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# PATHOPHYSIOLOGICAL STATUS OF SERUM ANTIOXIDANT, MACRO-MINERALS AND TRACE ELEMENTS IN PATIENTS WITH METABOLIC SYNDROME IN BANGLADESH

Mohammad Shahidur Rahman [1], Kamrul Hasan [2], Md. Saddam Hussain [2], Md. Shalahuddin Millat [2], Niloy Sen [2], Mohammad Safiqul Islam [*2], Md. Shahid Sarwar [2], Wahiduzzaman Noor [2], Auditi Kar [2], S. M. Naim Uddin [3] and Kazushige Yokota [1]

Department of Life Science and Biotechnology [1], Shimane University, 1060 Nishikawatsu-cho, Matsue - Shi Shimane 690 - 8504, Japan.
Department of Pharmacy [2], Noakhali Science and Technology University, Noakhali - 3814, Bangladesh.
Department of Pharmacy [3], University of Chittagong, Chittagong - 4331, Bangladesh.

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Metabolic syndrome, Antioxidant, Trace elements, Macro-minerals, Inter-element relationship

**Correspondence to Author:**
**Dr. Mohammad Safiqul Islam**

Professor and Chairman, Department of Pharmacy, Noakhali Science and Technology University, Noakhali - 3814, Chittagong, Bangladesh.

**E-mail:** research_safiq@yahoo.com

**ABSTRACT:** This study was undertaken to elucidate the serum antioxidant (Vitamin C), macro - minerals (Ca, Na, and K) and trace elements (Zn, Fe) concentration of in patients with metabolic syndromes. Metabolic syndrome was examined by following the definition developed by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII). Serum Vitamin C was estimated by phenyl-hydrazine spectrophotometry method, while Macro-minerals and trace elements were determined under coordination of two methods these was flame atomic absorption spectrometry (FAAS) and graphite furnace. This study found that Vitamin C is lowering significantly ($p < 0.05$) in patients with metabolic syndrome while compared with control subjects. Analysis of serum trace elements (Zn, Fe) and macro minerals (Ca, Na, K) explored low serum concentrations in examined patients significantly either than control group ($P < 0.05$). Pearson's correlation analysis revealed negative correlation between blood glucose and Fe, Triglyceride and Zn level were found statistically significant. On the basis of our present study it can be asserted that depletion of Vitamin C, Zn, Fe, Ca, K and Na levels is strongly associated with the metabolic syndrome pathogenesis.

**INTRODUCTION:** Metabolic syndrome is a bunch of several hazardous heart attack risk factors: diabetes and raised fasting glucose in plasma, high cholesterol, abdominal obesity and hypertension [1 - 4]. About 20 - 25% adult population are suffering from metabolic syndrome through the world and they are also at the three times greater risk for the development of heart attack as compared with people lack the syndrome.

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In addition, these patients are five times more prone to risk of developing type-2 diabetes while 230 million people already widely have lived with diabetes [5, 6], as a result it becomes one of the most widely spread chronic diseases and thus takes fourth or fifth place among narrative disorders causes death in developed countries.

On account of this, metabolic syndrome is now considering a new epidemic of cardiovascular disease because its pathological conditions are clustered with cardiovascular disease (CVD) risk factors that intensified abnormal heart condition. In Pacific and Middle East nations, it causes death to one out of four in adults (35 - 64 years) and also susceptible to the greater risk of developing type-II diabetes.

Type-II diabetes comprises about 90% of all diabetes and becomes one of the leading causes of illness, premature death and heart diseases, which in turn responsible for up to 80 percent of deaths globally [7, 8].

Either hampered glucose tolerances or type 2 diabetes status are liable for generating various risk factors that often take place altogether what are now literally denoted under the name of "metabolic syndrome". Mostly in same individual "clustering" of metabolic abnormalities occur and precipitated to confer substantial additional cardiovascular risk with the clustering of the risk consociated with each abnormality [9, 10]. However, it is ascertained that when a person is identified with diabetes, hyperglycemia and related changes in blood lipids (increased triglycerides and decreased HDL- C) also concurrently showing high blood glucose level which in turn ameliorates the risk of cardiovascular disease [9]. The rate of mortality due to cardiovascular arrest is highly proportional to the presence of components of metabolic syndrome [11]. Diabetes Generally diabetes is associated with cardiovascular difficulties and also responsible for blindness, amputation and kidney failure, which aggrandize social and economic burden related to diabetes [12]. The prediction, "the prevalence of diabetes will double by 2025" stipulates an analogous raise in cardiovascular disease following death related to enormous and incorrigible impacts on health systems throughout the world. The total medical costs of all diabetes in 25 countries of the European Union at 20 and 79 years old was estimated up to 64.9 billion Dollars (ID), equivalent to 7.2 percent of total health expense in these countries [7, 13].

