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A study between serum Magnesium, Zinc, and Copper levels in Egyptian patients of Metabolic Syndrome and its Component.

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ABSTRACT

Metabolic syndrome (MetS) is a major worldwide health problem. It is considered to be a significant risk factor for type 2 diabetes mellitus (T2DM) and cardiovascular disease. Magnesium (Mg), Zinc (Zn) and Copper (Cu) are essential trace elements which maintain metabolic body homeostasis through its antioxidant effect. The relationship between trace elements and MetS syndrome and its component is subtle. It may be mediated via oxidative stress. To investigate serum Mg, Zn, and Cu in MetS and its component in Egyptian patients. It included 15 MetS patients in-group (G)-II, 15 hypertensive in G-III, and 15 T2DM in G-IV. There were 15 healthy control in G-I. It carried out in Al Azhar University. Laboratory assessments of lipid profile, HbA1c, blood glucose level, serum Mg, Zn and Cu in all groups. serum Mg and Zn were significantly lower in G-II, G-III and G-IV, in compare to G-I. Cu was significantly high in G-II but significantly low in G-III & G-IV in comparative to G-I. Increased serum Cu level is associated with T2DM, but the decrease of it leads to MetS and hypertension. Mg or Zn deficiency may lead to MetS, T2DM and hypertension and should be supplemented.

Keywords: Metabolic Syndrome, Trace elements, Antioxidant, Oxidative stress, T2DM, Hypertension.

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INTRODUCTION

The metabolic syndrome (Mets) is a clustering of risk factors, which predispose an individual to cardiovascular disease and type 2 diabetes mellitus (T2DM) [1]. It is the most important health problem in the world, leading to mortality and morbidity [2]. Oxidative stress has been hypothesized as one of the main mechanisms leading to Mets [3], systemic hypertension and may contribute to the pathogenesis of diabetes mellitus [4] [3]. The relationship between degrees of oxidative stress and the number of MetS components is also valid. Previous study reported that there is a greater risk of exacerbation of oxidative stress in individuals who have more MetS components suggest that treatment for the reduction or disappearance of each component should reduce the pro-oxidant status in these patients [3].

Oxidative stress occurs when there is imbalance between antioxidant system and reactive oxygen species (ROS) [5]. Therefore, the decrease of antioxidant defense associated with increased of oxidative stress, which lead to metabolic upsets [3]. Hence, the antioxidant enzymes convert dangerous oxidative products to water in presence of cofactors called trace elements to protect cell from damage [6].

Trace elements such as magnesium (Mg), zinc (Zn), and Copper (Cu) perform many important physiologic functions to maintain proper metabolic state [7]. They are accepted as essential component of the antioxidant defense of the body against oxidative damage. Hence, the changes in trace elements levels reduce the efficacy of antioxidant defense mechanism causing increase the oxidative stress on cell integrity [2]. Therefore, impaired metabolism of trace elements are associated with MetS and its complications [8] [9].

Mg is cofactor of many enzymes involved in carbohydrates and lipids metabolism, hypomagnesaemia often coexists with hypertension, may lead to increased atherosclerosis, insulin resistance, T2DM, and its complications [9]. Many studies confirm the link between Mg status and the prevalence of MetS [10]. Zn is an essential trace element, which participate in the synthesis, storage and secretion of insulin [11]. In addition, Zn deficiency may cause insulin resistance, hyperglycemia, impaired glucose tolerance, and the development of diabetes [8]. Cu is yet another important trace element, which acts both a pro-oxidant and antioxidant [12]. In various studies, altered levels of serum Cu levels lead to development of diabetes mellitus and its associated complications. It has also been shown that excess of Cu lead to increase predisposition of vascular disease in diabetic patient [13]. Thus, the purpose of current study is to investigate changes in the levels of serum Mg, Zn, and Cu in MetS and its component in Egyptian patient.

MATERIAL AND METHODS

The present study was carried out during 2016 at Al Zahraa hospital; in the department of internal medicine. Type 2 diabetes mellitus (T2DM) was the final diagnosis in 15 patients(8 males and 7 females) their ages ranged between 37- 70 years, 15 patients with hypertension (10 males and 5 females) their ages ranged between 26 -70 years, and 15 patients having metabolic syndrome (11 males and 4 females), and their ages ranged 50 – 69. Another 15 persons of matching age were included as a volunteer after receiving information about the study; their ages ranged between 32-75 years, they having no history of diabetes, hypertension or any major illness. Smokers were included in this study but who received multivitamins were excluded. Also, patients with hepatic or renal disease were excluded.

Blood pressure was measured from the right arm with a mercury manometer in the sitting position after ten minutes of rest.

Metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP) Expert Panel on detection, Evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) [14].

Subjects with three or more of the below mentioned criteria were considered as having metabolic syndrome:

1. Waist circumference> 102 cm in men and>88 cm in women.
2. Serum triglycerides level≥ 150 mg/dl(≥1.7 mmol/dl)
3. HDL cholesterol<40 mg/dl(<1.04 mmol/l) in men and< 50 mg/ dl (<1.29 mmol/dl in women

4. Systolic blood pressure ≥ 130 mmhg and diastolic blood pressure ≥ 85 mmhg or on treatment for hypertension.
5. Serum fasting blood glucose level ≥110 mg/dl ≥6,1 mmol/l or on treatment for diabetes **[14].**

The patients and controls were divided into the following groups:

- Group I (G-I): consisted of 15 healthy subjects as controls.
- Group II (G-II): consisted of 15 patients having metabolic syndrome.
- Group III (G-III): consisted of 15 hypertensive patients.
- Group IV (G-IV): consisted of 15 patients with type 2 diabetes mellitus.

All studied participants were subjected to the following:

- Full history taking.
- Full clinical examination and electrocardiography were done.
- Laboratory investigations: All routine clinical tests were performed at Al Zahraa hospital and trace elements tests were performed at Al Azhar University center for virus research and studies.
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  - Complete blood picture.
  - Serum creatinine(normal:0.6 to 1.2mg/dl).
  - Serum sodium (normal: 135 to 145 mEq/l).
  - Serum bilirubin (normal: 0.2-0.7 mg\dl), SGOT (normal: 5 to 41 IU/L). SGPT (normal:4-37 IU\dl),serum Albumin (normal:3.4-4.7 g/dl).
  - Fasting blood glucose (FBS)(normal:70 to 110mg/dl) and HBA1C
  - Plasma concentration of :
  - \
    a) Total cholesterol (TC) (Normal: 150 to 200 mg/dl).
    b) HDL-C (normal :< 40 for male, <50 for female mg/dl) and LDL-C (normal:100 to 129 mg/dl).
    c) Triglycerides (TG) (normal: 100 to > 150 mg/dl).
  - Serum magnesium (Mg) (normal: 1.70 to 2.70 mg/dl).
  - Serum zinc (Zn) (normal: 109 to 167µg/dl).
  - Serum copper (Cu) (normal: 80 to 160µg/dl).

