ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of serum copper concentration and ceruloplasmin on lipid parameters leading to increased propensity to cardiovascular risk

Vivek R Joshi[1*], Ayaz K Mallick[1], Manjunatha Goud B K[1], Ravindra Maradi[2], Maheshwar G Reddy[2], Raghavendra Tey[3], Gaurav Shorey[4]

*[1]Department of Biochemistry, Melaka Manipal Medical College, Manipal University.
[2]Department of Biochemistry, Kasturba Medical College, Manipal University.
[3]Department of Biochemistry, Kasturba Medical College, Mangalore.
[4]Department of Pathology, Melaka Manipal Medical College, Manipal University.

ABSTRACT

Copper is one of the trace metals which has many important physiological functions, redox active element and for maintaining thyroid activity. Copper is known to effect lipid metabolism. Increase in serum copper level is associated with decrease in concentration of total cholesterol (TC), this is due to down regulation of both biosynthetic enzymes and regulatory proteins. The purpose of this study was to determine the effect of various levels of serum copper on total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein cholesterol. 61 subjects were divided into three groups of 13 hypothyroid, 23 hyperthyroid and 25 euthyroid based on thyroid stimulating hormone levels. The correlation between serum copper and low density lipoprotein (**$p < 0.01$**) **($r = -0.742$)**, and that between ceruloplasmin and low density lipoprotein was significant **($p < 0.05$) ($r = -0.429$**) with strong negative correlation. It can be concluded that serum copper has significant effect on lipid metabolism particularly in hypothyroidism. Dietary copper is essential for proper lipid metabolism. It is known that Copper and Ceruloplasmin lead to oxidation of lipids. This propensity to oxidation can lead to free radical damage and dyslipidemia leading to increased risk of atherosclerosis and cardiovascular diseases.

Kewords: Serum copper, dyslipidemia, atherosclerosis, low density lipoprotein cholesterol, ceruloplasmin, hypothyroidism.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The importance of thyroid gland in maintaining human health is well recognised and normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of thyroid hormones. Most inorganic microelements present at very low concentration in body fluids and tissues like iodine, iron, selenium, copper and zinc. Among the different trace elements copper is one of the trace metals which is not only very much abundant in our body but it also has many important physiological functions and redox active element [1, 2]. Copper levels in serum can be influenced not only by its metabolism which can be influenced by hormone levels but also by change in its transport protein in blood1. It is known that hormones have been shown to influence trace metal metabolism at several levels, including excretion and transport of trace metals [1]. Copper is a catalytic component of numerous enzymes and is also a structural component of other important proteins. It is seen that deficiency of copper (Cu) has been observed to affect the endocrine system adversely [3]. In plasma copper is bound mainly to ceruloplasmin and albumin [4].Thyroid hormones are known to have actions on protein metabolism and lipid metabolism. Serum copper has been shown to increase in hyperthyroidism but no statistically significant difference was found in plasma Cu concentrations between control subjects and patients with other thyroid diseases (including hypothyroidism), though in an animal study serum copper level has been shown to be lower than that of the controls [5, 6]. Copper status have also been linked to decreased plasma T3 concentrations in animals and man [7, 8]. Copper is known to effect lipid metabolism but details of its mechanism are still not clear. Increase in serum copper level is associated with decrease in concentration of total cholesterol (TC), this is due to down regulation of both biosynthetic enzymes and regulatory proteins. For example, mRNA for nine proteins involved in cholesterol biosynthesis was all significantly down-regulated; these include cholesterol-7-monooxygenase, farnesyl diphosphate, farnesyl transferase, HMG-CoA reductase, lanosterol synthase and others. The levels of triglycerides (TG), high density lipoprotein cholesterol (HDL-C) are also significantly reduced whereas the levels of low density lipoprotein cholesterol (LDL-C) remained unchanged in subjects with increased Cu concentration. But few authors have reported that copper enhance the formation of oxidised LDL-C due to formation of free radicals. Ceruloplasmin (Cp) is a α_2-Globulin that contains approximately 95% of the total copper found in serum. The primary physiological role of Cp involves plasma redox reactions. It can function as an oxidant or antioxidant depending on other factors, such as the presence of free ferric ions and ferritin binding sites. Ceruloplasmin is also important in the control of membrane lipid oxidation – probably by direct oxidation of cations – thus preventing their catalysis of lipid peroxidation. At the same time, in the presence of superoxide, Cp is a major contributor to LDL-C oxidation. Increased plasma Cp level has been reported in patients with hyperthyroidism [9]. But no data has been found relating serum ceruloplasmin level and hyperthyroidism.

Aim and objective

The purpose of this study was to determine the effect of various levels of serum copper on lipid metabolism and its transport protein and the consequence of such changes in subjects with thyroid dysfunction. To analyse the importance of trace metal like copper in treatment of subjects with thyroid dysfunction and there efficacy in it.

