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Original Research Article

Role of serum copper and lipid profile in cardiovascular diseases patients of Southern Rajasthan

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ABSTRACT

Background: It is clear that deficiencies of some trace elements cause marked alterations in lipid and lipoprotein metabolism. The mechanisms of their effects are not completely obvious and inspite of intense research, the role of these microelements need further elucidation. Thus, the objective of this study is to evaluate the correlations of serum concentrations of trace elements including copper with lipid profile parameters of adult men in Southern Rajasthan.
Methods: Present study was conducted in Cardiology and Biochemistry Department in GMCH, Udaipur during the year 2019. A total of 150 MI cases/controls were selected. Blood sample was collected by vein puncture using an aseptic technique. The blood was allowed to settle at 37ºC and then centrifuged at 3000 rpm for 15 min. Serum was then separated and stored at -20ºC until analysis.
Results: The study cases had significantly higher lipid values than those of controls ($p<0.001$). In cases the copper levels are higher than the controls. Copper levels were more in males 95.40±18.93 than in females 86.93±14.54 ($p<0.05$). Lipid levels were higher in urban population related to rural, TC (238.47 V/s 266.59), TG (219.83 V/s 202.40), HDL (64.39 V/s 54.19) and LDL (131.28 V/s 139.48) ($p<0.05$). Significant negative correlation was seen in study group between serum copper levels and TC ($r=-0.288$, $p<0.05$), TG ($r=-0.236$, $p<0.05$), HDL ($r=-0.946$, $p<0.05$) and VLDL levels ($r=0.102$, $p<0.05$). This indicates that as copper level reduces lipids increases.
Conclusions: Our findings indicate the possible effect of Cu level in serum lipid profile and this effect may be due to the role of Cu as an antioxidant. The correlations between the serum concentrations of trace elements with lipid profile in physiological concentrations may not be the same as the changes observed during deficiencies of the trace elements as in hyperlipidemic patients.

Keywords: Hyperlipidemia, Lipid profile, Lipoprotein, Serum copper, Trace elements

INTRODUCTION

The determination of trace elements in the blood is of increasing interest in many clinical and research laboratories due to their role in maintenance of health and development of optimal physiological function.[1] It is clear that deficiencies of some trace elements cause marked alterations in lipid and lipoprotein metabolism. The mechanisms of their effects are not completely obvious and inspite of intense research, the role of these microelements need further elucidation. Additionally, there are some contradictory findings regarding the relationship between serum trace elements with lipid and lipoproteins.[2] Thus, the objective of this study is to evaluate the correlations of serum concentrations of trace elements including copper with lipid profile parameters of adult men in Southern Rajasthan.

Oxidation is now thought to play an important role in the pathogenesis of coronary heart disease through oxidation of low density lipoprotein cholesterol and free radical formation. Oxidized low density lipoprotein cholesterol

facilitates the evolution of early arterial wall lesions into atherosclerotic plaques by promoting the formation of foam cells from macrophages as well as the recruitment and retention of monocytes in the arterial wall. Free radicals, whose effects are less well established, may contribute to atherogenesis by damaging arterial endothelium, promoting thrombosis, and interfering with normal vasomotor regulation.[3]

Because metals can cause oxidative modification of low density lipoprotein cholesterol and the formation of free radicals, the effect of several pro-oxidant metals, including copper, on cardiovascular disease has come under investigation. Results from ecologic, angiographic, cross-sectional and case-control studies support the possibility that decreased copper concentration may increase the risk for cardiovascular disease.[4]

Cardiovascular disease (CVD) prevention in Asia is an important issue for world health, because half of the world's population lives in Asia.[5] In all the Asian countries, there is a concomitant rise in the level of serum total cholesterol (TC), and with it a rise in CVD. Serum TC levels are also higher in the urban compared with the rural population.[6] Cardiovascular diseases, especially coronary heart disease (CHD), are epidemic in India. The Registrar General of India reported that CHD led to 17% of total deaths and 26% of adult deaths in 2001-2003, which increased to 23% of total and 32% of adult deaths in 2010-2013. Copper play an important role in cardiovascular diseases. Till date we have found very few studies with serum lipid profile, serum copper in association with cardiac in southern part of Rajasthan.

Authors conducted this study to determine levels of serum copper and lipid profile in cardiovascular disease patients.