Biochemical Investigations:

A Peripheral blood was collected by a single venipuncture from all subjects. All blood samples were subjected to the following:

Sampling:

A. Three ml without anticoagulant was centrifuged and serum was removed for assays of routine biochemical investigations ,lipids profile(total cholesterol ,triglyceride, HDL, LDL), FBS, HbA1c (All were done on Cobase c311 autoanalyzer using Roche reagent kits.
B. Two ml with anticoagulant EDTA was used for complete CBC ,TLC,DLC,ESR, those was done automatically on Sysmex KX21N Hba1c was done by, Bio-Rad D10 hemoglobin A1c program all routine investigations were done in AL-Zhraa Hospital AL- Azhar University.
C. Three ml blood without anticoagulant serum samples were allowed to clot for 2 hours at room temperature then centrifuged for 15 minutes at 1000xg. The samples were clear and were centrifuged to remove solids. The supernatant was collected and stored at-20oC until assays of serum ZINC, CUPPER, and MAGNESIUM.
D.

Zinc, copper, and magnesium level was assayed using colorimetric methods from biodiagnostic CO (Diagnostic and Research Reagents).

CAT. NO for copper 2010, magnesium 1610, and zinc 2120.

Tests were done in Virology center in faculty of medicine for girls Al- Azhar University by instrument CHEM-7 Serial NO.9503 Eeba Mannium

Principles of colorimetric methods for zinc, copper and magnesium

Zinc

Zinc present in sample is chelated by zinocon (2-carboxy- 2" –hydroxy-5-Sulfoformazyl- benzene) in the reagent at alkaline ph, the formation of this complex is measured at a wavelength of 610nm[15].

Copper

Copper is released from protein by hydrochloric acid. The protein is precipitated by triochloroacetic acid. Di ethyldi thio carbamate form golden yellow colored complex with copper, which can be extracted by n-butanol[16] [17].

Magnesium

Magnesium ions react in alkaline medium with the metallochrome dye calmagite to form a chromophore wich absorbs at 520nm.calcium is excluded from the reaction by complexing with ethylene glycol bis (B-aminoethyl ether)- N,N tetraacetic acid (EGTA) [18] [19].

Statistical analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric. The comparison between more than two groups regarding quantitative data with parametric distribution was done by using *One Way Analysis of variance (ANOVA)* followed by post hoc analysis using LSD test. *Spearman correlation coefficients* were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: $P > 0.05$: Non significant; $P < 0.05$: Significant and $P < 0.01$: Highly significant.

RESULTS

- Baseline Demographic, clinical and Biochemical data of the studied group:

The difference between clinical and biochemical data of the healthy control group (G-1), Patients with MetS (Group-II), hypertensive patients (G-III) and T2DM patients (G-IV) are shown in table (1).

As exhibited in table (1) & (2), G-I had mean age (52.20±11.92) years, G-II had mean age (60.47±4.47) years, and mean age of G-III was (53.27±11.58) years, while the mean age of G-IV was (55.80±8.73) years. The BMI, were significantly higher in all patients groups (G-II, G-III, G-IV) than G-I and were (35.26±2.08, 32.94±5.75, 35.17±4.06, 28.45±3.35), respectively and p value =0.000, =0.004 and =0.000, respectively.

The mean total cholesterol levels were found to be (183.93±17.11) mg in G-I,(247.93±17.82) mg\dl in G-II,(175.13±13.74) mg\dl in G-III and(206.00±16.28) mg\dl in G-IV. Highly significant differences (p value =0.000) between the three groups were obtained (Table1). Tables (1) and (2) also showed that, LDL-C and HBA1C were significantly higher in G-II and G-IV than G-I, and were (175.20 ± 16.25, 136.00 ±21.78, 103.43±26.23), respectively and p value=0.000 and =0.000, respectively, (8.11±1.01, 9.36±2.01, 5.22± 0.58), respectively and p value=0.000 and =0.000, respectively.

Serum triglyceride was significantly higher in G-II, G-III, G-IV in comparison to G-1(194.67±33.30, 130.33±10.08, 128.67±29.39, 97.80±8.41), respectively and (p value =0.000.=0.000, =0.001),respectively as shown in table 1and 2. Also, Triglyceride was significantly higher in G-III and G-IV in comparison to G-II

(130.33±10.08, 128.67± 29.39, 194.67±33.30), respectively (table 2). While, HDL- C was significantly lower in G-II, and G-IV than G-I (33.93±2.91, 53.20±2.51), respectively.

- Trace elements:

Serum Mg concentrations:

As exhibited in table (1) and (2) shows a highly significant decrease in concentrations of serum Mg in G-II, G-III and G-IV (all patients groups) in comparison with G-I and were Mean ± SD (1.03±0.31, 1.36 ± 0.39, 1.32 ± 0.41, 2.19 ± 0.31), respectively and (p value = 0.000, = 0.000, = 0.000), respectively . The lowest level is being in G-II (figure 3). Patients in G-II (1.03±0.31) had significantly lower serum Mg than G-III (1.36±0.39), (p value =0.015). Also, there was a significant difference between the mean serum Mg in G-II (1.03±0.31) and those in G-IV (1.32±0.41) (P value=0.033). Mean serum Mg did not differ significantly between G-III (1.39±0.39) and G-IV (1.32±0.41) (p value = 0.756). The correlation study of Mg and other parameters in all patients groups are shown in table (3). Which, showed a significant negative correlations between serum Mg and BMI of G-II (r = - 0.515, p value =0.049), between serum Mg and blood pressure of G- II (systolic; r = - 0.534, P value = 0.000 / diastolic; r = - 0.598, P value=0.000) and G- III (systolic; r = - 0.909, P value = 0.000 / diastolic; r = - 0.831, P value=0.000). In addition, there is a significant negative correlations between serum Mg and total cholesterol in G-II, G-III and G-IV (r= -0.535, P value= 0.000**/** r= -0.833, P value= 0.000 **/** r= -0.773, P value= 0.001). As well, there is a significant negative correlations between serum Mg and both fasting blood sugar and HbA1c (r= 0.522, P value=0.046 **/** r= -0.810, P value= 0.000)

Serum Zn concentrations:

Table (1) and (2) show that, the concentrations of serum Zn, were significantly lower in G-II, G-III, and G-IV than the G-I and were (89.60±14.02, 86.73±19.23, 78.93±23.23, 143.60±20.65), respectively and p value=0.000, =0.000, and =0.000, respectively. But, there were no significant difference in serum Zn level in comparisons between G-II (89.60±14.02) and G-III (86.73±19.23) P value was= 0.690 or between G-IV(78.93±23.23) and p value = 0.141. Comparatives study of Zn levels in all groups were shown in figure (2).