ISSN: 0975-8585

MATERIALS AND METHOD

We evaluated 61 subjects of which 13 were hypothyroid, 23 were hyperthyroid and 25 were euthyroid controls who would visit Kasturba Hospital. Hyperthyroid and hypothyroid patients were selected on the basis of serum thyroid stimulating hormone (TSH). TSH values between 0.30 mIU and 5.00 mIU (both inclusive) were taken to be within normal limits. TSH > 5.00 mIU were considered as hypothyroid and TSH < 0.30 mIU were considered as hyperthyroid. All the subjects were age and sex matched having excluded smokers, alcoholics, and subjects on lipid lowering agents, OCP users, pregnant females, diabetics, jaundiced patients and those diagnosed with thyroid carcinoma. The ethical clearance was duly obtained from the ethical review committee for the study.

Biochemical parameters

The various biochemical parameters were determined by auto analyser by enzymatic methods like CHOD – PAP method for TC [10], GPO – PAP [10] method for TG and enzymatic method for HDL-C [11]. LDL-C was determined by modified Friedwald's formula. Serum copper levels were estimated colorimetrically using bathocuproin disulphonate method [12]. Serum ceruloplasmin was estimated using immunoturbidimertric method in autoanalyser [13].

RESULTS AND DISCUSSION

Figure 1

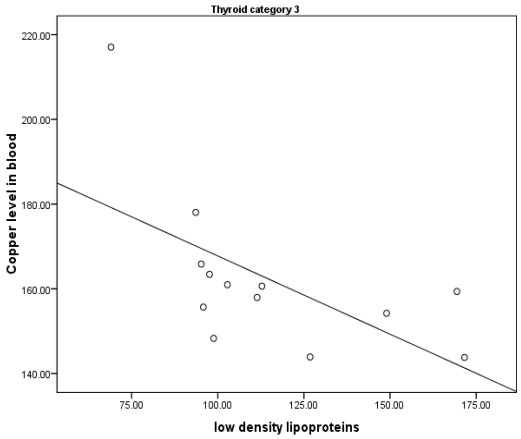
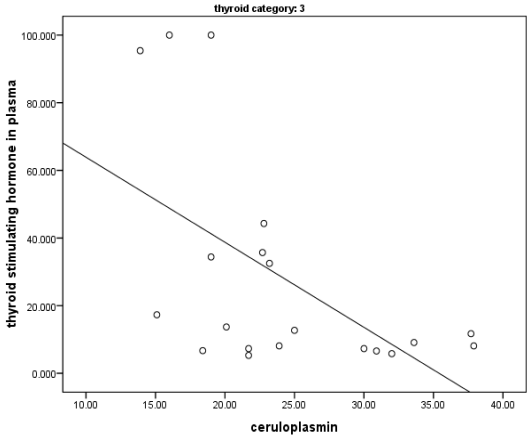


Figure 2



Full descriptive analysis of available data was performed using statistical package for social sciences (SPSS) version 17. The correlation analysis was done to test any possible correlation between plasma copper, HDL-C, LDL-C, TG, TC and ceruloplasmin. The correlation between serum copper and TC was not significant statistically **(p= 0.144)** with strong negative correlation in hypothyroid group **(r= - 0.429)** but it had no significance statistically with weak negative correlation in hyperthyroid and euthyroid group. The correlation between serum copper and TG had no statistical significance with weak negatively correlation in hypothyroid, euthyroid and hyperthyroid group. The correlation between serum copper and HDL-C in hypothyroid group had no significance statistically **(p= 0.172)** with strong negative correlation **(r= - 0.403)** whereas it had weak negative correlation with no statistical significance in euthyroid and hyperthyroid group. The correlation between serum copper and plasma LDL-C levels in hypothyroid group were significant (**p < 0.01**) (**Figure 1**) which had very strong negative correlation **(r = -**

0.742) (Table 1) but was not significant statistically with weak negative correlation in hyperthyroid and euthyroid group.

Table 1: Correlation between serum copper and TC,TG,HDL-C and LDL-C.

TC	r = - 0.429,p = 0.144	**r= - 0.242, p= 0.244**	**r= - 0.302, p = 0.161**
TG	**r= - 0.033, p= 0.915**	**r= - 0.060, p = 0.774**	**r = -0.040, p = 0.856**
HDL-C	**r= - 0.403, p= 0.172**	**r = - 0.170, p = 0.417**	**r = - 0.310, p = 0.149**
LDL-C	**r = -0.742, p < 0.01**	**r = - 0.276, p = 0,181**	**r= - 0.188, p = 0.391**

Table 2: Correlation between ceruloplasmin and LDL-C, TSH

LDL-C	**r = 0.525, p = 0.065**	**r = 0.043, p = 0.768**	**r = 0.037, p = 0.868**
TSH	**r = -0.429, p < 0.05**	**r = -0.448, p = 0.914**	**r = -0.924, p = 0.055**

