

CIRCULATORY LEVELS OF TRACE ELEMENTS IN METABOLIC SYNDROME PATIENTS IN A TERTIARY HEALTH CENTRE OF UTTAR PRADESH

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Abstract

Background: Metabolic Syndrome refers to a set of metabolic attributes that are correlated to each other. These metabolic attributes contribute to the development of cardiovascular diseases, Diabetes Mellitus Type II, etc. directly. Minerals are physiologically present in the human body at various concentrations. Metabolic syndrome being a cluster of metabolic disorders, participation of minerals is of paramount importance. This metabolic disorder might be associated with the disturbances in the metabolism of minerals viz; zinc, copper, iron, etc. **Aim:** The current study aimed to estimate the levels of serum Trace elements like CU, Zn and Fe among Metabolic Syndrome patients and compare with the levels in healthy controls. **Methods:** The study was conducted on a total 220 individuals out of whom two groups were divided. Metabolic Syndrome case group had 110 subjects between 25-55 age groups who were selected according to the ATP III guidelines under the consultation of experts in Medicine department. Estimation of trace elements viz; Serum Copper and Serum Zinc was estimated by colorimetric methodology. Serum Iron was estimated by Ferrozine method, colorimetric assay. **Result:** The mean level of serum copper was observed to be $521 \pm 376.7 \mu\text{g/dl}$ in MetS cases whereas the mean concentration was $232.9 \pm 138.25 \mu\text{g/dl}$ among the healthy subjects. An insignificant higher value of circulating Zn was observed among the MetS cases i.e. $131.54 \pm 92.7 \mu\text{g/dl}$ as compared to the healthy controls which was $116.55 \pm 62.81 \mu\text{g/dl}$ ($p > 0.05$). Serum Iron concentration when compared between the study groups, mean concentration of serum iron was found to be $243.76 \pm 154.4 \mu\text{g/dl}$ in MetS subjects which was significantly higher than in the healthy controls with mean concentration $188.87 \pm 129.2 \mu\text{g/dl}$ ($p < 0.05$). **Conclusion:** The MetS patients show higher values of Serum Copper, Zinc and Iron and are correlated significantly with few of the attributes of Metabolic Syndrome.

Keywords: Metabolic Syndrome, Insulin resistance, Trace elements, Copper, Zinc, Iron.

INTRODUCTION

Metabolic Syndrome refers to a set of metabolic attributes that are correlated to each other. These metabolic attributes contribute to the development of cardiovascular diseases, Diabetes Mellitus Type II, etc. directly. Metabolic syndrome is linked to a number of metabolic derangements such as Insulin resistance, hypertension, visceral adiposity, oxidative stress, dyslipidemia, hyper-coagulable state, genetical susceptibility, endothelial dysfunction, pro-inflammatory responses, etc. Metabolic Syndrome (MetS) is also known as Syndrome X.¹ Of the major contributors for underlying pathogenesis of Metabolic Syndrome, sedentary lifestyles, lack of physical activities and exercise, high-calorie intake and abdominal obesity hold the leading positions.

There are several diagnostic criteria proposed for the clinical diagnosis of the Metabolic Syndrome based on the clinical features and presentations. Among those diagnostic criteria, ATP III guideline is the most globally accepted one according to which hyperglycemia, hypertriglyceridemia, decreased HDL Cholesterol, hypertension and abdominal obesity forms the basis of diagnosis. Any individuals having any three of the five above mentioned clinical presentations are diagnosed as Metabolic Syndrome. The disease burden of Metabolic Syndrome varies widely in the globe ranging from less than 10% to

as much as 84% having multiple affecting factors such as, composition of population (age, sex and ethnicity), depending on region (urban and rural), etc. The susceptible population for Metabolic Syndrome generally has a history of high- calorie diet in excess and insufficient nutrients.²

Metabolic syndrome being a cluster of metabolic disorders, participation of minerals is of paramount importance. This metabolic disorder might be associated with the disturbances in the metabolism of minerals viz; zinc, copper, iron, etc.³ Minerals are physiologically present in the human body at various concentrations. There are numbers of studies investigating the biochemical functions of these elements and their association with metabolic disease. In this study, we have focused to study serum Zn, Cu and Fe among the minerals in Metabolic Syndrome patients.

