Original Article

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Evaluation of Serum Zinc, Copper Level and their Correlation with Cu/Zn Ratio and FT3/FT4 Ratio in Hypothyroidism

Biochemistry Section

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ABSTRACT

Introduction: Hypothyroidism is a condition characterised by deficiency of thyroid hormones due to defect in hormone synthesis pathway or development of resistance at the tissue level. It has been observed that trace elements may influence hormonal function at several levels. Various studies suggest that reduction in zinc and copper levels adversely affect the endocrine system.

Aim: To determine the serum zinc and copper levels in hypothyroidism and its effect on the progression of hypothyroidism.

Materials and Methods: The present case-control study was conducted among 80 hypothyroid patients and 80 apparently healthy individuals aged 18-55 years. Serum zinc (Zn) and copper (Cu) were measured by the colorimetric assay using semi-automated analyser. Free triiodothyronine (T3), free thyroxine (T4) and Thyroid stimulating hormone (TSH) were measured using the chemiluminiscence immunoassay method. Individual parameters by independent sample t-test and correlation was analysed by Pearson's correlation test. The p-value <0.05 was considered significant.

Results: No statistical difference was observed in the BMI among the two groups. Zinc levels in hypothyroid group (85.21 µg/dL) were significantly (p-value=0.002) lower as compared to the control group (100.83 µg/dL). However, copper levels in hypothyroid group (151.1 µg/dL) were increased as compared to the control group (140.5 µg/dL) but the difference was not statistically significant (p=0.09). In the hypothyroid patients, the Copper to Zinc (Cu/Zn) ratio was 1.7/1, which was higher than that of control group 1.3/1 (p-value=0.001). Zinc was found to be negatively correlated with TSH (r-value=-0.248; p-value=0.033) and positively correlated with FT4 (r-value=0.374; p-value=0.001). Cu/Zn ratio was found to have a weak positive correlation with FT3/FT4 ratio (r=0.0875; p-value=0.45).

Conclusion: This study proved the presence of imbalance of trace elements like zinc and copper among hypothyroid patients and thereby emphasises on their importance in maintenance of thyroid homeostasis.

Keywords: Hypothyroid, Hormone homeostasis, Micronutrients, Trace elements

INTRODUCTION

Trace elements play an important role in hormone secretion and activity, and their binding to the target tissue. Numerous enzymes and transport processes have to work in concert for the proper functioning and secretion of various hormones [1-4]. Prevalence data revealed that more than 100 million Indians are affected by different type of endocrine and metabolic disorders, among them 42 million people have thyroid abnormalities. Statistics reflect that 11% of Indians suffer from hypothyroidism as compared to only 2% and 3.7% of UK and U.S.A populations, respectively [5].

In 2008, Kotecha PV reported that daily diet of 70% of Indian population has insufficient quantity of essential micronutrients. The study also highlighted that nearly two-third of the two billion Indian population have zinc deficiency and are at high risk of developing associated metabolic disorders [6]. In India, although all age groups are equally susceptible to zinc deficiency development, young children, infants, pregnant and lactating women are most vulnerable. Zinc is an essential agent that affects and controls the synthesis of TRH (Thyrotropin releasing hormone). It acts as a catalytic agent that regulates the functioning of more than 200 enzymes in our body, and plays a cardinal role in cell-mediated immunity, wound healing, cell division and protein and DNA synthesis. Zinc supplementation has been reported to improve hyperthyroidism, and thyroid profile in diabetic patients apart from reducing the risk of obesity [7,8].

Copper is one of the vital minerals that is found in many basic oxidative enzymes such as Cu/Zn SOD (Superoxide dismutase) that neutralises the toxic superoxide, by breaking it in to O_2 and H_2O_2. It acts as an anti-oxidant and helps in eliminating free radicals and thus reduces oxidative damage. Literature suggests that it is an essential catalyst for synthesis of thyroid hormone, thyroxine and copper supplementation have a positive effect on treatment, complication management, and Progression of hypothyroidism [2].

Due to paucity of data on the correlation between thyroid hormones FT3, FT4 and TSH, and trace elements-zinc and copper, the present study was conducted to determine the serum zinc and copper levels in hypothyroidism.

