SERUM CERULOPLASMIN AND COPPER ACTIVITIES ALONG WITH THEIR CORRELATION OF LIPID PROFILE IN TYPE 1 AND 2 DIABETIC PAKISTANI POPULATION

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A.F. and I.A.S. designed the model and the computational framework. N.L. and Q. A. wrote the manuscript with the input from all the authors and carried out the implementation. N.L., A.F., I.A.S. and A.B. performed the calculations and analyzed the data. A.F., Q.A. and A.B. conceived the study and were in charge of overall direction and planning.

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ABSTRACT:

To estimate the serum levels of Ceruloplasmin (Cp), Copper (Cu) levels and their correlation with lipid profile in controls, type 1 and 2 diabetics. The case control study was conducted at the Baqai Institute of Diabetes and Endocrinology (BIDE), Karachi, Pakistan. Fasting venous blood was taken from total 348 subjects. 107 healthy subjects made a control group with age ranges from 6-60 years, diagnosed type 1 diabetic (T1D) group had 107 patients with age ranges from 6-25 years and diagnosed type 2 diabetic (T2D) group had 134 patients with age ranges from 26-60 years. The study found a significant decreased level of Cu in T2D as compared to control subjects while significant high level of serum Cp in T2D group only. In T1D group; Cp was found positively correlated with HbA1c = 0.358 r-value, total CHOL = 0.296 r-value, TGs = 0.308 r-value and Cu = 0.735 r-value. In T2D group; Cp was positively correlated only with Cu = 0.390 r-value. In T1D group; Cu was also positively correlated with HbA1c = 0.319 r-value, total CHOL = 0.366 r-value, TGs = 0.338 r-value and LDL = 0.200 r-value. In T2D group; Cu was positively correlated with HbA1c = 0.223 r-value, total CHOL = 0.220 r-value, and TGs = 0.487 r-value. The current study concluded that changes of Cu and Cp levels in diabetic patients may alter the anti-oxidation equilibrium of a subject; therefore, creating more oxidative stress which is responsible for increased per oxidation of lipids either in type1 or 2 diabetic individuals.

Keywords: Ceruloplasmin, Copper, Diabetes mellitus, Glycated Hemoglobin, Lipid profile.

INTRODUCTION:

Diabetes mellitus is a chronic metabolic disorder manifested by hyperglycemia which results in micro and macro-vascular complications. It is mainly classified into two types, Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM); T1DM is identified by lack of insulin secretion pancreatic β-cells while T2DM is due to lack of insulin sensitivity to the peripheral tissues (Skyler, 2017; Newman, 2017). Uncontrolled diabetes could leads to many micro and macro-vascular complications like heart diseases, retinopathies, kidney failure, amputation of extremities and comma (Kitabchi *et al*, 2009; De Ferranti *et al*, 2014). Hyperglycemia in diabetics varies the levels of microelements and increased micronutrients demands because of the induction of oxidative stress that leads to the formation of excessive reactive oxygen species (ROS) like hydroxyl (OH^-) and superoxide (O_2^-) radicals which adversely effects cellular physiology (Li *et al*, 2017) by lipid per-oxidation and poor the metal transportation (Kaur and Henry, 2014; Maret *et al*, 2017). The levels of glycated hemoglobin (HbA1c), showed to have an association with copper (Cu) (Atari-Hajipirloo *et al*, 2016; Naka *et al*, 2013). Approximately 80–95% of Ceruloplasmin (Cp) bound Cu accounts for 50% inhibition of lipid peroxidation (Orzheshkovskyi and Trishchynska, 2019). Serum Cu is reported to be increased in T1DM and associated with macro-vascular disease, hypercholesterolemia and hypertension (Lin *et al*, 2014). Cu deficiency is reported as a cause of glucose intolerance (Forte *et al*, 2013). Therefore, the purpose of current research was to estimate Cp and Cu levels in both T1DM and T2DM and evaluate their relation with lipid profile.

MATERIALS AND METHODS:

The current case-control study was conducted from 2nd February, 2019 to 17th February, 2020 at the Department of Physiology, Baqai Medical University (BMU) and clinical recruitment was done at Baqai Institute of Diabetology and Endocrinology (BIDE).

Ethics Consideration:

The study protocols were prior approved by the ethics committee of BMU dated 8th October, 2018, Ref: BMU-EC/2018-03.

Patient and Public Involvement Statement:

In a fasting state, diagnosed T1D and T2D patients were recruited while controls were recruited on the basis of HbA1c at BIDE. The informed consent form was taken from each participant prior to the sample collection in which detailed description regarded to the rational, possible outcomes and significance of this study was explained in an understandable language. The results were disseminated via hardcopy.