Among the cluster of metabolic risk factors, oxidative stress has been considered as one of the main mechanisms to develop clinical symptoms related to the MetS. Since Copper and Zinc are cofactor of antioxidant enzymes, studies have been conducted in order to find out possible differences in these mineral levels in subject with or without MetS. 4,5,6

Iron is an essential element required for heme synthesis and synthesis of heme containing enzymes like Cytochromes. Some studies have shown that increased iron level could affect insulin synthesis and secretion in pancreas. It has also been reported that iron overload could elevate lipid peroxidation which decrease glucose consumption and activate gluconeogenesis pathway in the liver. These lead to the development of insulin resistance. There are evidences of the involvement of iron in obesity and the onset of NAFLD (non alcoholic fatty liver), two pathogenesis related to MetS.⁷ In view of the fact that mineral metabolism influences the metabolic processes in the body and it might have some direct impact in development of Metabolic Syndrome, the present study was planned to measure the serum levels of Copper, Zinc and Iron and to correlate it with the attributes of Metabolic Syndrome.

MATERIAL AND METHODS

Study Set up and Subject selection

The present study is a case-control study. The study set up was at Teerthanker Mahaveer Medical College & Research Centre, Moradabad in collaboration with Santosh Medical College, Ghaziabad. The study was conducted on a total 220 individuals out of whom two groups were divided. Metabolic Syndrome case group had 110 subjects between 25-55 age groups who were selected according to the ATP III guidelines under the consultation of experts in Medicine department. Healthy group had age and sex matched 110 individuals. All the subjects were asked to give their written consent before inclusion in the study groups. Institutional Ethical clearance was taken prior to the start of this research study. The subjects with pregnancy, malabsorption syndrome, gastrectomy and having chronic diseases such as Tuberculosis, Cancers, etc. were excluded from the study. The demographic details, family history of illness and medication history were recorded while selecting the subjects.

Sampling and Methodology

After overnight fasting, plain and fluoride blood sample was drawn under aseptic conditions and was centrifuged at 3000 rpm for 5 minutes. Plasma blood sample was used for the analysis of fasting blood sugar and serum sample was used for estimation of Total Cholesterol, Triglycerides, HDL Cholesterol, Copper, Zinc and Iron. The blood estimations were performed on Semi-Autoanalyser (make- Horiba) using reagents from Tulip Diagnostics P. Ltd, Coral Clinical Systems.

Estimation of trace elements viz; Serum Copper and Serum Zinc was estimated by colorimetric methodology.^{8,9} Serum Iron was estimated by Ferrozine method, colorimetric assay.¹⁰ Other baseline parameters like height, weight and blood pressure were also recorded from each study participants.

RESULTS

Table 1: Comparison of Anthropometric measures, Attributes of MetS and Trace elements between Metabolic Syndrome patients and Healthy subjects