MATERIALS AND METHODS

The observational case-control study was carried out over a period of six months (November 2017 to April 2018) at Central Clinical Laboratory of Chettinad Academy of Research and Education, Chennai, Tamil Nadu, India. Before commencement of the research work, ethical approval was obtained from the Institutional Human Ethics Committee (Proposal no: 351-A/IHEC/10-17). Written informed consent was taken both from cases and control subjects. Personal details and anthropometric details of the participants were recorded.

The study included 80 hypothyroid (13 males and 67 females, aged 18 to 55 years) patients and 80 age-matched controls (7 males and 73 females), with power of study was 80% with 95% confidence interval. Clinically diagnosed hypothyroid patients with TSH >4.5 µIU/mL, FT3 <2.5 pg/mL and FT4 <0.58 ng/dL were selected. Patients with chronic diseases such as diabetes mellitus, cancer, renal disease, liver and psychiatric disorders, and antenatal mothers, smokers and patients on multivitamin supplements were excluded from the study.

BMI was calculated using the formula BMI=weight (kg)/height (m^2). For biochemical analysis, 5 mL of morning blood sample was collected aseptically from the subjects. The serum was obained

by centrifugation at 2500-3000 rpm for 5 minutes using a REMI centrifuge (Mumbai). The sample was stored at -80°C and analysed within 1-2 months of sample collection. Estimation of FT4, FT3 and TSH was carried out by chemiluminiscence immunoassay, thyroid function test kits were obtained from Beckman Coulter (FT3 catalogue No: -A13422, FT4: 33880 and Hypersensitivity TSH: 33820). Serum zinc and copper were measured by colorimetric assay using semiautomated analyser and kits from Coral Diagnostics (zinc-catalogue number: Zn (Col):01(P), copper-Catalogue Number: CPR (Col):01(P) [9].

STATISTICAL ANALYSIS

Statistical analysis was carried out using IBM Statistical Package for Social Sciences. (SPSS; version 21.0). Individual parameters were compared by Independent sample t-test and correlation was analysed by Pearson's correlation test. The $p<0.05$ was considered significant.

RESULTS

The serum TSH (p-value=0.02) levels were significantly increased in hypothyroid patients as compared to the control group. Copper levels were higher among hypothyroid patients but the difference among groups was not statistically significant (p=0.09). The serum FT3, FT4 and zinc levels were significantly lowered in hypothyroid patients as compared to the control group [Table/Fig-1].

	Group				Independent samples t-test	
	Hypothyroid		Normal			
	Mean	SD	Mean	SD	t-value	p-value
Age	35.275	9.699	35.500	10.976	-.137	0.891
Height (in cm)	157.588	8.583	157.188	10.971	.257	0.798
Weight (in Kg)	71.963	12.199	68.975	12.155	1.552	0.123
BMI (kg/m^2)	29.148	5.454	28.170	5.594	1.120	0.264
Copper (µg/dL)	151.113	51.015	140.500	21.223	1.718	0.088
Zinc (µg/dL)	85.206	36.112	100.825	23.865	-3.227	0.002*
TSH (µIU/mL)	14.215	29.074	4.610	22.026	2.350	0.020*
FT3 (pg/mL)	2.893	1.273	3.345	0.475	-2.962	0.004*
FT4 (ng/dL)	0.736	0.308	1.999	9.287	-1.201	0.232
FT3/FT4 ratio	3.7	2.21	3.6	0.85	0.56	0.28
Copper/Zinc ratio	1.7	0.275	1.3	0.194	-3.290	0.001*

[Table/Fig-1]: Mean±SD of biochemical parameters of hypothyroid and control groups. TSH: Thyroid stimulating hormone, fT3: Free T3, fT4: Free T4, BMI: Body mass index. Independent sample t-test; *p<0.05 statistically significant

The Cu/Zn ratio of hypothyroid group was (1.7/1), which was significantly (p-value=0.001) higher than that of control group (1.3/1). Cu/Zn ratio was found to have a weak positive correlation with FT3/FT4 ratio and the result was not statistically significant (r=0.0875) (p=0.45).

Zinc was found to be negatively correlated with TSH (r-value=-0.248; p-value=0.033) and positively correlated with FT4 (r-value=0.374; p-value=0.001). Copper was negatively correlated with TSH (r=-0.071, p=0.53) and positively correlated with FT3 (r=0.374, p=0.45) and FT4 (r=0.074, p=0.58) [Table/Fig-2].

