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Full Length Research Article

Plasma Copper Status in Hypercholesterolemic Patients

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ABSTRACT

There has been inconsistent association between low copper (Cu) status and hypercholesterolemia (Hypercholesterolemia is a known risk factor in coronary heart disease). Most of these earlier studies have been predominantly in experimental models; very few reports have examined human subjects. We investigated the relationship between Cu status and hypercholesterolemia in human subjects and if this relationship is established it may be amenable to nutritional interventions. Seventy four (74) randomly selected plasma samples from patients on which cholesterol (Chol) estimations had been previously performed were included. The plasma samples were classified into three (3) categories according to the cholesterol concentration based on the reference range at UCH, Ibadan as at the time of analysis. The study groups included the following, hypercholesterolemic group (group1) (Chol level, > 250mg/dl), normocholesterolemic group (group2) (Chol level, 150 = 250mg/dl); and hypocholesterolemic group (group 3) (Chol level, 87-149mg/dl). The mean values of Cu in groups 1, 2, 3 were 103.39±8.58 µg/dl, 122.67±14.69µg/dl and 123.82±10.15µg/dl respectively. The mean concentration of Cu in hypercholesterolemia was significantly different from the normocholesterolemia ($p < 0.0001$) and the hypocholesterolemia ($p < 0.0001$) respectively. The plasma Cu level of the hypercholesterolemic group was the lowest; while the levels in the normocholesterolemic and the hypocholesterolemic groups were similar. The low level of Cu in the hypercholesterolemic group was significantly lower than the levels in groups 2and 3 ($p<0.0001$) in both cases. There was a significant inverse correlation between cholesterol and Cu levels ($r = - 0.4909$; $p < 0.0001$). These data support some previous reports that hypercholesterolemia is associated with decrease Cu status and this may be manipulated to control hypercholesterolemia and associated disorders.
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Key Words:
hypercholesterolemia, hypocholestrolemia, normocholesterolemia, copper, coronary heart disease.

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INTRODUCTION

The transition metal copper is an essential human micronutrient for enzymes that catalyze oxidation - reduction reactions (Linder and Hazegh-Azam, 1996). Copper has also been described as an antioxidant nutrient for cardiovascular health (Allen *et al*, 1994). One of the nutrients associated with copper metabolism is cholesterol. The latter plays a central role in many biomedical processes but is best known for its association with cardiovascular disease. Hypercholesterolemia is considered a major risk factor for the development of atherosclerosis (Gotto, 1986). Diets low in copper also has been suggested as an explanation for much of the epidemiology and pathophysiology of ischemic heart disease (Klevay, 2000). For some authors, absolute copper deficiency could be a risk factor in the etiology of cardiovascular diseases by altering lipid metabolism (Thuiller- Jeteau *et al*, 1987). Furthermore copper deficiency was claimed to be the only nutritional insult that elevates cholesterol (Klevay *et al*, 1984). Hypercholesterolemia was also found to be one of the similarities that exist between animals deficient in copper and people with ischemic heart disease (Klevay, 1983). Copper deficiency is therefore offered as the simplest and most general explanation for ischemic heart disease (Klevay, 2000).

A substantial decrease in liver copper concentration has been demonstrated after feeding rats with a cholesterol-rich diet (Abu-el-Zahab 1991). Moreover feeding rats a Copper – deficient diet resulted in hypercholesterolemia (Al-Othman *et al* 1994; Carr *et al* 1990). Biochemical correlate of copper insufficiency included hyper-cholesterolemia when over 30 men and women were depleted of copper carefully with diets made with conventional foods containing 0.65 to 1.02mg/day (Klevay *et al*, 1984; Reiser *et al* 1987). Copper deficiencies from several species have produced hypercholesterolemia in at least 22 independent laboratories worldwide (klevay, 2000). Many people consume slightly less than the "safe and adequate range" of copper, 1.5 – 3.0mg/ day though, frank copper deficiency is uncommon. Deficiency can occur in people using zinc supplement without increasing copper intake because zinc interferes with copper absorption (Sandstead, 1995).