In recent years, there has been a notable increase in the number of people with the metabolic syndrome. It is become an epidemic disease and acknowledge globally with other life style disease such as type-2 diabetes and cardiovascular disease. It is with an increasing prevalence and leading cause of death in adults and children world widely and considered as the one of the most health squeezing disease for 21st century. Not much work has been done to find out the possible reasons for the development of metabolic syndrome, which is counted to be very significant for the treatment of this syndrome. Thus an attempt has been made to examine serum antioxidant levels (Vitamin C); macro minerals (Ca, Na, K) and trace element concentrations (Zn, Fe) s, and to clinch the correlation between the serum levels of these components of this metabolic patient.

## METHODS AND MATERIALS:

**Materials and Chemicals:** This study was conducted with some well-organized instrumental setup which was HPLC machine, Flame atomic absorption spectroscopy, pH meter, ultrapure water system and some other auxiliary instrument to facilitate this whole experiment. All of this instruments were run and handled by following the SOP provided by respective manufacturer.

Chemicals were used for the determination of serum antioxidant, trace-elements and macro-molecules level was maintained in standard grade and purchased mostly from Merck, Germany. For antioxidant determination Metaphosphoric acid, N-(1-napthyl) ethylene diamine dihydrochloride was purchased from Loba Chemie, India, while Trichloroacetic acid (TCA) and Copper sulphate was purchased from Guangdong and Uni-chem laboratory of China respectively. Standards for macromolecule and trace element determination were sourced from Buck Scientific, USA.

**Study Design:** This case-control study was carried out in Laxmipur Diabetic Hospital, Noakhali, Bangladesh. Ethical permission was taken from ethical committee of the respective hospital. For the study purpose, 100 patients with metabolic syndrome were recruited as cases and for comparison 65 healthy volunteers were selected as control subjects.

**Data Collection:** Detailed patient history was taken with a well-designed questionnaire by regularly attending to Laxmipur Diabetic Hospital. Following data's were collected from patients with metabolic syndrome and healthy volunteers: age, sex, blood pressure, plasma glucose level, serum triglyceride level (TG), serum high-density lipoprotein (HDL) cholesterol level and body mass index (BMI).

**Blood Sample Collection and Processing:** 5 ml venous blood samples was drawn from each patient and control in a metal-free sterile tube. The blood sample was kept at room temperature for about 30

minutes to clot and centrifuged at 3000 rpm for 15 minutes to extract the serum. Then the serum was taken in Eppendorf tube and was stored at - 80 °C until the study day. These samples were then used for determining the serum level of antioxidant (Vitamin C), macro-minerals (Na, K, Ca) and trace elements (Zn, Fe).

**Determination of Serum Antioxidant (Vitamin C) Level:** Serum Vitamin C was estimated by phenyl-hydrazine spectrophotometry method [14]. Absorbance of sample and standard were read against reagent blank at 520 nm in the spectrophotometer (UV - 1800, Shimadzu Corporation, Japan). The concentration of ascorbic acid in the serum was calculated by using the formula used by M. S. Sarwar *et al*., [15]

**Determination of Serum Macro-Minerals and Trace Elements:** By using Czuprynetal method, macro-minerals (Na, K, Ca) and trace elements (Fe, Zn) were determined through Flame Atomic Absorption Spectrometry (Shimadzu AA 6800) as well as graphite furnace [16]. By using deionized water, samples were diluted by a dilution factor of 10. Different concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 mg/L) of trace elements were used for calibration of standard graphs. For Zinc, Calcium, Iron, Sodium and Potassium determination, at 213.9 nm, 422.7 nm, 248.3 nm, 589.0 nm, 766.5 nm absorbance were taken respectively. To verify the assay accuracy and to maintain quality, the standard solutions were run for every ten test sample. A software package (Wizard AA software) was used to calculate the concentration of Zinc, Calcium, Iron, Sodium and Potassium.