Table (4) showed a significant negative correlation between serum Zn and blood pressure of G-II (systolic; r = - 0.611, P value = 0.000 / diastolic; r = - 0.725, P value=0.000) and G- III (systolic; r = - 0.718, P value = 0.000 / diastolic; r = - 0.637, P value=0.000). Additionally, there is a significant negative correlations between serum Zn and both fasting blood sugar and HbA1c (r= 0.688, P value=0.000 **/** r= -0.627, P value= 0.002).

Serum Cu concentration:

Table (1) , (2) and figure (2) showed that, the mean serum Cu level was a significantly lower in G-II (69.73±14.69) when compared with G-I(105.93±30.56) and p value = 0.000. But, there was no statistically significant difference found between mean serum Cu level of G-III (98.07±19.55) and G-I(105.93±30.56),P VALUE = 0.411. And there was a significantly increased of serum Cu in G-IV (192.53± 34.34) than G-I (105.93±30.56), p value = 0.000.

Table (5) showed a significant negative correlations between serum Cu and blood pressure of G- III (systolic; r = - 0.602, P value = 0.008 / diastolic; r = - 0.545, P value=0.036). However, there is a significant positive correlations between serum Zn and both fasting blood sugar and HbA1c (r= 0.690, P value=0.000 **/** r= 0.701, P value= 0.000).

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Table (1): Baseline Demographic, Clinical and Biochemical data of all subjects.

		G- I	G- II	G- III	G- IV	One Way ANOVA test	
		No. = 15	No. = 15	No. = 15	No. = 15	P-value	Significant
Age / years	Mean ± SD Range	52.20 ± 11.92 32 – 75	60.47 ± 4.47 50 – 69	53.27 ± 11.58 26 – 70	55.80 ± 8.73 37 – 70	0.100	NS
Sex (M/F)	Female Male	5 (33.30%) 10 (66.70%)	4 (26.70%) 11 (73.30%)	5 (33.30%) 10 (66.70%)	7 (46.70%) 8 (53.30%)	0.707	NS
Smoker (cigarette)	Negative Positive	11 (73.30%) 4 (26.70%)	11 (73.30%) 4 (26.70%)	10 (66.70%) 5 (33.30%)	8 (53.30%) 7 (46.70%)	0.615	NS
BMI kg/m2	Mean ± SD Range	28.45 ± 3.35 21.41 – 34.2	35.26 ± 2.08 30.20 – 38.10	32.94 ± 5.75 25.95 – 45.09	35.17 ± 4.06 27.68 – 39.79	0.000	HS
Systolic BP/ (mmHg)	Mean ± SD Range	114 ± 6.32 110 – 130	150.67 ± 12.23 130 – 170	150.33 ± 13.69 130 – 180	124.40 ± 12.49 110 – 146	0.000	HS
Diastolic BP/ (mmHg)	Mean ± SD Range	71.33 ± 3.52 70 – 80	98.67 ± 10.43 80 – 115	91.33 ± 9.90 80 – 110	77.00 ± 6.49 60 – 85	0.000	HS
Serum TC mg/dl	Mean ± SD Range	183.93 ± 17.11 150 – 200	247.93 ± 17.82 200 – 270	175.13 ± 13.47 150 – 195	206.00 ± 16.28 170 – 230	0.000	HS
Serum LDL-c mg/dl	Mean ± SD Range	103.43 ± 26.23 30 – 128.4	175.20 ± 16.25 140 – 195	96.60 ± 13.7 72 – 118	136.00 ± 21.78 113 – 199	0.000	HS
Serum HDL-c mg/dl	Mean ± SD Range	53.20 ± 2.51 49 – 57	33.93 ± 2.91 30 – 40	52.47 ± 3.11 49 – 59	50.80 ± 2.81 45 – 55	0.000	HS
Serum TG mg/dl	Mean ± SD Range	97.80± 8.41 88 – 120	194.67 ± 33.30 130 – 250	130.33 ± 10.08 110 – 140	128.67 ± 29.39 80 – 200	0.000	HS
FBS (mg/dl)	Mean ± SD Range	92.40 ± 10.43 80 – 117	182.40 ± 22.01 150 – 220	93.73 ± 12.41 75 – 110	207.87 ± 67.82 75 – 331	0.000	HS
HbAIc	Mean ± SD Range	5.22 ± 0.58 4.4 – 6.2	8.11 ± 1.01 6.5 – 10	5.66 ± 0.52 4.6 – 6.3	9.36 ± 2.01 6.5 – 13.2	0.000	HS
Serum Mg mg/dl	Mean ± SD Range	2.19 ± 0.31 1.64 – 2.9	1.03 ± 0.31 0.64 – 1.66	1.36 ± 0.39 1 – 2.34	1.32 ± 0.41 0.73 – 1.88	0.000	HS
Serum Zn (µg/dl)	Mean ± SD Range	143.60 ± 20.65 118 – 175	89.60 ± 14.02 62 – 121	86.73 ± 19.23 59 – 145	78.93 ± 23.23 38 – 128	0.000	HS
Serum Cu (µg/dl)	Mean ± SD Range	105.93 ± 30.56 82 – 168	69.73 ± 14.69 53 – 96	98.07 ± 19.55 80 – 134	192.53 ± 34.34 105 – 260	0.000	HS

BMI: body mass index; TC: total cholesterol; LDL-c: low-density lipoprotein-c; HDL-c: high-density lipoprotein-c; TG: triglyceride; FBS: fasting blood surge; HbAIc: hemoglobin A1c.

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Table (2): Comparative study between all groups by Post Hoc analysis.