S.N	Variables	Mets (n=110)	Non- Mets (n=110)	P-value
1.	Age (years)	44.42 ± 9.26	40.88 ± 8.17	> 0.05
2.	BMI (kg/m ²)	27.46 ± 2.84	24.52 ± 3.04	< 0.05
3.	Waist circumference (cm)	97.90 ± 8.27	86.88 ± 8.18	< 0.05
4.	HbA1c (%)	7.35 ± 1.42	5.0 ± 0.49	< 0.05
5.	Fasting Plasma Glucose (mg/dl)	139.98 ± 30.75	96.94 ± 4.98	< 0.05
6.	Systolic Blood Pressure (mm/Hg)	132 ± 10	118 ± 3	< 0.05
7.	Diastolic Blood Pressure (mm/Hg)	84 ± 6.0	80.5 ± 2.8	< 0.05
8.	HDL Cholesterol (mg/dl)	35.07 ± 8.89	76.57 ± 8.44	< 0.05
9.	Triglycerides (mg/dl)	226.12 ± 67.59	123.58 ± 9.71	< 0.05
10.	Total Cholesterol (mg/dl)	176.36 ± 52.31	87.34 ± 13.12	< 0.05
11.	Serum Copper (µg/dl)	521.64 ± 376.7	232.9 ± 138.25	< 0.05
12.	Serum Zinc (µg/dl)	131.54 ± 92.7	116.55 ± 62.81	> 0.05
13.	Serum Iron (µg/dl)	243.76 ± 154.4	188.87 ± 129.2	< 0.05