	TSH		FT3		FT4		FT3/FT4 ratio	
	r-value	p-value	(r-value)	p-value	(r-value)	p-value	(r-value)	p-value
Zinc	-0.248	0.033*	0.051	0.72	0.374	0.001*	-0.11	0.33
Copper	-0.071	0.525	0.374	0.45	0.074	0.58	0.213	0.06
Copper/Zinc	0.071	0.54	0.077	0.36	-0.128	0.35	0.088	0.45

[Table/Fig-2]: Pearson correlation analysis between variables in hypothyroid patients. TSH: Thyroid stimulating hormone, fT3: Free T3, fT4: Free T4. Pearson Correlation Test; *p<0.05 statistically significant

DISCUSSION

Thyroid related disorders are highly prevalent in the Indian population [10]. In the present study, the mean levels of zinc were reduced in hypothyroid patients as compared to control group. These findings were similar to Baltaci AK et al., and Gupta RP et al., studies on animal models, that reported decreased levels of zinc and elevated levels of copper in newly diagnosed cases of hypothyroidism [11,12]. Rashid NF et al., studied the mean zinc level of 50 hypothyroid patients on thyroxin 100 µg supplementation which were observed to be significantly lower than the control subject [13]. Baltaci AK et al., Zhang et al., and Yoshida K et al., showed that hypothyroidism had a negative influence on gastrointestinal absorption of zinc [11,14,15] and Kochupillai N and Ruz MI et al., reported sequestration of zinc by the liver as another cause. Zinc facilitates the secretion of thyroid hormones by acting as a catalytic agent which stimulates the tissue. It has been further observed that zinc acts as a cofactor for Iodothyronine Iodinase (IDI) enzyme and regulates the genesis and function of TRH [10,16]. Freake HC et al., showed that it is a vicious cycle where zinc deficiency can lead to hypothyroidism and hypothyroidism in turn leads to zinc deficiency [17]. Nishiyama S et al., reported that serum levels of total T3, free T3 (FT3) and reverse T3 (rT3) can be normalised in hypothyroidism by oral zinc supplementation that highlights the importance of trace elements in maintenance of thyroid homeostasis [18].

In the present study, serum copper level (151.1 µg/dL) were (p-value=0.88) higher in hypothyroid patients as compared to the control group (140.5 µg/dL). Copper was positively correlated with FT3 and negatively correlated with TSH, suggesting that elevated serum copper levels are a risk factor for development of thyroid cancer. Our results were in accordance with Akcay G et al., study and can be explained by the fact that zinc deficiency leads to increased absorption of copper from the intestine [19]. Copper act as a co-factor in the angiogenesis mechanism in tumor cells, and copper toxicity can lead to the development of thyroid cancer [20, 21]. In contrast to our findings, Rashid NF et al., and Jinger SK et al., reported decreased levels of copper among hypothyroid patients [13,22]. While Mohammed HIY et al., and Al-Juboori IA et al., studies reported that there was no significant difference in the copper levels between hypothyroid and control group [23,24].

Tomita H et al., showed that the normal value of copper/zinc ratio should be less than 1.5 among normal individuals and lower levels can be associated with various disorders [25]. In the present study, the Copper/Zinc ratio was found to be higher among hypothyroidism patients as compared to control group. These findings were similar to Rashid NF et al., study [13]. This indicates that zinc deficiency is associated with copper toxicity.

In our study, a positive correlation between FT3/FT4 ratio and Cu/Zn ratio among hypothyroid patients was observed, which indicated that FT3/FT4 ratio is a better diagnostic biomarker than FT3 and FT4 parameters individually to differentiate the causes of chronic thyroid diseases. Studies done by Izumi Y et al., and Yoshimura Noh J et al., reported the usefulness of FT3/FT4 ratio in differentiation of graves' disease and painless thyroiditis [26, 27]. Studies suggest that Cu/Zn ratio is an inflammatory marker and the ratio is highly specific in diagnosing chronic thyroid diseases, than the elements individually [28,29].

LIMITATION

The Sample size was relatively smaller, further studies on larger population are required to determine the diagnostic utility of these trace elements in screening and planning treatment for thyroid disorders.

CONCLUSION

Trace element deficiencies often lead to the development of thyroid hormone deregulations and thyroid disorders that in turn affects the trace element homeostasis. The study highlights the importance of the

daily intake of micronutrients rich diet essential for the prevention of thyroid disorders.

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