Parameters	Post Hoc analysis by LSD test					
	G-I vs G-II	G-I vs G-III	G-I vs G-IV	G-II vs G-III	G-II vs G- IV	G-III vs G- IV
Age / years	0.023	0.763	0.311	0.046	0.191	0.475
Sex (M/F)	0.690	1.000	0.456	0.690	0.256	0.456
Smoker (cigarette)	1.000	0.690	0.256	0.690	0.256	0.456
BMI kg/m2	0.000**	0.004**	0.000**	0.120	0.950	0.135
Systolic BP/ (mmHg)	0.000**	0.000**	0.017**	0.937	0.000**	0.000**
Diastolic BP/ (mmHg)	0.000**	0.000**	0.060	0.016**	0.000**	0.000**
Serum TC mg/dl	0.000**	0.144	0.000**	0.000**	0.000**	0.000**
Serum LDL-c mg/dl	0.000**	0.356	0.000**	0.000**	0.000**	0.000**
Serum HDL-c mg/dl	0.000**	0.483	0.025**	0.000**	0.000**	0.114
Serum TG mg/dl	0.000**	0.000**	0.001**	0.000**	0.000**	0.844
FBS (mg/dl)	0.000**	0.921	0.000**	0.000**	0.062	0.000**
HbAIc	0.000**	0.316	0.000**	0.000**	0.006**	0.000**
Serum Mg mg/dl	0.000**	0.000**	0.000**	0.015**	0.033**	0.756
Serum Zn (µg/dl)	0.000**	0.000**	0.000**	0.690	0.141	0.280
Serum Cu (µg/dl)	0.000**	0.411	0.000**	0.004**	0.000**	0.000**

BMI: body mass index; TC: total cholesterol; LDL-c: low-density lipoprotein-c; HDL-c: high-density lipoprotein-c; TG: triglyceride; FBS: fasting blood surge; HbAIc: hemoglobin A1c. **: significant.

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Table (3): Correlation study between Mg and other elements in all patients groups.

	Mg					
	G- II		G- III		G- IV	
	r	P-value	r	P-value	r	P-value
Age / years	-0.325	0.237	0.298	0.281	0.222	0.427
BMI kg/m2	-0.515**	0.049	0.146	0.603	-0.032	0.909
Systolic BP/ (mmHg)	-0.534**	0.000	-0.909**	0.000	-0.453	0.090
Diastolic BP/ (mmHg)	-0.598**	0.000	-0.831**	0.000	-0.315	0.219
Serum TC mg/dl	-0.535**	0.000	-0.833**	0.000	-0.773**	0.001
Serum LDL-c mg/dl	-0.341	0.214	-0.394	0.147	-0.006	0.982
Serum HDL-c mg/dl	-0.063	0.825	-0.256	0.356	-0.420	0.119
Serum TG mg/dl	0.16	0.569	0.092	0.745	0.121	0.666
FBS (mg/dl)	0.235	0.399	0.299	0.280	-0.522**	0.046
HbAIc	0.126	0.654	-0.007	0.980	-0.810**	0.000

BMI: body mass index; TC: total cholesterol; LDL-c: low-density lipoprotein-c; HDL-c: high-density lipoprotein-c; TG: triglyceride; FBS: fasting blood surge; HbAIc: hemoglobin A1c. **: significant.

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Table 4: Correlation study between Zn and other elements in all patients groups.

	Zn					
	G- II		G- III		G- IV	
	r	P-value	r	P-value	r	P-value
Age / years	-0.135	0.632	0.393	0.148	0.371	0.173
BMI kg/m2	-0.282	0.309	0.068	0.810	-0.057	0.839
Systolic BP/ (mmHg)	-0.611**	0.000	-0.718**	0.000	0.163	0.561
Diastolic BP/ (mmHg)	-0.725**	0.000	-0.637**	0.000	-0.092	0.745
Serum TC mg/dl	-0.183	0.515	0.221	0.428	0.203	0.468
Serum LDL-c mg/dl	-0.156	0.578	0.134	0.633	-0.046	0.869
Serum HDL-c mg/dl	-0.034	0.904	-0.053	0.850	-0.156	0.580
Serum TG mg/dl	-0.119	0.673	0.068	0.809	0.559**	0.030
FBS (mg/dl)	0.277	0.311	0.392	0.148	-0.688**	0.000
HbAIc	0.054	0.815	-0.434	0.106	-0.627**	0.002

BMI: body mass index; TC: total cholesterol; LDL-c: low-density lipoprotein-c; HDL-c: high-density lipoprotein-c; TG: triglyceride; FBS: fasting blood surge; HbAIc: hemoglobin A1c. **: significant.

Table 5: Correlation study between Cu and other elements in all patients groups

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	Cu					
	Group II		Group III		Group IV	
	r	P-value	r	P-value	r	P-value
Age / years	- .432	0.108	0.385	0.157	0.272	0.326
BMI kg/m2	0.035	0.900	0.363	0.184	-0.017	0.952
Systolic BP/ (mmHg)	-0.182	0.517	-0.602**	0.008	0.260	0.435
Diastolic BP/ (mmHg)	0.011	0.968	-0.545**	0.036	0.441	0.100
Serum TC mg/dl	-0.034	0.905	-0.306	0.268	0.160	0.568
Serum LDL-c mg/dl	-0.203	0.469	-0.192	0.494	-0.219	0.433
Serum HDL-c mg/dl	0.243	0.383	-0.035	0.901	0.253	0.363
Serum TG mg/dl	0.282	0.309	-0.242	0.384	0.361	0.187
FBS (mg/dl)	-0.026	0.926	0.376	0.167	0.690**	0.000
HbAIc	-0.146	0.603	-0.118	0.676	0.701**	0.000

BMI: body mass index; TC: total cholesterol; LDL-c: low-density lipoprotein-c; HDL-c: high-density lipoprotein-c; TG: triglyceride; FBS: fasting blood surge; HbAIc: hemoglobin A1c. **: significant.

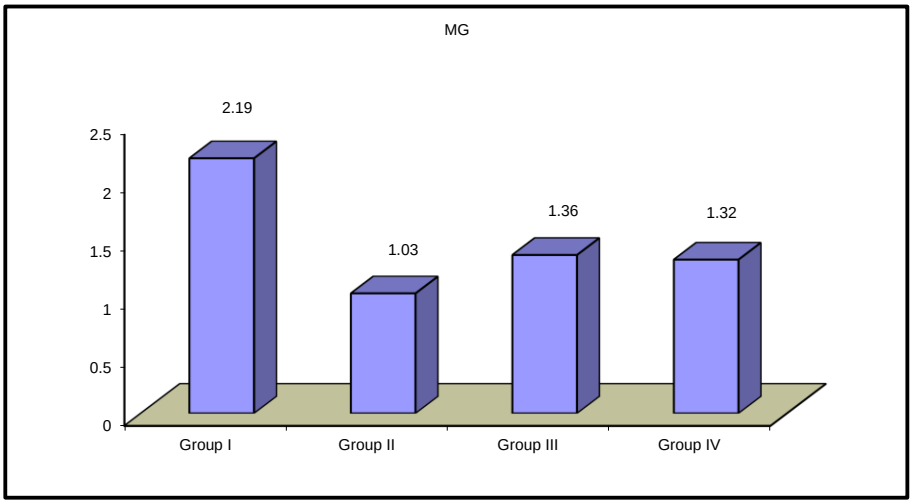

Figure (1): Comparative study of Mg level in all groups.