Figure: Comparison of Trace elements among MetS and Non-MetS subjects

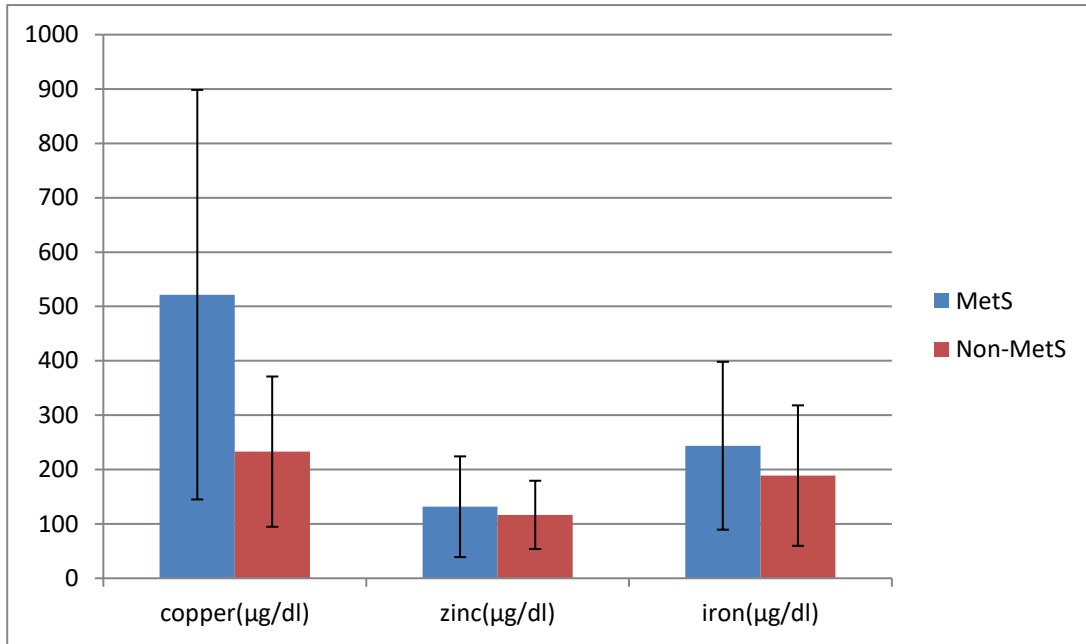


Figure: Comparison of attributes of MetS among study groups

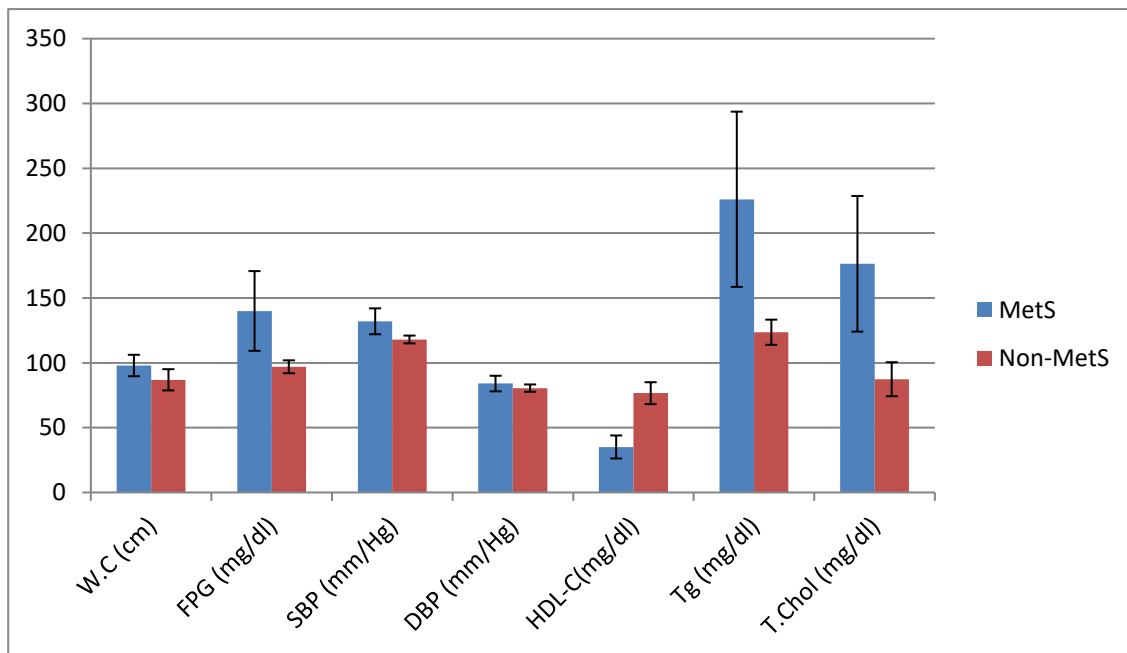


Table 2: Correlation of Serum Copper with parameters

S.N	Variables	r-value	p-value
1.	Waist Circumference (cm)	0.29	<0.05

2.	BMI (kg/m ²)	0.31	<0.05
3.	HbA1c (%)	0.511	<0.05
4.	Fasting Plasma Sugar (mg/dl)	0.72	<0.05
5.	Systolic Blood Pressure (mm/Hg)	-0.37	>0.05
6.	Diastolic Blood Pressure (mm/Hg)	0.01	>0.05
7.	HDL Cholesterol (mg/dl)	0.09	>0.05
8.	Triglycerides (mg/dl)	0.67	<0.05
9.	Total Cholesterol (mg/dl)	0.02	>0.05

Table 3: Correlation of Serum Zinc with parameters

S.N	Variables	r- value	p- value
1.	Waist Circumference (cm)	0.29	<0.05
2.	BMI (kg/m ²)	0.37	<0.05
3.	HbA1c (%)	0.58	<0.05
4.	Fasting Plasma Sugar (mg/dl)	0.84	<0.05
5.	Systolic Blood Pressure (mm/Hg)	0.01	>0.05
6.	Diastolic Blood Pressure (mm/Hg)	0.02	>0.05
7.	HDL Cholesterol (mg/dl)	-0.16	>0.05
8.	Triglycerides (mg/dl)	0.63	>0.05
9.	Total Cholesterol (mg/dl)	0.02	>0.05

Table 4: Correlation of Serum Iron with parameters

S.N	Variables	r- value	p- value
1.	Waist Circumference (cm)	0.05	>0.05
2.	BMI (kg/m ²)	0.09	>0.05
3.	HbA1c (%)	0.07	>0.05
4.	Fasting Plasma Sugar (mg/dl)	0.08	>0.05

5.	Systolic Blood Pressure (mm/Hg)	0.25	<0.05
6.	Diastolic Blood Pressure (mm/Hg)	0.26	<0.05
7.	HDL Cholesterol (mg/dl)	0.26	<0.05
8.	Triglycerides (mg/dl)	0.12	>0.05
9.	Total Cholesterol (mg/dl)	-0.06	>0.05