The correlation between serum Cp and plasma LDL-C in hypothyroid group was not significant with strong positive correlation **(r = 0.525) (Table 2)** in hypothyroid group but weak positive correlation in hyperthyroid and euthyroid. The correlation between serum Cp and plasma TSH was significant statistically **(p < 0.05)** with strong negative correlation **(Figure 2)** in hypothyroid group**(r = -0.429**) (**Table 2**) but it was not significant with strong negative correlation in hyperthyroid and euthyroid**.**

DISCUSSION

Thyroid disorders are the most common among all endocrine diseases in India. On the basis of countrywide study and other related studies, it can now be estimated that total burden of significant thyroid diseases in the country is approximately 42 million [14]. Many micronutrients are associated with various biochemical function of which copper is one of the important essential micronutrient. Copper plays an essential role in human physiology. It serves as a cofactor of key metabolic enzymes and is required for embryonic development, neuronal myelination, radical detoxification, and numerous other physiological processes. Cu is a redox-active transition metal that may cause oxidative damage to lipids, proteins and DNA molecules. Most plasma Cu (approximately 93%) is bound to ceruloplasmin and a small fraction to albumin (6 to 7%) or is chelated to amino acids (<1%), which is diffusible. It has been reported by few authors that copper selectively up-regulates molecular machinery associated with the cell cycle and chromatin structure and down-regulates lipid metabolism, particularly cholesterol biosynthesis. It is known that the mean concentration of plasma Cu was significantly higher in patients with hyperthyroidism than in control or hypothyroid subjects. . Copper status has also been linked to decreased plasma T3 concentrations in animals and man. As mentioned previously copper has significant effect on lipid metabolism but the mechanism of action of alteration in Cu concentration on lipid parameters is still not clear with varying results. Ceruloplasmin which is bound to copper too shows change with differing thyroid hormones level. It was reported that plasma Cp level were increased in patients with hyperthyroidism. In our study we studied a relatively large group and demonstrated the correlation between trace metal like serum copper and plasma TC, TG, HDL-C, LDL-C. We also studied the correlation between serum Cp and LDL-C, TSH.

As reported earlier copper affects lipid metabolism by down regulating biosynthesis of cholesterol. Our study demonstrated that relationship between serum copper and plasma TC varied in all three groups, it was not significant statistically in any group and correlated negatively but strength of correlation was different in all groups with strong negative correlation in hypothyroid group and weak

negative in other groups. Similar findings have been demonstrated in other studies too but not in subjects with thyroid dysfunctions [15].

It has been reported in the previous studies that elevated copper has significant effect on genes involved in lipid metabolism and hence on their plasma concentration [16]. In our study we observed that there was no significant change in levels of plasma TG in hypothyroid, euthyroid and hyperthyroid group. All the groups showed weak negative correlations which were not significant.

Denovo lipogenesis is reduced in hypothyroidism particularly in the liver, and this is corrected by thyroid hormone [17]. TH action on lipid metabolism and adipose tissue has also been demonstrated in human, wherein stimulation of same relevant gene and lipolysis has been documented [18, 19]. In our study we observed that there was no significant change in levels of plasma HDL-C. The correlation was negative with no statistical significance. It was reported that the levels of HDL-C are usually unchanged or elevated in hypothyroidism and may be reduced in hyperthyroidism.

It is known that most of the cholesterol is carried in LDL-C and hence alteration in cholesterol metabolism may lead to varying levels p of LDL-C. We observed that the LDL-C levels in hypothyroid group had very strong strength of correlation which was significant statistically but showed weak negative strength of correlation which had no significance statistically.

Ceruloplasmin is the major copper binding protein in blood. Its main physiological role is in redox reaction. Increased plasma Cp level has been reported in patients with hyperthyroidism [9]. But no data has been found relating serum Cp level and hyperthyroidism. In our study the level of Cp showed variation in all the groups. The correlation between Cp and TSH was significant statistically but showed strong negative correlation but was found to have very strong negative strength of correlation but no statistical significance in euthyroid and hyperthyroid group. Ceruloplasmin is one of the major contributors in oxidation of LDL-C in body [20, 21]. It was seen that the correlation between serum Cp and plasma LDL-C in hypothyroid group was not significant with strong positive correlation but had weak positive strength of correlation in other groups. Similar findings have been observed in previous studies too.

CONCLUSION

It can be concluded that serum copper has significant effect on lipid metabolism particularly on HDL-C and LDL-C in hypothyroidism. Also it can be concluded Cp levels vary significantly with TSH levels but not much with that of LDL-C. It shows that dietary Cu is essential for proper lipid metabolism and also it has significant effect on maintaining plasma concentration by regulating the catabolic and anabolic pathways of lipids. It is evident that Cu and Cp are redox elements and lead to oxidation of serum lipids. This propensity to oxidation can lead to free radical damage and dyslipidemia leading to increased cardiovascular diseases risk.

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