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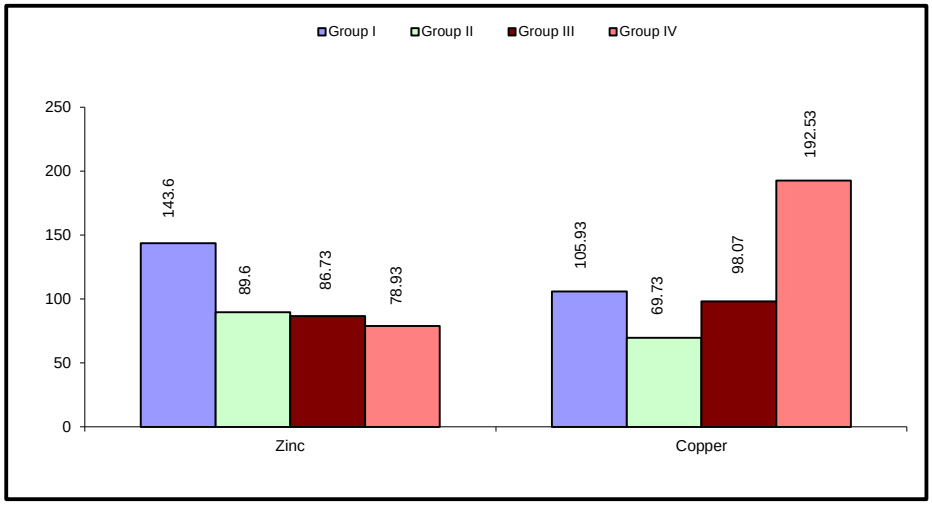

Figure (2): Comparative study of both Zn and Cu levels in all groups.

DISCUSSION

Our observation revealed a significant lowering of serum Mg in patients with T2DM as compared to healthy group. This observation was in coordination with khubchandani et al. study, which demonstrated decrease of serum Mg in diabetic patients, especially in patients who had diabetic complication [20]. Additionally, numerous studies have proven that Type 2 diabetic patients often have a low serum Mg level and the ARIC study demonstrated a relationship between serum Mg and the development of diabetes in the general population [21, 20, 22]. The exact cause of diabetic hypomagnesaemia is still unknown, but an increased urinary loss of Mg may contribute to Mg depletion [20]. Plasma Mg levels were found inversely correlated with urinary Mg excretion rate and with fasting blood glucose values, suggesting that the tubular reabsorption of Mg is decrease in presence of sever hyperglycemia and this deficiency may take the form of chronic latent Mg deficit rather than an overt clinical hypomagnesaemia [23, 21]. Furthermore, the ability of insulin once bound to receptor to activate tyrosine kinase is reduced in hypomagnesaemia states [24] so that hypomagnesaemia is associated with insulin resistance [23, 21] and insulin resistance can decrease the tubular absorption of Mg. Finally, a vicious circle formed by mutual influence between insulin resistance and hypomagnesaemia results in development and progress of diabetic complications [23, 21]. As well, we showed that a statistically significant lower of Mg in MetS patients as compared to healthy subjects. A study done by Nock et al. found a statistically significant potentially protective effect with high Mg intake and MetS [25]. The meta –analysis done by Boroujeni et al. regarding the association between dietary Mg intake and MetS, also found that higher consumption of Mg is associated with lower risk of MetS, so there is an inverse association between Mg intake and MetS [26]. This can be explained by the hypomagnesaemia elevates circulating pro - inflammatory cytokines levels that trigger the activation of low-grade chronic inflammatory response and there is a strong association between Mg deficiency and increased oxidative stress [27]. Hence, the inflammation and oxidative stress have been proposed to be a possible link between Mg deficit and insulin resistance /MetS [21]. Additionally, our study showed that low serum Mg levels in hypertensive patients when compared to healthy subjects. That result is in line with the findings of singh et al. study where revealed low serum Mg in patients with hypertension from North India [28]. However, another study where follow-up for eight years did not reveal a significant association between hypomagnesaemia and occurrence of hypertension [29]. Many studies reported that low Mg concentrations are associated with endothelial dysfunction, increased vascular reactivity, increased vascular tone, and elevated blood pressure, whereas increased Mg levels are associated with opposite effects. In addition, studies reported that a high magnesium intake ranging from 500 to 1000 mg/day might reduce blood pressure by functioning as a calcium channel blocker [30].

In our study, we found a highly significant decrease in serum Zn level in T2DM patients as compare with normal subjects. Similarly, several studies done suggested that Zn deficiency is a common phenomenon in diabetic patients [31, 32, 33]. The possible explanation of low serum Zn level in diabetic patients may be an increased in urinary Zn excretion [34]. Because the hyperglycemia was responsible for increased Zn execration through interfere with the active transport of Zn back in the tubular cells, which may induce a deficiency of these elements in blood of persons with diabetes [35]. Another important aspect is that, Zn is a potent

physiological regulator of insulin signal transduction and it is critical for insulin biosynthesis and storage. In Zn-deficient states, there is a clear decrease in islet cell insulin content [36]. As regarding MetS, we observed a significant decrease in serum zinc levels when compared to healthy subjects. This finding is in line with the observation of Marjami et al. study was observed that mean serum Zn level was significantly low in MetS as compared to without MetS [37]. Also, a study have observed a decrease Zn concentration in the MetS group than that of the normal group but not statistically significant [38]. Furthermore, serum Zn levels decreased as the number of MetS components increased [39]. Some studies have reported that high intake of Zn protects against MetS risk [40]. Therefore, dietary zinc intake was inversely associated with MetS. [41]. This may be due to the insulin resistance is known to play a key role in the development of MetS [39] and in previous study suggesting that Zn deficiency plays an important role in insulin resistance [42]. Moreover, decrease Zn levels may play an important role in the development or aggravation of MetS [39]. Notably, Zn supplementation decreased insulin resistance, oxidative stress, inflammation, blood sugar, cholesterol, and body mass index [43]. Hence, the insulin resistance is the link between MetS and low Zn. On the other hand, oxidative stress, which occurs when ROS exceed the antioxidant capacity, may play an important role in development of MetS and Zn, a cofactor for antioxidant enzymes, decreases ROS generation, suggesting that a decrease in body Zn status may contribute to the development or aggravation of MetS. [39]. This study also showed that the mean serum levels of Zn in subjects with hypertension was significantly lower than that in the control subjects, this results is in accordance with Solanki et al., who revealed that hypertensive patients had lower Zn level than normotensive ones [35]. Similarly, the study done by Okoduwa et al. showed that the levels of Zn decreased in the blood of both persons with T2DM and hypertension [44]. Furthermore, kaur et al. suggested that low serum Zn levels have been associated with higher incidences of coronary artery disease, diabetes mellitus and several related risk factors including hypertension, hypertriglyceridemia, and other factors indicative of mild insulin resistance in urban subjects [45]. Regarding the possible role of low serum Zn in hypertension is due to the low Zn diminished nitric oxide activity with consequent elevation of blood pressure. Hence, Zn deficiency may play an etiological role in subjects with primary hypertension [46]. While this result disagree with the studies of Onuegbu et al. and Singh who found that the mean serum levels of Zn in subjects with hypertension was significantly higher than that in the control subjects [47, 48].