Sample Size:

The total of 348 subjects include in study, which was divided into three groups. According to calculation, the minimum sample size required for control groups, there were 107 patients in Group1with diagnosed T1D and age ranged from 6-25 years, furthermore, 134 patients with diagnosed T2D required for group 2 with age ranged 26-60 years.

Methodology:

Fasting venous blood samples were taken from all the participants and the serum was separated for analyzing Cp, Cu, HbA1c, Total cholesterol (CHOL), High density lipoprotein (HDL), Triglycerides (TGs) and Low density lipoprotein (LDL). Serum Cp was determined by Immunoturbidimetric method (Hammadah *et al*, 2014) on Selectra ProS-Merck using Cp kit, DIALAB, Austria. Serum Cu levels were measured by Photometric method (He *et al*, 2020) using Cu kit, DIA LAB Austria on Selectra ProS-Merck which automatically read the absorbance at 580 nm. Lipid profile was analyzed through Selectra ProS-Merck using commercially available kits. HbA1c was determined by Bio-Rad analyzer (Basit *et al*, 2018). For measuring weight and height, the weighing scale took to the closest of 0.1 kg while subjects were shoeless, wore light clothing and stood in the middle of the floor with wall mounted stadiometer and stood in an upright position with their back to the ruler for height. BMI calculated in kg/m², and blood pressure (BP) measured as mmHg in a sitting position by standard auscultatory method (Basit *et al*, 2018).

STATISTICAL ANALYSIS:

Data was analyzed in SPSS (Statistical software for social sciences) version 21. Gender distribution was measured in term of frequency and percentages. Mean ± Standard Deviation (SD) were used for quantitative variables. However the data was not normally distributed, nonparametric test was applied. Mann-Whitney test was applied on individually all t variables of parameter to assess significant difference in the dependent variable by a single dichotomous independent variable. Rank Spearman correlation test was applied to study the association between variable Cp and Cu among each group. P-value ≤ 0.05 was considered significant.

RESULTS:

A total of 348 individuals were included in the study. For T1D group, 107 confirmed diagnosed T1D patients were taken with 29 age-matched health controls; while for T2D group, 134 confirmed diagnosed T2D patients were taken with 78 age-matched healthy controls. Age and sex distributions of cases and controls were similar due to matching (A1. Table 1)

The comparison of study variable including clinical parameters and lipid profile between T1D and T2D are mentioned in A1. Table 1. It was observed that height, weight, BMI, systolic blood pressure**,** HbA1c and HDL were significantly different in T1D, while, weight, height, HbA1c,

T1D patients had significantly higher levels of HbA1c and HDL compared to controls, whereas, T2D patients had significantly higher levels of weight, BMI, HbA1c, Cp and triglycerides compared to controls. However, serum Cu (p-value <0.001) and HDL (p-value <0.001) were found significantly lower in cases in contrast to controls in patients with T2D.

Correlation analysis was done to observe the association of Cp levels with study variable in cases and controls. It was found that Cp levels showed significant positive association with Cu level in both groups, however, the Cp and Cu levels were both significantly elevated in patients with T1D as compared to patients with T2D (r = 0.735 vs. r = 0.390, p-value <0.001). Moreover, Cp levels also found to be positively correlated with HbA1c (r = 0.358), total CHOL (r = 0.296) and TGs (r = 0.308) and had significantly negative correlation with height (r = -0.283) in patients with T1D. For patients with T2D, the Cp level had significantly negative correlation with height (r = -0.346) (A 2. Table 2).

The correlation analysis was used to observe the association of Cu levels with study variables between cases and controls as mentioned in A 3. Table 3. It was found that serum Cu had significantly positive correlation with HbA1c (r= 0.319), total CHOL (r= 0.366), triglycerides (r= 0.338) and LDL (r= 0.200) and had significantly negative correlation with height (r= -0.263) and DPB (r= -0.200) in patients with T1D. T2D patients had significantly positive correlation of Cu level with BMI (r= 0.197), HbA1c (r= 0.223), total CHOL (r= 0.220), and triglycerides (r= 0.487) and had negative correlation with height (r= -0.318).