**Statistical Analysis:** All values were expressed in the form of mean ± SEM (standard error mean). Statistical analysis was calculated by using the statistical software package named SPSS, version 16.0 (SPSS Inc., Chicago, IL). Independent sample t-test was undertaken to determine the level of significance of various parameters between studied and control groups. Finally, correlation among the study parameters was done by using Pearson's correlation analysis.

## RESULTS:

**Socio-demographic Profile of the Study Population:** This study comprised of 100 patients with metabolic syndrome as cases and 65 normal healthy adult as controls. All data are expressed as mean ± SEM. Socio-demographic findings for the patients and controls groups are represented in **Table 1**. It was observed that mean age of the patients with metabolic syndrome and controls were 53.43±1.28 and 54.75±1.63 years respectively and difference between age of this two group was not found Statistical significant (p = 0. 0.522). In this study, it was observed that women with metabolic syndrome had higher prevalence than men. The relative percentages of women and men with metabolic syndrome were 54% and 46% respectively.

**TABLE 1: SOCIO-DEMOGRAPHIC PROFILE OF THE STUDY POPULATION**

| Variables | Patient group | Control group | p value |
|---|---|---|---|
| | Value ± SEM | | |
| Age (years) | 53.43 ± 1.28 | 54.75 ± 1.63 | $0.522^{NS}$ |
| | Value, n (%) | | |
| Sex | | | |
| Male | 46 (46%) | 38 (58.46%) | |
| Female | 54 (54%) | 27 (41.54%) | |

NS = Not significant

**Anthropometric, Clinical and Biochemical Evaluations of the Study Population:** Anthropometric, clinical and biochemical evaluations of the patients and controls are represented in **Table 2**. Statistical analysis of below parameters showed that the patients (38.64 ± 0.45 kg/m$^2$) with metabolic syndrome had significantly ($p < 0.05$) higher level of BMI in comparison to control subjects (21.67 ± 0.26 kg/m$^2$). The patients had significantly higher level of blood glucose level (mmol/L) in comparison to control subjects ($p < 0.05$) with a mean value of 14.09 ± 0.46 and 6.46 ± 0.09 mmol/L respectively. Mean value of triglyceride (mg/dl) of the patients and controls were found 312.28 ± 17.50 and 161.63 ± 5.04 mg/dl respectively, where patients with metabolic syndrome had significantly higher level either than control subjects ($p < 0.05$).

It was also observed that mean value of HDL (mg/dl) of the patients and controls were and respectively. Finally, patients (44.35 ± 0.72 mg/ml) showed significantly ($p < 0.05$) lower level of HDL (mg/dl) while comparing with control group (54.09 ± 0.86 mg/dl).

**TABLE 2: ANTHROPOMETRIC, CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE STUDY POPULATION**

| Parameters | Values (Mean ± SEM) | | |
|---|---|---|---|
| | Patient group | Control group | p value |
| BMI (kg/m$^2$) | 38.64 ± 0.45 | 21.67 ± 0.26 | 0.000** |
| Blood Glucose level (mmol/L) | 14.09 ± 0.46 | 6.46 ± 0.09 | 0.000** |
| Triglyceride (mg/dl) | 312.28 ± 17.50 | 161.63 ± 5.04 | 0.004** |
| HDL (mg/dl) | 44.35 ± 0.72 | 54.09 ± 0.86 | 0.006** |

**p < 0.05 (Significant difference between patient and control groups at 95% confidence interval)

**Antioxidant Status (Vitamin C):** The patients and controls Serum level of Vitamin C are represented in **Table 3**. Serum level of Vitamin C in patients and control groups was found 13.26 ±0.45 and 16.47 ±0.98µmol/L respectively. Statistical analysis reveals that the patients showed significantly lower serum concentration of Vitamin C in comparison to control subjects ($p < 0.05$).