Our study also showed that there is a significant increase in serum Cu level in T2 DM patients than in control group. This result is in agreement with the studies of Santa et al. and Olaniyan et al. they found increase levels of Cu in diabetic patients compared with normal human subjects [49, 50]. Similarly, Kheradmand et al. observed significantly higher levels of Cu in the patients with T2DM compared with the control group [51]. Furthermore, result from recent study has indicated that diabetes might cause two- threefold increment in extracellular matrix Cu [52]. Conversely, the concentration of Cu were significantly lower in patients with T2DM than in healthy control which is consist with the findings of Yeasmin et al. they found that Cu level was significantly lower in T2 DM group than in control group [53]. The possible explanation of observed elevated Cu level in diabetic patients is related to the prolonged hyperglycemia, which initiates non-enzymatic glycosylation of ceruloplasmin "the primary Cu- binding protein in human serum" [52] lead to its fragmentation [54] and release of free Cu into circulation [34]. Cu in its free form is a potent cytotoxic element causes further accelerates the oxidative stress [55]. Moreover, it has been reported that Advanced Glycation End Products (AGES) formation is accelerated by hyperglycemia [54]. AGEs themselves bind to free Cu and potentiate their toxic effects [56]. As mention, we believe that the excess of Cu is involved in the pathogenesis of some complications of NIDDM. The current data concludes that the serum concentration of Cu in patients having MetS was significantly low when compared to healthy subjects. Our result is supported by Byul etal., who found that the concentrations of Cu in the MetS group were significantly lower than those of the normal group [46]. Another one showed that dietary Cu intake was inversely associated with the risk of MetS in women [57]. In addition, Robberecht et al. found that the intake of Cu was low in the patients having MetS [58]. Moreover, Cu deficiency has been linked to cardiovascular disease and features of the MetS including hypertension, atherogenic dyslipidemia, and high serum triglyceride levels. [59]. In addition, Cu deficiency can lead to impairs glucose tolerance, insulin resistance and MetS [60]. Our result of low serum Cu in MetS group is in disagreement with the studies done by Sayiner et al. [61]. Who detected the Cu levels in patients with MetS and its components were significantly high. Nevertheless, some other studies found a lack of association between serum Cu and MetS risk [62, 63]. As regard systemic hypertension, we found that the mean level of serum Cu concentrations in hypertensive group was slightly decreased than the healthy subjects; however, the existence of such differences did not show a statistically significant difference. Similarly, study done by Onuegbu et al. found that no significant changes in the concentrations of Cu in patients with hypertension compared to subjects without hypertension [47]. However, another study done by Suryana, et al. [64] showed a

significantly deficiency of serum Cu level of the hypertensive group than normotensive group. A study done by Mohanty et al. reported that there was a significantly higher in serum Cu levels in hypertensive cases as compared to controls [12].

CONCLUSION

From the above study, following conclusion could be drawn.

- The levels of serum Mg and Zn were decreased in MetS, hypertension and T2DM.
- The level of serum Cu was decreased in MetS and hypertension but increased in T2DM.
- Hence, low Mg and Zn levels may play an important role in the pathogenesis of these diseases by the involvement of these elements in the oxidative stress response.
- Any alternation of serum Cu (decrease or increase) level might be associated with the risk of Mets, hypertension and T2DM.

Recommendation:

Because of the important role of Mg and Zn in these diseases, it is suggested that an adequate supply of these trace elements must be taken in pre-diabetic, pre-hypertensive, obese subjects as well as MetS, hypertensive and T2DM.

Further studies need to be carried out to determine the role of these trace elements in the development in the complications of these diseases. Estimate these trace elements in urine and intracellular.

Declaration:

This study conforms to the principles outlined in The Declaration of Helsinki. Approval of the local ethical committee of faculty of medicine, Al- Azhar University was obtained and informed consents were obtained from all subjects.

REFERENCES

[1] Music M., Dervisevic A., Lepara O., Fajkic A., Ascic-Buturovic B. and Tuna E. Metabolic syndrome and serum liver enzymes level at patients with Type 2 Diabetes Mellitus .Med Arh.2015;69(4):251-255.

[2] Yanardag A. D., Mescigil P. F. and Sayiner Z. A. Do copper and zinc levels predict metabolic syndrome and metabolic syndrome's parameters as hs-CRP does ? Gaziantep Med J .2015; 21 (3):196-199.

[3] Avelar T. M.T., Storch A. S., Castro L. A., Zevedo G. V. M. M., Ferraz L. , Lopes P. F. Oxidative stress the pathophysiology of metabolic syndrome :which mechanisms are involved ? J Bras Patol Med Lab.2015; 51, (4): 231 – 239.

[4] Tajaddini M. H., Keilkha M., Razzazzadeh A. and Kelishadi R. A systematic review on the association of serum selenium and metabolic syndrome . J Res Med Sci 2015 ;20 :782-9.

[5] Sarbijani H.M., Khoshinia M. and Marjani A. The association between metabolic syndrome and serum levels of malondialdehyde and interleukin – 6 in Gorgan . J Posgrad Med Inst 2015 ; 29 (4) :264-9.

[6] Nimse S.B. and Pal D. Free radical, natural antioxidants and their reaction mechanisms. The Royal Society of Chemistry 2015;5 :2798 – 28006.

[7] Alghadir A. H., Gabr S. A., AL- Eisa E. S. and Alghadir M. H . Correlation between bone mineral density and serum trace elements in response to supervised aerobic training in older a dults. 2016; (11):265-273.

[8] Marjani Aaj, Fatima A. A. and Eshghinia S. Association Between Trace Elements And Metabolic Syndrome Among Type 2 Diabetes Mellitus Patients In Gorgan . Asian J Pharm Clin Res . 2015 ; 8 (3):358-362.