DISCUSSION

In the present study, estimation of the levels of major trace elements in the patients of Metabolic Syndrome was done. Among the various risk factors involved in the causation of Metabolic Syndrome, oxidative stress is considered as one of the most potential factors. The trace elements like Copper, Zinc and Iron have biochemical significance of being used as cofactors for number of antioxidant enzymes. All these trace minerals show their involvement in various metabolic pathways.^{11,12} The maintenance of metabolic homeostasis requires all these trace elements. A comparison of all the metabolic attributes and circulatory levels of Copper, Zinc and Iron was done in the present study with and without Metabolic Syndrome. Inconsistent findings of serum levels of trace elements among Mets from the previous studies lead to conceptualize the present study.

The circulatory status of Cu, Zn and Fe in our study was found to be elevated in the Mets subjects in comparison to the Non-Mets subjects. The mean level of serum copper was observed to be 521 ± 376.7 $\mu\text{g/dl}$ in MetS cases whereas the mean concentration was 232.9 ± 138.25 $\mu\text{g/dl}$ among the healthy subjects as shown in table 1. These biochemically most significant trace elements were also correlated individually with the attributes of Metabolic Syndrome. Serum Copper on correlating with the components of MetS, it was observed that Copper concentration is positively and significantly correlated with fasting plasma glucose ($r=0.72$, $p<0.05$) and HbA1C ($r=0.51$, $p<0.05$) as mentioned in table 2. Zhang H et al in 2021 studied an association between trace elements and MetS and concluded that there is an association of trace elements like Cu and Zn with MetS components which favors the findings of our study.¹³

This finding from the present study describes that serum Copper influences glucose metabolism. It has been explained through the studies that higher Copper levels are associated to hyperglycemia. Copper circulates in the circulation in a conjugated form with ceruloplasmin which accounts for about 85 to 95% of total circulating Cu. Ceruloplasmin, an acute phase reactant is a mediator of inflammation. Higher levels of Copper proportionate the higher ceruloplasmin concentration which promotes the inflammatory response which is one of the pathological event occurring frequently in Type II Diabetes Mellitus.^{14,15}

In the present study, serum Zinc concentration was also analyzed using colorimetric method. An attempt to compare the levels of serum zinc among the study groups was made. An insignificant higher values of circulating Zn was observed among the MetS cases i.e. 131.54 ± 92.7 $\mu\text{g/dl}$ as compared to the healthy controls as 116.55 ± 62.81 $\mu\text{g/dl}$ ($p>0.05$) which has been displayed above in table 1. Meanwhile, an attempt to correlate serum zinc with Metabolic Syndrome was also made in this study. It was observed that fasting plasma glucose and HbA1c are significantly and positively correlated with zinc levels in circulation with $r=0.84$ & $r=0.58$ respectively ($p<0.05$ in both cases). Similarly, serum Zinc too showed a significant correlation with BMI and Waist circumference as presented in table 3 ($p<0.05$). Zinc is considered crucial for insulin synthesis, storage and release and thus has significance in lipid and glucose metabolism. Also, Serum Zn is supposed to regulate inflammation by reducing oxidative stress.¹⁶ Chia Wen-Lu et al in 2021 proposed opposing findings showing positive associations of serum Zinc with MetS.¹⁷ Another study by Seo JA et al in 2014 found similar observation to that of our study and stated that no significant association exists between serum Zn levels and the prevalence of MetS in either men or women.¹⁸