**TABLE 3: SERUM LEVEL OF VITAMIN C IN THE STUDY POPULATION**

| Parameter | Values (Mean ± SEM) | | |
|---|---|---|---|
| | Patient group | Control group | p value |
| Vitamin C (µmol/L) | 13.26 ± 0.45 | 16.47 ± 0.98 | 0.001** |

**P < 0.05 (Significant difference between patient and control groups at 95% confidence interval)

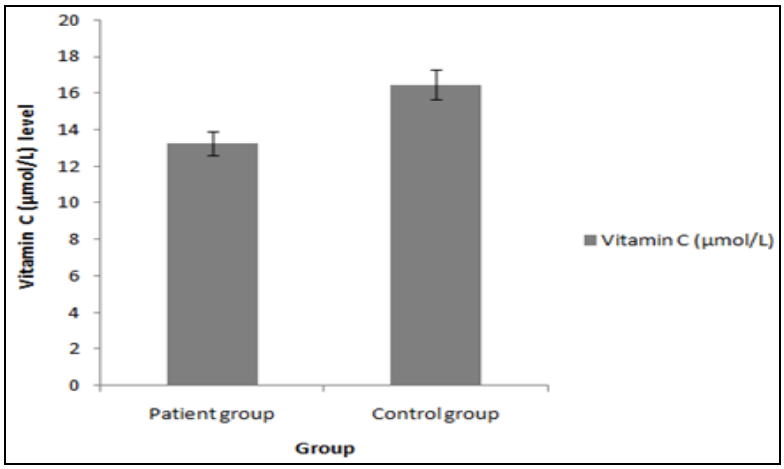


**FIG. 1: SERUM LEVEL OF VITAMIN C IN PATIENT AND CONTROL GROUPS**

**Macro Minerals Status (Ca, K, Na):** Serum level of macro minerals (Ca, K, Na) in patients and control groups are represented in **Table 4**. Statistical analysis reveals that the patients had significant ($p < 0.05$) lower level of Ca with a mean value of 16.47 ± 0.98 mg/L in comparison to control subjects (45.39 ± 2.19). K was found 61.23 ± 3.03 and 167.41 ± 2.80 mg/L in patient and control groups respectively. Similar lower levels of K, Na were found in patients with metabolic disorder while control subjects had higher level for these two respective macro molecules which was found statistically significant ($p < 0.05$) as well. But serum level of Na was found at higher value either than of other two macromolecules in patient group.

**TABLE 4: SERUM LEVEL OF Ca, K AND Na IN THE STUDY POPULATION**

| Parameters | Values (Mean ± SEM) | | |
|---|---|---|---|
| | Patient group | Control group | p value |
| Ca (mg/L) | 45.39 ± 2.19 | 86.24 ± 2.49 | 0.000** |
| K (mg/L) | 61.23 ± 3.03 | 167.41 ± 2.80 | 0.000** |
| Na (mg/L) | 2821.2 ± 75.78 | 3210.8 ± 30.66 | 0.000** |

**P < 0.05 (Significant difference between patient and control groups at 95% confidence interval)

**Trace Elements Status (Zn, Fe):** Serum level of trace elements (Zn, Fe) in both of patient group and control group are represented in Table 5. Mean value of Zn and Fe level (0.30 ± 0.01 and 0.36 ± 0.01 mg/L) was found significantly lower than their concentration in control groups (0.79 ± 0.03 and 0.78 ± 0.03 mg/L), where between this two Fe concentrations showed more prominent value in patient group.

**TABLE 5: SERUM LEVEL OF Zn AND Fe IN THE STUDY POPULATION**

| Parameters | Values (Mean ± SEM) | | |
|---|---|---|---|
| | Patient group | Control group | p value |
| Zn (mg/L) | 0.30 ± 0.01 | 0.79 ± 0.03 | 0.000** |
| Fe (mg/L) | 0.36 ± 0.01 | 0.78 ± 0.03 | 0.000** |

**P < 0.05 (Significant difference between patient and control groups at 95% confidence interval).

**Correlation of Age and BMI with Serum Vitamin C, Macro Minerals and Trace Elements in the Patient and Control Groups:** Pearson's correlation analysis was carried out to determine the relationship between different variables **Table 6**. There was found an inverse relationship while age of patient and control group was compared with HDL and BMI. A positive relationship noticed for both of this group when age was compared with Vitamin C, Zn, Fe and Na respectively. Triglyceride level of Patient group showed negative correlation with age but control group didn't depict this. Control group depicted a significant negative correlation while age compared with Ca, age of this group also showed negative relation with blood glucose but it was not statistically significant.