METHODS

The present study was conducted in Cardiology Department and Biochemistry Department in Geetanjali Medical College, Udaipur during the year 2019 from February to May fulfilling inclusion criteria till sample size is achieved or end of study period whichever is earlier.

Inclusion criteria

A total of 150 MI cases/controls were selected attending cardiac department of Geetanjali Medical College and Hospital, Udaipur, Rajasthan.

Group I

It consisted of healthy adult of age 25-70 yrs (control subjects) n=75. By routine examination and tests, we ensured that all the subjects were healthy and there are no signs and symptoms or history of MI and other chronic diseases.

Group II

It consisted of MI patients of age 25-70 yrs subjects (n=75).

Patients in both groups with age, height, weight and body mass index were matched. Other factors affecting trace elements levels (eating disorders, trace elements medication, alcoholism, smoking, kidney disease, type 2 diabetes mellitus, liver disease, pancreas disease), pregnant ladies and diagnosed cancer patients were excluded by history.

Intervention / assessment

Informed consent was obtained from all subjects participating in the study. Blood sample was collected by vein puncture using an aseptic technique. Serum was separated from the sample and was analyzed for following biochemical parameters:

- Serum copper was estimated by BR-PAESA method on semi autoanalyzer.[7]
- Lipid profile on Cobas-6000 fully automate analyzer.[8]

About 10 mL of blood was obtained from each patient in both groups. The blood was allowed to settle at 37ºC and then centrifuged at 3000 rpm for 15 min. Serum was then separated and stored at -20ºC until analysis. These parameters were analyzed from all samples - Total cholesterol (mg/dl), HDL-C (mg/dl), LDL-C (mg/dl), Triglyceride (mg/dl), VLDL (mg/dl).

Linearity

This procedure is linear upto 500 µg/dl. If the value exceeds this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

Chelating agents such as EDTA, Oxalate and Citrate, present even in traces, prevent the formation of the colour complex, hence necessary care should be taken during the assay.

Highly lipemic samples could interfere and should be cleared by centrifugation or filtration before use. The assay can be run at 600 nm however the absorbance's would be approx. 30% lower as compared to 570 nm.

Statistical analysis

The data of all the subjects (control and the subjects) were statistically calculated & compared and their level of significance and the correlation coefficient was calculated. Summary statistics was done by proportions, mean, median, and standard deviation. The inferential statistics were done by, Student's 't' test and Pearson correlation. All measurements were analyzed using SPSS

version 16.0. 'p' value <0.05 was considered as statistically significant.

RESULTS

There were no significant differences between the two groups accept SES (Table 1).

Table 1 shows biochemical lipid profile characteristics (TC, TG, LDL-C, HDL-C and LDL-C/HDL-C ratio) and copper levels in study and control cases. There is statistically highly significant difference between mean lipid profile characteristics of controls and cases. The study cases have significantly higher lipid values than those of controls. In cases the copper levels are higher than the controls, showing imbalance in their levels (Table 2).

Table 1: Descriptive physical characteristics of controls and cases.

Physical characteristics		Cases		Controls		p value
		No.	%	No.	%	
Age	(Mean ±SD yrs)	54.15	±13.35	50.29	±16.10	0.11 (NS)
Diet	Non Vegetarian	42.00	56.00%	38.00	50.67%	0.51 (NS)
	Vegetarian	33.00	44.00%	37.00	49.33%	
According to area	Rural	28.00	37.33%	30.00	40.00%	0.78 (NS)
	Urban	47.00	62.67%	45.00	60.00%	
Smoking habit	Non Smokers	49.00	65.33%	46.00	61.33%	0.61 (NS)
	Smokers	26.00	34.67%	29.00	38.67%	
Socio Economic Status (SES)	High	26.00	34.67%	38.00	50.67%	0.04*
	Middle	49.00	65.33%	37.00	49.33%	

*Significant; NS- Non Significant

Table 2: Descriptive Biochemical characteristics in controls and cases.

Biochemical characteristics	Cases (n=75)		Controls (n=75)		p value
	Mean	SD	Mean	SD	
Cholesterol (mg/dl)	251.50	42.59	169.21	45.59	<0.001**
Triglycerides (mg/dl)	213.32	24.71	138.27	30.15	<0.001**
HDL (mg/dl)	61.19	13.29	46.03	12.76	<0.001**
LDL (mg/dl)	137.54	21.77	92.31	18.37	<0.001**
VLDL (mg/dl)	60.40	24.17	28.09	14.57	<0.001**
LDL-C/HDL-C (mg/dl)	2.78	1.41	3.87	2.24	<0.001**
Cu (µg/dl)	92.35	22.68	149.23	30.07	<0.001**

**Highly Significant

Table 3: Descriptive biochemical characteristics across genders in cases.