[9] Rotter I., Kosik-Bogacka D., Dołęgowska B., Safranow K., Lubkowska A. and Laszczyńska M. Relationship between the Concentrations of Heavy Metals and Bioelements in Aging Men with Metabolic Syndrome. Int. J. Environ. Res. Public Health. 2015; (12): 3944- 3961.

[10] Rotter I., Kosik-Bogacka D., Dołęgowska B., Safranow K., Lubkowska A. and Laszczyska M. Relationship between serum magnesium concentration and metabolic and hormonal disorders in middle - aged and older men. Magnesium Research 2015; 28 (3): 99-107.

[11] Alwan I. F. and Hamood A. M. Serum Trace Elements in Patients with Type 2 Diabetes Mellitus. Mesop. Environ. j. 2017; C :16 -23.

[12] Asha G. and Mohanty S. A Study Of Serum Calcium, Magnesium And Copper Levels In Patients Of Essential Hypertension World Journal Of Pharmacy And Pharmaceutical Sciences. 2015; 4(09):521-530.
[13] Sonny Bherwani, Ashok Kumar Ahirwar, Saumya A. S., Sitendu Kumar Patel, Sandhya A. S., Brijesh Prajapat, Srushtee Bipin Jibhkate, Ritu Singh and Ghotekar L. H. Effect Of Serum Copper Levels In Type 2 Diabetes Mellitus With Nephropathy: A Case Control Study In North Indian Population. *Int. J. of Adv. Res.* 2017; 5 (1) : 420- 424.
[14] Expert Panel on Detection. Evaluation and Treatment of high blood cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education program (NCEP) expert panel III) .J.Am . Med.Asso 2001;(285): 2486-97.
[15] Hayakawa R. and Jap J. Toxic Environ. Health. 1961; 8, 14-18.
[16] *Ventura s.* and King E. J. Determination of copper and zinc in blood serum. Biochem. J. 1951; 48 (5): lxi – lxii.
[17] Eden A. and Green H. H. 1940; Biochem. J. 34, 1202.
[18] Gindler E. Clin. Chem., 1971; 17, 662.
[19] Teidz N.W. Clinical Guide to Laboratory Teses.W.B. Saunders .Co.1983
[20] Khubchandani A. S. and Hiren S. Study of serum Magnesium and HBA1C in diabetic patients with changes in their lipid profiles. Indian journal of clinical practice. 2013; 23 (11): 717-719
[21] Barbagallo M. and Dominguez L. J. Magnesium and type2 Diabetes: An update. Int J Diabetes Clin Res. 2015; 2:1.
[22] Kao WHL, Folsom A. R., Nieto F. J., Mo J. P., Watson R. L. and Brancati F. L., Serum and Dietary Magnesium and the Risk for Type 2 Diabetes Mellitus: The Atherosclerosis Risk in Communities Study. Arch Intern Med. 1999; 159(18):2151-2159.
[23] Rao P. P. and Shariff M. G. Serum Magnesium levels in type 2 Diabetic patients with Microalbuminuria and Normoalbuminuria. Int J Sci Stud | july 2015; 3 (4):11-15.
[24] Prabhu R. and kunche S. Study of Serum Magesium and HbA1C in type 2 Diabetes Mellitus patients, IJSR, 2015; 4 (6): 2522-2524.
[25] Moore-Schiltz L., Albert J. M., Singer M. E., Swain J. and Nock N. L. Dietary intake of calcium and magnesium and the metabolic syndrome in the National Health and Nutrition Examination (NHANES) 2001-2010 data. British Journal of Nutrition. 2015; 114 (6):924-935.
[26] Sarrafzadegan N., khosrayi-Boroujeni H., lotfizadeh M., pourmogadds A., Salehi-Abargouei A. Magnesium status and the metabolic syndrome: A systemic review and meta-analysis. Nutrition 2016; 32(4): 409-417.
[27] Guerrero-Romero F. and Rodriguez-Moran M. Hypomagnesaemia. Oxidative Stress, Inflammation, and Metabolic Syndrome. Diabetes Metab Res Rev. 2006; 22(6): 471-476.
[28] Singh R. B., Rastogi V., Niaz M A., Sharma J.P., Raghuvanshi R. and Moshira M. Epidemiological study of magnesium status and risk of hypertension in a rural population of north India. Magnes Res 1996 ;9:173-81.
[29] Khan A. M., Sullivan L., McCabe E., levy D., Vasan R. S. and Wang T. J. Lack of association between serum magnesium and the risks of hypertension and cardiovascular disease. Am Heart J 2010; 160 (4) :715-20.
[30] Choi M. k. and Bae Y. J. Association of Magnesium intake with High blood pressure in Korean Adults: korea. National Health and Nutrition Examination Survey 2007- 2009. PLoS ONE 2015; 10 (6):1.
[31] Wang S., Liu G. C., Wintergerst K. A., Cai L. Metals in Diabetes: Zinc Homeostasis in the Metabolic Syndrome and Diabetes Molecular. In *Molecular* Nutrition and Diabetes, 2015: A volume in the molecular Nutrition series .9169-182.
[32] Makhough A., Makhlough M., Shokrzadeh M., Mohammadian M., Sedighi S. O., Faghihan M. Comparing the levels of Trace of Elements in patients with Diabetic Nephropathy and healthy individuals. Nephro Urol Non. 2015; 7(4): 28576.
[33] Kumar D. A., Priya V. S., Jaiprabhu J., Swaroopa R. Low serum magnesium and zinc levels in type 2 diabetes mellitus: A pilot study. Jour of Med Sc & Tech. 2015; 4(2): 148-151.
[34] Al-Dohan J. A., Haddad N. S., Al-Rubaye H. and Jawad M. M. The Relation between Trace Elements levels and some cardiovascular Risk Factors in patients with obstructive coronary Artery Disease in Basra. Biol Med 2015, S3: 010.
[35] Viral solanki, Meghana solanki, Asha khubchandani, vijay parmar, Utsav parmar, and parin shah. Significance of serum levels of copper and zinc in hypertensive patients. Int J Res Med. 2015; 4(2): 137-139.
[36] Prasad A. S. Discovery of human Zinc Deficiency: Its Impact on Human Health and Disease. Adv Nutr 2013; 4:176-190.
[37] Marjami A., Akbari F. A., and Eshghinia S. Association between trace elements and metabolic syndrome among type 2 diabetes mellitus patients in gorgan , (AJPCR) Asian J pharm Clin Res 2015; 8 (3): 358 - 362.
[38] Park S. B., Choi S. W. and Nam A. Y. Hair Tissue Mineral Analysis and Metabolic Syndrom , Biol Trace Elem Res (2009); 130: 218-228.
[39] Seo J. A., Song S. W., Han K., and Kim H. N. The associations between serum zinc levels and metabolic syndrome in the Korean population; findings from the 2010 korean. National Health and Nutrition Examination survey. PloS ONE. 2014;9(8): e105990.
[40] Rotter L., Kosik-Bogacka D., Dolegowska B., Safranow K., Lubkowska A. and laszczyriska M. Relationship between the Concentrations of heavy Metals and Bioelements in Aging Men with Metabolic Syndrome. Int.J.Environ.Res. Public Health. 2015; 12: 3944 -3961.