Relationship of BMI with serum Vitamin C, macro minerals and trace elements in the patient and control groups showed totally a different features. Where, BMI of both group depict positive correlation with triglyceride, Zn, Fe and K respectively. Here, we also found a negative correlation of BMI with blood glucose, HDL, and Ca level in patient group while BMI of control group showed positive correlation with all of this respective serum component. On the other hand BMI of control group was shown a negative correlation only with triglyceride level but patient group showed a positive BMI and triglyceride relationship. None of this data was found to be statistically significant.

**TABLE 6: CORRELATION OF AGE AND BMI WITH SERUM VITAMIN C, MACRO MINERALS AND TRACE ELEMENTS IN THE PATIENT AND CONTROL GROUPS**

| Correlation Parameters | Patient group | | Control group | |
|---|---|---|---|---|
| | R | P | R | P |
| Age and BMI | -0.049 | 0.629 | -0.020 | 0.876 |
| Age and Blood glucose | 0.000 | 0.999 | -0.237 | 0.058 |
| Age and Triglyceride | -0.088 | 0.381 | 0.041 | 0.748 |
| Age and HDL | -0.205 | 0.808 | -0.085 | 0.500 |
| Age and Vitamin C | 0.123 | 0.224 | 0.054 | 0.671 |
| Age and Zn | 0.145 | 0.150 | 0.102 | 0.418 |
| Age and Fe | 0.078 | 0.441 | 0.090 | 0.474 |
| Age and Ca | 0.041 | 0.688 | -0.264 | 0.034** |
| Age and K | 0.027 | 0.787 | -0.191 | 0.128 |
| Age and Na | 0.087 | 0.392 | 0.041 | 0.743 |
| BMI and Blood glucose | -0.159 | 0.114 | 0.038 | 0.766 |
| BMI and Triglyceride | 0.087 | 0.391 | -0.015 | 0.904 |
| BMI and HDL | -0.073 | 0.472 | 0.191 | 0.127 |
| BMI and Zn | 0.028 | 0.781 | 0.045 | 0.724 |
| BMI and Fe | 0.016 | 0.872 | 0.242 | 0.052 |
| BMI and Ca | -0.066 | 0.513 | 0.014 | 0.914 |
| BMI and K | 0.113 | 0.264 | 0.023 | 0.854 |
| BMI and Na | 0.134 | 0.183 | -0.084 | 0.504 |
| BMI and Vitamin C | 0.050 | 0.623 | -0.154 | 0.248 |

r = Correlation co-efficient; p = Significance; Values with negative sign indicate an inverse correlation; **Correlation is significant at 0.05 level (two-tailed).

**Correlation of Blood Glucose Level and Triglyceride with Serum Vitamin C, Macro Minerals and Trace Elements in the Patient and Control Groups:** Pearson's correlation data of blood Glucose level and triglyceride with serum Vitamin C, macro minerals and trace elements in the patient and control groups shown in **Table 7**. In the patient group blood glucose level showed a negative correlation with Vitamin C, Zn, Fe, K and Na respectively, but depict positive relationship only with HDL and Ca. Contrary Glucose level of control group showed negative correlation with all of this serum elements except Ca, Na, K, where negative correlation of blood glucose level with Fe found statistically significant. Interestingly this result was almost different while blood triglyceride level compared with in the patient and control groups. A negative correlation was found when serum triglyceride level compared with serum Vitamin C, macro minerals and trace elements except HDL, Vitamin C and iron. A slightly different data was found for control group where serum Ca, k and Na level only showed positive correlation among all of these with only a statistical significant negative correlation between Triglyceride and Zn level.

**TABLE 7: CORRELATION OF BLOOD GLUCOSE LEVEL AND TRIGLYCERIDE WITH SERUM VITAMIN C, MACRO MINERALS AND TRACE ELEMENTS IN THE PATIENT AND CONTROL GROUPS**