The study aims at investigating copper status in hypercholesterolemic patients and to determine the relationship between copper and cholesterol metabolism, and if this relationship is established copper deficiency may be amenable to nutritional interventions.

MATERIALS AND METHODS

Selection of Subjects.

The study was carried out on seventy-four (74) randomly selected plasma samples obtained from the Clinical Chemistry Laboratory of the Department of Chemical Pathology, University College Hospital (UCH), Ibadan. These samples were among the biological materials of subjects referred to the laboratory for cholesterol estimation. The details of the patients' states of health, features such as age and sex were then traced to their case records. Some of these could not be retrieved. The samples were classified into three groups. Group 3 was made up of samples with cholesterol values less than 150mg/dl (hypocholesterolemic) while group 2 consisted of samples with cholesterol values within the local reference range as at the time of analysis; these are values between 150 and 250 mg/dl (normocholesterolemic). Group 1 included samples with cholesterol values greater than 250mg/dl (hypercholesterolemic). Group 1, the hypercholesterolemic group, serves as the study group while groups 2 and 3 were included for comparison.

Methods

Plasma cholesterol estimation was performed using essentially the enzymatic method of Trinder (1969) on Boehringer Mannheim Hitachi 704 Autoanalyser. (Boehringer Mannheim GmbH. D-68298 Mannheim Germany). Plasma copper level was determined using atomic absorption spectrophotometer (AAS), on Pye Unicam S.P. 90A series 2 (Pye Unicam Ltd.,

Cambridge, England). Plasma total protein level was determined by the Biuret method (Reinhold, 1953) and albumin level in plasma was estimated using the method of Doumas *et al* (1971). Statistical analyses were carried out using Statpac Gold Statistical Analysis package. Student's t- test was used to compare means of two groups, while ANOVA was used to compare means involving three groups. Pearson's correlation coefficient was used to establish relationship between variables. Values of $P<0.05$ were considered statistically significant.

RESULTS

Table1 shows the mean copper levels in hyper-cholesterolemic group 1, normocholesterolemic group 2 and hypo-cholesterolemic group 3. The mean copper level ranges from 103.39±8.58µg/dl to 123.85±10.75µg/dl. Group 1 demonstrated the lowest mean copper value while group 3 demonstrated the highest mean copper value, thus displaying an interesting trend of decrease in mean plasma copper level from group 3 to1. When the mean copper levels of the various groups were compared, a highly significant difference was exhibited ($P < 0.0001$). Table 2 shows further comparison of the mean copper level between groups 1 and 2, a significant difference was observed, $p<0.0001$. Similarly a significant difference was observed between groups 1 and 3 $p<0.0001$. Correlation of copper with cholesterol exhibited a highly significant inverse relationship ($r = -0.4909$; $p<0.0001$) as shown in table 3. Table 4 shows the correlation between Age, cholesterol and Copper.

Table 1
Comparison of copper levels among hypercholesterolemic, normocholesterolemic and hypocholesterolemic subjects (groups 1, 2 and 3)

Groups/ sample No	copper level (µg/dl)*	F value	P value
hypercholesterolemic (Group 1) (n=18)	103.39 ±8.58	15.85	p< 0.0001
normocholesterolemic (Group 2) (n=43)	122.67 ±14.69		
hypocholesterolemic (Group3) (n=13)	123.82 ±10.15		

* Values are mean ± SD; n=sample number

Table 2
Comparison of copper levels between hypercholesterolemic and normocholesterolemic subjects; and between hypercholesterolemic and hypocholesterolemic subjects.