As shown in table 1, Serum Iron concentration when compared between the study groups, mean concentration of serum iron was found to be 243.76 ± 154.4 $\mu\text{g/dl}$ in MetS subjects which was significantly higher than in the healthy controls with mean concentration 188.87 ± 129.2 $\mu\text{g/dl}$ ($p<0.05$). A similar finding have been mentioned by Hoseini NT et al. in which the investigators found an association between high dietary iron intake that is oftenly related with high calorie intake and adiposity. The authors also have explained that serum ferritin level is too associated with fasting blood glucose and insulin

levels. The individuals with iron overload tend to decrease insulin sensitivity.¹⁹ A statistical analysis correlation to establish a relationship between status of iron and components of Metabolic Syndrome was done in our study. We observed a significant correlation between serum iron status and HDL- Cholesterol with $r= 0.26$ ($p < 0.05$) along with systolic blood pressure and diastolic blood pressure ($p < 0.05$) as depicted in table 4. The findings from our study do not accord with few other studies done to examine the relationship between MetS and Iron status. These studies have demonstrated no relationship between iron status and MetS. Marjani A et al in 2015 while studying association between trace elements and MetS among type II Diabetes Mellitus patients have shown that only Zinc concentration is affected by HDL- Cholesterol (HDL-C), Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) as components of MetS whereas they found no relationship between Cu and Fe concentration and MetS components.²⁰ In contrast to our observations, Athanasia et al in 2011 noted that there is no difference in the trace elements levels between subjects with or without Metabolic Syndrome.²¹ One of the mechanisms behind progression of MetS is insulin resistance which might be induced due to iron overload resulting in decreased insulin sensitivity either directly or via hepatic dysfunction.²² Serum iron status in the current study significantly correlated with HDL-C, SBP and DBP while none other components of MetS showed any relationship with serum iron.

Metabolic Syndrome has affected a wide number of populations globally and has been a major concern of public in the recent days. Our findings can be of significance in improving the daily lives of those persons who are suffering from MetS. It has been observed clearly in our study that the levels of major trace elements like Copper, Zinc and Iron in the blood circulation are deranged among the cases of Metabolic Syndrome. The excess of Copper and Zinc in the individuals are reported to promote several mechanisms that result in MetS. Inflammatory responses, damage to DNA molecules, mitochondrial damages and induction of oxidative stress are some of the pathologies that might be promoted due to overload of Copper and Zinc in the the systemic circulation. Meanwhile, dietary iron overdose leads to iron toxicity influencing liver tissues, skeletal muscles and other tissues as well and thus might play a role in development of Metabolic Syndrome. Therefore, a monitored consultation of nutritionists might help to keep a check over adequate intake of these trace elements so that there is neither a deficiency nor overdose of such nutrients.

LIMITATIONS

Although the study included an average number of subjects, a large sample sized prospective cohort study is suggested to pin- point the cause and effect relationship between trace elements and Metabolic Syndrome. A wider range of minerals having antioxidant property like Selenium and Manganese were not analyzed in the present study which limits the study in finding the role of trace elements overall. Also, the methodology for estimating serum levels of Cu, Zn and Fe applied was colorimetric kit method; instead flame photometric estimation could be suggestive for more accuracy in estimation.

CONCLUSION

The present study demonstrates significant differences in the levels of trace elements like Copper and Iron among Metabolic Syndrome patient and a normal healthy subjects whereas insignificant difference in the serum Zinc level. The MetS patients show higher values of Serum Copper, Zinc and Iron and are correlated significantly with few of the attributes of Metabolic Syndrome. On analyzing the association between trace elements and Metabolic Syndrome, it was observed that serum Copper correlates significantly with BMI, fasting blood glucose and serum triglycerides whereas serum Zinc correlates significantly with BMI and waist circumference which are the markers for obesity. On the other hand, serum Iron correlates significantly with systolic blood pressure, diastolic blood pressure and HDL Cholesterol among the attributes of Metabolic Syndrome. Therefore, it is a subject of concern to have an adequate intake of these trace elements through diet to control the initiation of metabolic disorders and its progression to associated complications.

CONFLICT OF INTEREST

There was no conflict of interest among any of the authors involved in this study.

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