| Correlation Parameters | Patient group | | Control group | |
|---|---|---|---|---|
| | R | P | R | P |
| Blood Glucose and Triglyceride | 0.119 | 0.240 | 0.199 | 0.112 |
| Blood Glucose and HDL | 0.068 | 0.505 | -0.021 | 0.867 |
| Blood Glucose and Vitamin C | -0.094 | 0.354 | -0.145 | 0.248 |
| Blood Glucose and Zn | -0.143 | 0.155 | -0.179 | 0.153 |
| Blood Glucose and Fe | -0.156 | 0.122 | -0.245 | 0.049** |
| Blood Glucose and Ca | 0.102 | 0.310 | 0.226 | 0.070 |
| Blood Glucose and K | -0.015 | 0.885 | 0.181 | 0.149 |
| Blood Glucose and Na | -0.021 | -0.837 | 0.000 | 0.998 |
| Triglyceride and HDL | 0.016 | 0.871 | -0.043 | 0.733 |
| Triglyceride and Vitamin C | 0.039 | 0.698 | -0.085 | 0.502 |
| Triglyceride and Zn | -0.045 | 0.658 | -0.280 | 0.024** |
| Triglyceride and Fe | 0.036 | 0.720 | -0.134 | 0.289 |
| Triglyceride and Ca | -0.003 | 0.976 | 0.009 | 0.940 |
| Triglyceride and K | -0.023 | 0.822 | 0.123 | 0.330 |
| Triglyceride and Na | -0.021 | 0.837 | 0.087 | 0.488 |

r = Correlation co-efficient; p = Significance; Values with negative sign indicate an inverse correlation; ** Correlation is significant at 0.05 level (two-tailed)

**Inter-Element-Correlations between Macro Minerals and Trace Elements:** The present study depict an inter-element-correlations for the analyzed elements (macro minerals and trace elements) between patient and control subjects which exhibited a positive (direct) or negative (inverse) correlations for selected elements. The correlation coefficient and the statistical confidence levels at which the correlations were determined are presented in **Table 8**. Here, Zn ions showed a positive inter elemental correlation with other macro minerals for both of these groups except with K ion, where control group also depicts a negative correlation with Ca ion too. While for both group Ca ion showed negative correlation with K and Na but narrate positive correlation with Fe. On the other hand Fe showed positive and negative correlation with K and Na ion respectively, while K and Na ion itself depicts negative correlation between them for both of this group. But none of this data seems to be statistically significant.

**TABLE 8: INTER-ELEMENT-CORRELATIONS BETWEEN MACRO MINERALS AND TRACE ELEMENTS**

| Correlation Parameters | Patient group | | Control group | |
|---|---|---|---|---|
| | R | P | R | P |
| Zn and Fe | 0.024 | 0.813 | 0.101 | 0.425 |
| Zn and Ca | 0.056 | 0.582 | -0.036 | 0.778 |
| Zn and K | -0.094 | 0.354 | -0.026 | 0.835 |
| Zn and Na | 0.156 | 0.122 | -0.076 | 0.547 |
| Ca and Fe | 0.030 | 0.769 | -0.024 | 0.849 |

| | | | | |
|---|---|---|---|---|
| Ca and K | -0.057 | 0.573 | -0.070 | 0.580 |
| Ca and Na | -0.010 | 0.924 | -0.035 | 0.784 |
| Fe and K | -0.003 | 0.975 | 0.007 | 0.958 |
| Fe and Na | 0.085 | 0.403 | -0.190 | 0.130 |
| K and Na | -0.052 | 0.608 | -0.082 | 0.517 |

r = Correlation co-efficient; p = Significance; Values with negative sign indicate an inverse correlation.

**DISCUSSION:** Conceptualized as a grouping of several metabolic cardiovascular conditions [17,] metabolic all of this following syndrome including blood pressure, abdominal obesity, hyperglycemia, fasting TG and low HDL-cholesterol (HDL-C) and two types of dyslipidemia [18]. Other conditions for metabolic syndrome include elevated level of C-reactive protein, high homeostatic model assessment insulin resistance (HOMA-IR), high hemostasis and hyperuricemia. Several studies suggested type 2 diabetes and cardiovascular disease have found a mark able association with metabolic syndrome this two disease state is responsible for mortality to [19 - 24].

Present study was conducted to understand the role and status of antioxidants, minerals, macro and trace elements in metabolic patients, by comparing the serum level of Vitamin C, calcium (Ca), potassium (K), sodium (Na), Zinc (Zn) and iron (Fe) in the normal and patient group. A recent study found that FRAP (Ferric reducing ability of plasma) levels were significantly found in lower level in the study group compared to controls ($p = 0.001$) [25]. FRAP is a measure of the antioxidant potency, based on ferrous ions reduced by the effect of the reducing power of plasma constituents, produced by low molecular weight antioxidants of a hydrophilic and hydrophobic character particularly Vitamins C and E, serum bilirubin and uric acid in serum. Therefore, in order to have more biologically and clinically validated data for antioxidant capacity of biological sample FRAP will be the most acknowledged approach. This depicts the dynamic equilibrium between pro- and antioxidants in plasma [26]. Metabolic patients have been shown to have depleted levels of antioxidant Vitamins [27, 28]. This depletion has been shown to be more in obese patients compared with non-obese patients with metabolic syndrome [28, 29].