Biochemical characteristics	Males (n=49)		Females (n=26)		p value
	Mean	SD	Mean	SD	
Cholesterol (mg/dl)	236.05	11.28	274.44	11.14	<0.001**
Triglycerides (mg/dl)	211.16	17.33	217.39	10.81	0.05*
HDL (mg/dl)	52.62	13.02	68.16	13.53	<0.001**
LDL (mg/dl)	135.28	21.08	146.78	22.82	0.036*
VLDL (mg/dl)	57.28	24.35	66.26	23.16	0.121
LDL-C/HDL-C	2.57	1.30	2.15	1.62	0.257
Cu (µg/dl)	95.40	18.93	86.93	14.54	0.034*

*Significant; **Highly Significant

The biochemical characteristics across genders in study (cases) patients were significantly higher in females than in males. Females had higher lipid values than males.

Copper levels were more in males (95.40±18.93) than in females (86.93±14.54) which was statistically significant (p<0.05) (Table 3).

There was statistically significant difference in lipid levels among smokers and nonsmokers among TC (257.96±36.41 v/s 239±39.79), TG (232.47±15.11 v/s 204.93±15.40), HDL (55.15±13.77 v/s 62.27 v/s 12.60) and LDL levels (134.00±20.61 v/s 144.21±18.73). When copper levels were compared there was no statistically significant difference.

Above table shows the biochemical characteristics according to area in cases patients. TC, TG, HDL and LDL showed statistically significant difference in rural and urban according to area in cases. Vitamin C levels (0.56 V/s 0.49) shows statistically significant difference ($p<0.05$). Though the copper levels are higher in rural than urban but are statistically non-significant (Table 4).

Authors didn't found any significant correlation between serum copper levels and lipid profile levels in control group.

In present study the results indicate that in cases (study) group there was a significant negative correlation between serum copper levels and TC ($r=-0.288$, $p<0.05$), TG ($r=-0.236$, $p<0.05$), HDL ($r=-0.946$, $p<0.05$) and VLDL levels ($r=0.102$, $p<0.05$). This indicates that as copper level reduces lipids increases (Figure 1).

Table 4: Descriptive biochemical characteristics according to area in cases.

Biochemical characteristics	Rural (n=28)		Urban (n=47)		p value
	Mean	SD	Mean	SD	
Cholesterol (mg/dl)	238.47	40.11	266.59	46.77	0.007*
Triglycerides (mg/dl)	219.83	25.28	202.40	23.79	0.004*
HDL (mg/dl)	64.39	13.71	54.19	12.53	0.002*
LDL (mg/dl)	131.28	12.08	139.48	11.21	0.005*
VLDL (mg/dl)	57.92	23.20	64.55	25.60	0.253
LDL-C/HDL-C	2.91	1.45	2.56	1.35	0.303
Vitamin-C (mg/dl)	0.56	0.15	0.49	0.14	0.049*
Cu (µg/dl)	93.80	18.82	90.17	16.07	0.397