Groups	Copper (µg/dl)*	t-value	P value
Hypercholesterolemic (Group 1)	103.39 ±8.58	5.35	p<0.0001
Normocholesterolemic (Group 2)	122.67 ±14.69		
Hypercholesterolemic (Group1)	103.39 ±8.58	4.38	p<0.0001
Hypocholesterolemic (group3)	123.82 ±10.15		

* Values are mean ± SD

Table 3
Simple Correlation matrix between cholesterol, copper, total protein and Albumin

Biochemical variables	Cholesterol	Total Protein	Albumin
Total Protein	r = 0.3131		
	P = NS (0.007)		
Albumin	r = 0.0782	r = 0.3232	
	P = NS (0.508)	P = NS (0.005)	
Copper	r = -0.4909	r = 0.1111	r = -0.0601
	P =0.0000*	P = NS (0.346)	P = NS (0.611)

*r = correlation coefficient; P = P-value; *= Significant; NS= Not-significant*

Table 4
Correlation between Age, cholesterol and Copper

Age	Cholesterol	Copper
Age	r = 0.0138	r = 03795
(Mean = 42.23 ± 15.05)	P = NS	P = NS

r = correlation coefficient; P = P-value; NS= Not-significant

DISCUSSION

Association between low copper status and hypercholesterolemia has been documented consistently in experimental models (Allen and Klevay 1978; Harvey and Allen 1981; Croswell and Lei, 1985; Carr *et al* 1990; Al- Othman *et al* 1994; Bureau *et al* 2003). Clinical pursuits to ascertain similar effects (or otherwise) among patients that are hypercholesterolemic is scanty.

This study was designed therefore to examine copper status in hypercholesterolemic patients so as to provide a basis for consideration of nutritional interventions, which have been suggested by some investigators (Alarcon-Corredor *et al* 2004; Galhardi *et al*, 2005). This study is important because hypercholesterolemia is a risk factor of coronary heart disease, which is a leading cause of death among many populations (Anon, 1967; Gotto, 1986; Strain 1994; Anon, 2000; AIHW, 2002). It is therefore a relevant factor in the pursuit of strategies for prevention or reduction of coronary heart disease through nutritional interventions.

The inverse relationship between copper and cholesterol levels observed in this study is consistent with other studies (Klevay *et al* 1984; Klevay 1990a, 1990b, and 2000). Klevay has consistently demonstrated an inverse relationship between cholesterol and copper metabolism. However several other investigators have not been able to reproduce this association. Thuiller – Jeteau *et al* (1987) reported increased Cu status in hypercholesterolemia. Abiaka *et al* (2003) observed that unlike in animal studies, copper excess in humans is associated with hypercholesterolemia and therefore will predispose to atherosclerosis. Aoyama *et al* (1999) observed that serum cholesterol did not increase in rats fed with copper-deficient diets, though copper in serum decreased markedly in rats. Bergomi *et al* (1997) found an inverse correlation between lysyl oxidase activity in serum and both systolic and diastolic blood pressure in untreated, mild essential hypertension. This can be said to be indirectly consistent with this study because lysyl oxidase is a copper enzyme (among many others) Klevay (2000) and hypercholesterolemia is a risk factor of hypertension.

From past studies, copper deficiency developed in experimental animals and also in man, had resulted in hypercholesterolemia (Klevay *et al*, 1984; 2000). Copper supplementation had also been found to reduce total cholesterol in rats (Galhard *et al* 2005). According to Alarcon-Corredor *et al* 2004, copper supplementation decreased serum total cholesterol in man. This study had demonstrated reduced copper status in hypercholesterolemia, hence it substantiates the fact that reduced copper status is associated with hypercholesterolemia. There is therefore need to establish if with adequate copper nutriture, hypercholesterolemia can be prevented, thus probably reducing the risk of coronary heart disease.

One of the suggested mechanisms of action between reduced copper status and hypercholesterolemia is that copper deficiency increases the activity of ß-hydroxyl-ß-methyl-glutarylCoA (HMG-CoA) reductase, This enzyme catalyzes the rate limiting step in the biosynthetic pathway of cholesterol from acetyl-CoA. The increased activity of the enzyme in the liver in copper deficient rats corresponds with increased cholesterogenesis. It was thought that more of this newly synthesized cholesterol may be channeled for the synthesis of lipoproteins and their subsequent release into circulation thus causing increase in plasma level. (Valzala *et al* 1987).

The results of this study show an inverse relationship between copper and cholesterol levels. The findings support the hypothesis that hypercholesterolemia is associated with reduced copper status.

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