Furthermore, the high concentration of serum uric acid observed in metabolic patients, can result pro-oxidant effects, causing a further depletion in the antioxidant capacity of plasma [30].

Our present finding showed depleted level of Vitamin C in metabolic patients than the healthy subjects which concomitantly endorses the hypothesis on how oxidation becomes to act as a causative factor during pathogenesis of metabolic disorder. Antioxidant supplementation may be recommended to scavenge the action of free radicals which may be useful as secondary therapy to prevent oxidative damage in the tissue of metabolic patients. Handsome number of trace elements (Fe, Zn, Cu, Se, $I_2$, Mn, Mb, Cr, Co *etc.*) has been weighed to be important for the nutritional requirement of the human. High undersupply of these trace elements catalyze a number of diseases such as breast, colon, lung and prostate cancers, leukemia, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), arteriosclerosis, osteoporosis, arthritis, diabetes, edema, Alzheimer's disease, Attention Deficit Disorder (ADD), bi-polar, influenza heart and cardiovascular diseases, many allergies and birth defects [30, 31].

Trace elements are acting as a substrate molecules for enzymes which attract and facilitate conversion of particular component to specific end products. Some donate or accept electrons in the reduction and oxidation reactions resulting in the generation and use of metabolic energy. Gastrointestinal tract is the most common site for the absorption of trace elements, where these are absorbed more freely than any other site. While homeostasis of trace elements are maintained most frequently through urine some other mechanisms are also identified these are excretion through bile, sweat, and breathing. Another mechanism of regulation of serum trace element concentration is storage of trace element in inactive sites or inactive forms (ferritin iron), which itself than prevents inappropriate quantities of reactive trace elements to be present elsewhere.

On the other hand, deficiency of trace elements are being prevented through releasing trace elements from storage site. The interest in clinical and biochemical consequence of the metabolism of

trace elements has been constantly increasing. Trace elements when present at low concentrations shown significant physiological impacts other than those associated with classical toxicity. Some recent hypothesis also suggested that diabetes sometimes manifested in the chemical modification of several trace elements [33, 34]. Zinc is a fundamental trace element and adequately relay on maintaining normal cells processing (cell division and apoptosis) and many of the important biochemical pathways such as transcription, translation, and cell division [35]. Zinc also for initiating catalytic activities of more than 300 enzymes, thus elimination of Zn from catalytic site is directly manifested on the loss of enzyme activity [36]. Moreover, About 70% of Zn is bound with albumin, so alteration of serum Zinc levels is being occurred in any kinds of pathological anomaly of albumin [37]. Several disorders such as dermal disorders, gastrointestinal, neurological and immunological disorders are manifested due to malabsorption of Zinc [38].

Recently published studies show that type - 2 diabetic patients have developed hypozincemia in the blood due to increased urinary depletion [38, 39]. Zinc also boosts the uptake of glucose through assisting the storage and secretion of insulin [39, 40]. Thus, depletion of plasma Zinc level adversely effects on the production and secretion of insulin through rendering the competency of the islet cells [40, 41]. Another hypothesis suggested that Zinc conveyer (ZnT8) is considered as the main protein for maintaining and controlling insulin secretion from pancreatic β - cells [42]. Very recently a mutation in ZnT8 conveyer was found and has partnered with T2D [42]. In short we can say Zinc become act as a pioneer in metabolic regulation and all these evidences also suggest magnitude of Zinc in the sustentation and integration of insulin hexamer. Fe is indispensable for proper metabolism, including normal cell function and thyroid activation. Obesity-induced inflammation increases a hormone called hepcidin, which has the damning side effect of altering iron metabolism in our body. Hepcidin is a hormone produced in the liver that regulates iron homeostasis.