*Significant; **Highly Significant

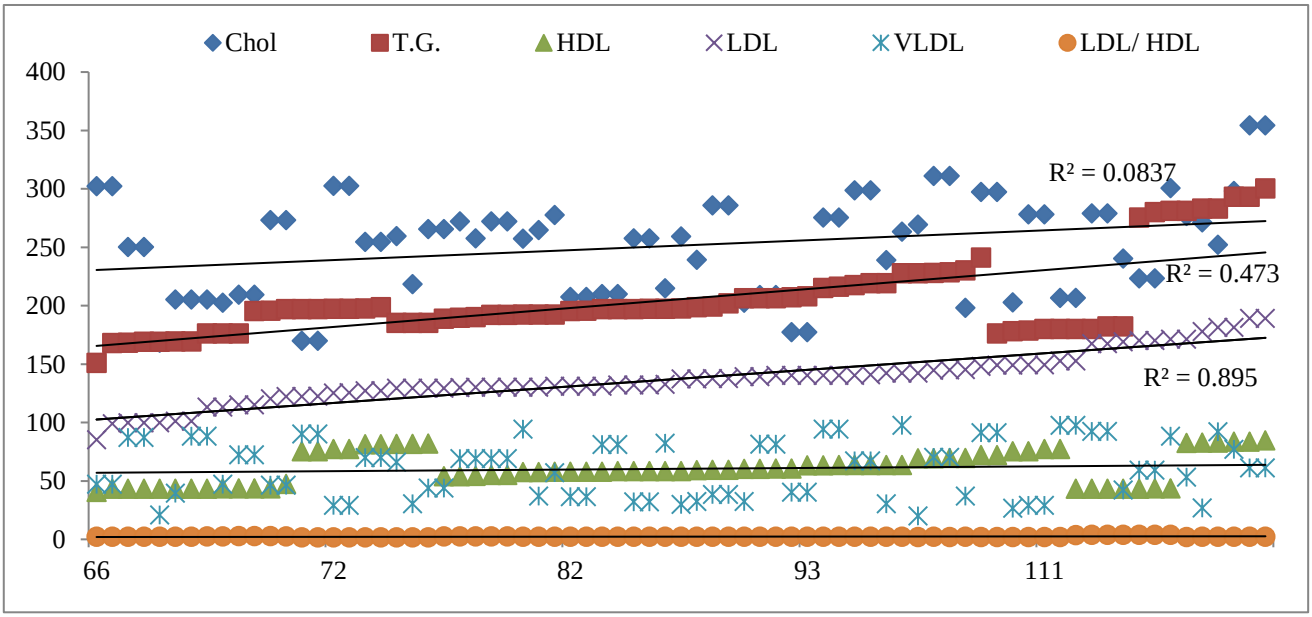

Figure 1: The correlations of serum concentrations of Copper with lipid profile parameters in cases.

DISCUSSION

This study was conducted on 150 subjects, divided into two groups (a) Normal control subjects (75) and (b) Cases (75), who visited cardiology department for the treatment/ diagnosis/ management of cardiovascular disease at Geetanjali Medical College and Hospital, Udaipur and their blood samples were evaluated in the central laboratory of Biochemistry department. The data

so obtained was analyzed and is being compared with other studies.

The mean age of case patients was 54.15±13.35 yrs and of controls was 50.29±16.10 yrs. There were 42(56%) non vegetarians and 33(44%) vegetarians in cases as compared to 38(50.67%) non vegetarians and 37 (49.33%) vegetarians in controls. There were 28 (37.33%) rural and 47(62.67%) urban in cases and 30 (40%) rural and 45(60%) urban in controls. There were 49(65.33%) nonsmokers and 26(34.67%) smokers in cases and 46(61.33%) nonsmokers and 29(38.67%) smokers in controls and 26(34.67%) high SES and 49(65.33%) middle SES in cases and 38 (50.67%) high SES and 37(49.33%) middle SES in control group patients. In our study there were no significant differences between the two groups for the above characteristics (Table 1).

There was statistically highly significant difference between mean lipid profile characteristics of controls and cases. The study cases have significantly higher lipid values than those of controls (Table 2). Similar to study by Zhao et al, (2018) in which lipid abnormality was more prevalent in nonelderly patients (74% vs. 67%, $P<0.05$).[9] The proportion of double- abnormality in nonelderly patients was significantly higher than that in elderly patients (27% vs. 21%, $P<0.05$), whereas no difference was seen. The copper levels were higher in controls, showing imbalance in their levels. There was a significant increase in serum TC, TG, LDL-C and a highly significant decrease in HDL-C when the patient group was compared to the control group.

In study group there was statistically significant difference in Cholesterol, TG, HDL, LDL and LDL-C/ HDL-C ratio among males and females. Females had higher lipid values than males. When vitamin C levels were compared between males and females, males had non-significant difference than females. Copper levels were more in males (95.40±18.93) than in females (86.93±14.54) which were statistically significant ($p<0.05$) (Table 3). It was increasingly recognized that there were differences between male and female in relation to stroke. Evidence indicated that compared with male stroke patients, female stroke patients tended to have worse outcomes, for example, more severe disability.[10] Epidemical studies showed that over 80% strokes occurred in the elderly (>65 years) and this could be explained by the presence of greater risk factors that increased with age, such as atrial fibrillation and hypertension.[11] It was reported that TC level in women increased with age, whereas in men tended to remain stable.[10]

Findings similar to those of present study shows gender differences in lipid/lipoprotein metabolism reported by Russo et al, Kolovou et al, Wang et al, and others.[12-14] Males having higher levels of LDL than females have been reported by Russo et al.[12] Females having higher levels of HDL than males have been reported by Russo et al. and Kolovou et al.[12,13] The HDL comparison for abnormal values requires understanding of the different cut-offs used. Since we normally think of females as having favorable cardiovascular risk profiles until the age of menopause, the population data may provide additional detail to inform clinical risk factor considerations.