[41] Khayyatzadeh S. S., Moohebati M., Mazidi M., Avan A., Tayefi M., Mohammed S., Parizadeh R., Ebrahimi M., Heidari-Bakavoli A., Parizadeh S. M. R., Ferns G. A., Neamaty M., Safarian M. and, Ghayour-mobarhan M. Nutrient patterns and their relationship to metabolic syndrome in Iranian adults. Eur J Clin Invest. 2016; 46 (10): 840-852.
[42] Miao X., Sun W., Fu Y., Miao L. and Cai L. Zinc homeostasis in the metabolic syndrome and diabetes. Front .Med. 2013; 7 (1):31-52.
[43] Rathnayake K. M., Silva K. and Jayawardena R. Effect of zinc supplementation on obesity; study protocol for a randomized controlled clinical trial Trials. 2016; 17:534.
[44] Okoduwa S. R., Umar I. A., Ibrahim S., Bello F. and Habila N. Age-dependent alteration of antioxidant defense system in hypertensive and type-2 diabetes patients. Journal of Diabetes &Metabolic Disorders. 2015;14:32.
[45] kaur K., Gupta R., Saraf S. A., and Saraf S. K. Zinc: The Metal of Life. Comprehensive Reviews in Food Science and food safety. 2014; 13: 358 -376.
[46] Miriam M. Cortese-Krotta, Larissa Kulakova, Christian Oplander, Victoria Kolb-Bachofenb, Klaus D. Kroncked, and Christoph V. Suschek. Zinc regulates iNOS – derived nitric oxide formation in endothelial cells. Redox biology. 2014; 16 (2):945-54.
[47] Onuegbu A. J., Ayodele O. E., Ayelagbe O. G., Olisekodiaka M. J., Abiola R. A., Amah U. K., and Ukeh I. L. Evaluation of selected trace metals in some hypertension subjects in a tertiary health institution in Southwest Nigeria. Biokemistri . 2013; 25 (1):6-11.
[48] Singh K. B. Molecular Basis of hypertension: A Systemic Review on the role of Metal Ions for Increase Prevalence of Hypertension In Hypertension In India. Journal of Biosciences and Medicines. 2016; 4:12-22.
[49] Santa S., Swati B., kanika M. C., Santasmita P., Aruna B., Gargi S., and Soma G. Status of serum magnesium, zinc &copper in patients suffering from type-2 diabetes mellitus. Journal of Drug Delivery & Therapeutic. 2014; 4(1):70-72.
[50] Olaniyan O. O., Awonuga M. A. M., Ajetunmobi A. F., Adeleke A., Fagbolad O. J., Olabiy K. O., Oyekanmi B. A., and Osadolor H. B. Serum copper and zinc levels in Nigerian type 2 diabetes patients. African Journal of Diabetes Medicine. 2012; 20 (2): 36-38.
[51] Atari-Hajipirloo S., Valizadeh N., Mohammad- Hassan, Khadem- Ansari, Ramsmi Y. and Kheradmand F. Altered concentration of copper; Zinc ,and Iron are associated with increased levels of Glycated Hemoglobin in patients with Type 2 Diabetes Mellitus and their first – Degree Relatives, Int J Endocrinol Metab . 2016; 14 (2):e33273.
[52] Raghav A., Ahmad J., Alam K., Noor S. and Ozair M. Non-Enzymatic Glycation: A link between chemistry and biology, diabetes and obesity international journal. 2016; 1(7): 000138.
[53] Yeasmin R., Muttalib M. A., Sultana N., Bhutyan N. H. and Alam R. A status Of Some Trace Elements In Type 2 Diabetic Patients And Its Relationship With Lipid Profiles. J, Bangladesh Acad Sci. 2016; 40 (1): 79-85.
[54] Sadowska-Bartosz I. and Bartosz G. Prevention of protein Glycation by Natural Compounds Molecules, 2015;20:3309-3334.
[55] Devi T. R., Hijam D., Dubey A., Debnath S., Oinam P., Devi N. G. T. and Singh W. G. study of serum Zinc and copper levels in type 2 Diabetes Mellitus. 2016; 3 (4): 50- 43.
[56] Bhattacharjee D., Chakroborti G., Bhattacharya G. C., and Ravi B. V. Study of Serum Sialic acid and copper as inflammatory Markers in type 2 Diabetes Mekllitus. Indian Medical Gazette. 2015.
[57] Choi M. K. and Bae Y. J. Relationship Between Manganese and Copper Intakes and Metabolic Syndrome Diagnostic Components in Korean adults. Biol Trace Elem Res. 2013; (27) 1: 634-7.
[58] Robberecht H., Bruyne T. D., and Hermans N. Biomarkers of the metabolic syndrome: Influence of the minerals, oligo- and trace elements. Journal of Trace Elements in Medicine abd Biology Available online 21 october 2016.
[59] Aigner E., Strasser M., Haufe H., Sonnweber T., Hohla F., Stadimayr A., Solioz M., Tilg H., Patsch W., Weiss G., Stickel F. and Datz C. A role for low Hepatic Copper Concentrations in Nonalcoholic Fatty liver Disease. Am J Gastroenterol advance. 2010; 105(9):1978-85.
[60] Klevay L. M. Metabolic interactions among dietary cholesterol, copper, and fructose. American Journal of physiology – Endocrinology and Metabolism. 2009; l (298) 1: 138 - 139.
[61] Yanardag Acik D., et al. Do copper and zinc levels predict metabolic syndrome and metabolic syndrome's parameters as hs-CRP does? Gaziantep Med J. 2015; 21(3):196-199.
[62] Marjani A., Akbari F. A., And Eshghina S. Association Between Trace Elements And Metabolic Syndrome Among Type 2 Diabetes Mellitus Patients In Gorgan. Asian J Pharm Clin Res. 2015; 8 (3): 358-382.
[63] Papazafropoulou A., et al Serum Copper. Zinc and Selenium levels in Subjects with and without Metabolic syndrome. J Endocrinol Metab. 2011; 1(2):92-93.
[64] Suryana A. L., Wirjatmadi B. and Adriani M. Zinc and copper levels in patients with primary hypertension and normotension. Makara J.Health Res, 2015,19(2):67-74.