One of the ways that hepcidin regulates the iron level is by determining how much iron to let into our body. High levels of hepcidin reduce or prevent iron intake in the diet, regardless of the iron content of the food. A new study shows the serious metabolic consequences of low iron status [43]. This shows that low iron turns on genes in the liver and muscles that elevate fat storage and cause unusual blood sugar elevation precisely what is wrong with the metabolism leading to metabolic syndrome. Our study explored that metabolic patients have low serum concentration of Zinc and Fe than the normal individuals. Statistical analysis reveals that the patients had significantly lower level of Zn and Fe in comparison to control subjects ($p < 0.05$) which supports the hypothesis that depleted level of trace elements may be a causative factor in the pathogenesis of metabolic syndrome. There is found an inverse relationship between consuming a higher intake of potassium with blood pressure and cardiovascular disease [44]. There is found a muscular relationship between the thiazide-induced hypokalemia and glucose intolerance in thiazide-treated subjects. Again, when potassium depletion have been manifested from a low potassium diet thus impairs insulin secretion and finally turns into glucose intolerance [45].

Another study revealed that potassium ion is responsible for the regulation or increasing of insulin secretion in humans [46]. It is confirmed essential hypertension as well as in type- 2 diabetes is a common feature of potassium deficiency [47]. A study over hypertensive Japanese patients revealed Low potassium intake is significantly associated with increased systolic blood pressure and diastolic blood pressure in hypertensive patients, while same pathological condition was found in the prevalence of metabolic syndrome in Japanese women [48].

Our study explored that metabolic patients have low serum concentration of K than the normal individuals. Statistical analysis reveals that the patients had significantly lower level of K in comparison to control subjects ($p < 0.05$) which supports the hypothesis that depleted level of trace elements may be a causative factor in the pathogenesis of metabolic syndrome. So, Potassium supplementation may be recommended to reduce the risk of metabolic syndrome.

Potassium supplements was found to reduce salt-induced high blood pressure [49, 50], and to improve salt-induced insulin resistance in patients with

hypertension [51]. In consistent with these findings, composition of the diet of fruits and vegetables, which are rich sources of potassium has been considered to lower lipid-induced oxidative stress in obese people and also lower blood pressure and fasting glucose in hypertensive patients [52, 53]. Thus findings like that suggested about a diet rich in food sources of potassium with salt restriction, is the first-line therapy for the treatment of metabolic patients [52]; this finding placed an better statement when combined with physical activity, while a research was conducted over group of Korean women and an assumption was generated that a higher intake of dietary potassium was found associated with a lower risk metabolic syndrome [54].

Calcium is an important trace elements which is used throughout the life cycle of an organism to control various biological processes that is why it is considered as an all-round intracellular messenger [55]. A common defect is evidence for diabetes and cardiovascular diseases and this defect could be divalent cation metabolism, which may include calcium [56].

A recent study suggests changes in calcium metabolism, even within the physiological range, were linked with glucose intolerance, hypertension, and hyperlipidemia [57]. A survey in the population of Newfoundland, Canada, though this relationship remained even after adjustment for 25-OH Vitamin D and PTH, but it was suggested that impaired fasting serum glucose, insulin resistance, and the function of B cells is resulted when calcium homeostasis is being altered [58].

A study suggested that except HDL-cholesterol serum calcium levels were involved in all metabolic syndrome components [59]. So, metabolic syndrome should be taken into account when any interruption is being occurred in serum levels of calcium. Our study reveals that the patients had significantly lower level of Ca and Na in comparison to control subjects ($p < 0.05$) which indicates clear involvement of Ca and Na level in metabolic syndrome.

**CONCLUSION:** The present study suggests a strong association between the pathogenesis of the metabolic syndrome with the level of depletion of Vitamin C, Zn, Fe, Ca, K and Na. Dietary supplementation with antioxidants, macro-minerals and trace elements may drive the treatment of metabolic syndrome and thus reduce its complications. Moreover, changes in lifestyle and therapeutics may reduce adiposity could provide the benefit of preventing obesity-related morbidity and mortality.

**Ethics Declaration:** The total study workout, the protocol of the study and the consent forms of the volunteers were approved by the Ethical Review Committees of Lakshmipur Diabetic Hospital. To eliminate metal contamination during blood collection and storage, the National precaution criteria from the Committee of Clinical Laboratory Standards (NCCLS) were followed.

**Informed Consent:** Informed consent was obtained from all individual participants included in the study.

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