinc from the diet: Watery diarrhea, seborrheié skin lesions acrally and around body orifices, alopecia, immunodeficiency, and impaired healing [19].

METHODS

Subject
The study design included 30 subjects presenting to clinical laboratory, global hospitals. The subjects were divided into 2 Groups. The Group 1 consists of 15 healthy individuals and Group 2 consists of 15 anemic patients. The subjects suffering from any systemic disorder, smokers, pregnant women, and those taking antibiotics 6 months before research were excluded. All these conditions were considered to possibly influence the hemoglobin count. Furthermore, each patient received a detailed explanation regarding the study procedure and gave their oral consent to participate in the study.

Sample collection
Venous blood samples were collected and transferred to sterile 5 cm ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes and hemoglobin levels of anemic patients were measured using automated cell counter for analysis. Hemoglobin levels between 6 and 8 were taken for the current study. Venous blood samples were collected for analysis of zinc and copper which were drawn into sterile tubes nontreated with heparin, EDTA, citrate, etc. Serum was separated from cells by centrifugation at 3500 rpm for 10 minutes. The serum samples were stored at 18°.

Determination of serum zinc
Serum zinc was estimated as described by nitropaps method colorimetrically using a kit supplied by Agappe. 1 ml of working reagent of was mixed with the serum sample of 0.05 ml. Then, the solution was mixed well and incubated at 25°C for 5 minutes. The absorbance of standard and test samples was measured against the blank within 20 minutes in a semi-auto analyzer to get the zinc level.

Determination of serum copper
Serum copper was estimated as described by Di-Br-PAESA method colorimetrically using a kit supplied by Agappe. 1 ml of working reagent was mixed with 0.05 ml of serum sample. Then, the solution was mixed well and incubated at 25°C for 10 minutes. The absorbable of standard and, test samples were measured against the blank within 30 minutes in a semi-auto analyzer to get the copper level.

RESULTS AND DISCUSSION

Statistics
The given sample was analyzed statistically as given below.

This research was conducted on 15 anemic patients who were diagnosed as anemic and 15 healthy individuals. Mean values of zinc are 47.04 µg/dl in anemic patients which is significantly lower than the control group. The mean value of copper is 76.327 µg/dl in anemic patients which is significantly lower than the control group. Hence, it is evident that the serum zinc and copper in anemic patients are lower when compared to control groups.

Nutrition, physiological features in different life periods and underlying pathological conditions also affect trace elements status. It has been shown that children in infancy and early childhood are particularly susceptible to deficiency of iron and zinc and copper. This vulnerability is due to increased requirement for rapid growth which is frequently met by the diet [20-25]. Anemia often shows association with low serum zinc level as well as zinc deficiency status [26-28]. In our study, obtained values for serum zinc in patients with anemia were also lower in comparison to controls shown in Table 1. The patient with less body weight and significantly lower red cell zinc when compared with controls. Zinc deficiency in patients with sickle cell anemia probably is due to continued hemolysis and hyperzincuria [29] has been documented by several studies with strong indications that zinc deficiency associated with impaired T helper functions, cell-mediated

immunity, and reduced interleukin 2 and increased rate of bacterial infection vaso-occlusive crisis, growth retardation [30-33].

Table 1 shows that the serum copper in anemic patients is lower when compared to control individuals. In recent study of USA which states that anemia is typically normocytic or macrocytic and rarely microcytic with copper deficiency and is of variable severity depending on the degree of copper deficiency [8]. It is not clear how anemia is related with copper deficiency. However, some authors have suggested that copper enzymes are critical to the maturation of hematopeietic cells and copper deficiency can impair effective iron utilization. Our study noted that there is a higher frequency of copper deficiency in anemia [34].

CONCLUSION

Thus, the study concludes that the serum zinc and copper in anemic patients is significantly less when compared to the control individuals. Hence, it is important to add adequate amount of zinc and copper rich food in their diet to control the prevalence of anemia. Due to the decreased level of zinc and copper in anemic patients in addition to iron patients are more prone to the risk of other diseases too. Hence, proper medical care must be provided for those patients to improve their health.

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