In their study TC (238.47 V/s 266.59), TG (219.83 V/s 202.40), HDL (64.39 V/s 54.19) and LDL (131.28 V/s 139.48) showed statistically significant difference in rural and urban according to area in cases. Copper levels were higher in rural than urban but were statistically non-significant (Table 4). Rosa de Groot (2018) revealed that LDL and TC and triglyceride levels are consistently less favorable in urban areas as compared with rural areas.[15] No overall differences in HDL-cholesterol were found between urban and rural areas. Anyway, differences in urban and rural areas are likely to become even more relevant as it is projected that 70% of the world's population will reside in urban areas by 2050.[16] Possible explanation is that in urban areas, occupations often involve office work that generally requires less physical activity as compared with labour in rural, agricultural settings. Also, less heterogeneity might exist between urban and rural areas at the level of occupation-related physical activity, food availability and dietary habits and social-economic status. Another potential limitation is that there is no generally accepted definition of urban and rural.[17]

Our results indicate that in cases group there was a significant negative correlation between serum copper levels and TC, TG, HDL and VLDL levels (Figure 1). The results indicate that there was a significant higher level of serum Cu in hyperlipidemic patients compared with the control group, and that there was a significant correlation between serum Cu and VLDL and a significant positive correlation existed between serum Cu and TG and HDL-C in the patient group while the control group showed no such correlation. Therefore, these findings indicate the possible effect of Cu level in serum lipid profile and this effect may be due to the role of Cu as an antioxidant.

Thus, the decrease in Cu level in patients may lead to increased lipid peroxidation and leading to increased levels of TC, TG and LDL-C according to the results of previous studies. These results support the hypothesis that cholesterol stored in the lipid droplets of the adipose tissue cells is released into plasma and is the chief source of the hypercholesterolemia observed during stress.[18]

According to the results of the present study, serum Cu had significant positive correlations with TG only and no significant correlation was exhibited with other parameters in hyperlipidemic patients. The reason for this result may be due to the sharing of Cu indirectly in formation of TG in serum of patients by contributing to

lipid peroxidation, however, the subject needs further studies in the future to be more precise.[19] Cu had no significant correlations with lipid profile parameters in the control group. This finding is in agreement with previous studies which showed no significant correlation between the serum Cu and TC levels in non-copper deficient.[20]

The results indicate that there are different correlations between trace elements and lipid profile in hyperlipidemic patients while there is no such correlation in healthy men. The cause of these findings suggest that the correlations between the serum concentrations of trace elements with lipid profile in physiological concentrations may not be the same as the changes observed during deficiencies of the trace elements as in hyperlipidemic patients.

CONCLUSION

The physical characteristics such as residence, smokers, nonsmokers, SES, showed significant difference in the controls and cases. Women seemed to have higher proportion of lipid abnormality than men. Copper levels were more in males than in females. As per WHO and recommended copper doses per day is 0.05-1.5 mg/L. Copper can help in increasing HDL (good cholesterol) and decrease LDL (bad cholesterol). The right balance lowers the chances of cardiovascular diseases. These findings would be instructive for lipid management and reduce dyslipidemia in stroke patients.

It is concluded from this study that, there is no doubt that mineral such as copper definitely play an important role in the maintenance of balance in various fractions of lipid profile such as HDL, cholesterol, TG and LDL, which are protective as well as risk factors.

Authors have also observed in their early childhood that their grant grandparents used to store drinking water in the copper utensils and used to drink the same water in the night as well as in the morning. It is because that definitely copper has some beneficial effects in our body, firstly it act as antibacterial and prevent many infections and anemia, improves immunity and heart health.

Now-a-days also copper charged technology is based upon electrochemical dissolution of copper in water. Consumption of two liters of copper charged RO water everyday will also be helpful for improving the normal function of the body.

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