	T1DM			T2DM		
Characteristics	**Controls**	**Cases**		**Controls**	**Cases**	
	Mean ± SD	**Mean ± SD**	**p-value**	**Mean ± SD**	**Mean ± SD**	**p-value**
Age (Years)	19.59 ± 4.93	18.89 ± 4.02	0.358	46.13 ± 9.99	47.72 ± 9.51	0.186
Height (Cm)	166.16 ± 8.98	158.53 ± 11.71	0.002**	159.47 ± 19.37	161.94 ± 9.22	0.757
Weight (Kg)	64.38 ± 12.74	53.70 ± 13.29	<0.001***	71.94 ± 13.23	75.60 ± 12.65	0.018*
BMI (Kg/ m^2)	23.25 ± 3.87	21.24 ± 3.70	0.030*	27.61 ± 5.26	28.89 ± 4.70	0.020*
SBP (mmHg)	109.66 ± 12.88	103.05 ± 11.40	0.014*	113.72 ± 17.10	116.11 ± 16.25	0.256
DBP (mmHg)	72.41 ± 9.22	70.25 ± 7.50	0.316	75.26 ± 9.00	78.05 ±11.10	0.079
HbA1c (%)	5.06 ± 0.65	9.81 ± 2.58	<0.001***	5.60 ± 0.70	8.94 ± 2.52	<0.001***
Cp (mg/dl)	33.59 ± 5.37	32.78 ± 6.40	0.183	35.60 ± 6.08	37.64 ± 6.80	0.030*
Cu (µg/dl)	112.48 ± 38.93	114.80 ± 31.65	0.291	127.96 ± 40.75	105.12 ± 18.20	<0.001***
Total CHOL (mg/dl)	160.48 ± 37.06	166.39 ± 32.18	0.375	181.64 ± 41.33	174.96 ± 48.35	0.332
TGs (mg/dl)	115.07 ± 97.68	98.66 ± 49.25	0.913	140.65 ± 72.80	200.64 ± 128.71	<0.001***
LDL (mg/dl)	88.52 ± 35.46	96.43 ± 27.81	0.322	106.06 ± 37.95	113.02 ± 37.04	0.180
HDL (mg/dl)	30.45 ± 9.39	35.27 ± 18.02	0.044*	30.05 ± 6.88	25.87 ± 6.20	<0.001 ***

p-value calculated using Mann Whitney U test

Significant (p-value≤0.05); **Very significant (p-value≤0.01);Highly significant (p-value≤0.001)**

A.1 Table 1: Study variables compared between cases and controls (n=348)

Cp								
	T1DM				T2DM			
Variables	Controls		Cases		Controls		Cases	
	r	p-value	r	p-value	r	p-value	r	p-value
Age (Years)	-0.060	0.755	-0.020	0.841	-0.006	0.957	-0.181	0.038*
Height (Cm)	-0.177	0.358	-0.283	0.003**	-0.314	0.005**	-0.346	<0.001***
Weight (Kg)	0.140	0.468	-0.054	0.585	-0.178	0.121	-0.070	0.427
BMI (Kg/ m^2)	0.305	0.108	0.168	0.087	-0.013	0.908	0.159	0.069
SBP (mmHg)	0.018	0.925	-0.113	0.258	-0.012	0.925	0.017	0.843
DBP (mmHg)	0.191	0.324	-0.092	0.356	-0.081	0.483	0.001	0.993
HbA1c (%)	0.217	0.257	0.358	<0.001***	-0.083	0.475	0.054	0.536
Cu (µg/dl)	0.302	0.111	0.735	<0.001***	0.341	0.002**	0.390	<0.001***
Total CHOL (mg/dl)	-0.069	0.721	0.296	0.002**	0.252	0.027*	0.037	0.678
TGs (mg/dl)	-0.079	0.685	0.308	0.001**	0.138	0.231	-0.021	0.815
LDL (mg/dl)	-0.081	0.676	0.163	0.097	0.165	0.151	0.083	0.341
HDL (mg/dl)	-0.068	0.727	-0.020	0.841	0.092	0.427	0.005	0.952

p-value calculated using Spearman correlation analysis

Significant (p-value≤0.05); **Very significant (p-value≤0.01);Highly significant (p-value≤0.001)**

A.2 Table 2: Correlation analysis of Cp compared study groups (n=343)

Cu

	T1DM				T2DM			
Variables	**Controls**		**Cases**		**Controls**		**Cases**	
	r	**p-value**	**R**	**p-value**	**r**	**p-value**	**R**	**p-value**
Age (Years)	0.246	0.198	0.109	0.267	-0.196	0.086	-0.158	0.068
Height (Cm)	0.106	0.584	-0.263	0.007**	-0.291	0.010*	-0.318	<0.001***
Weight (Kg)	0.251	0.191	-0.041	0.679	0.126	0.273	-0.033	0.706
BMI (Kg/ m^2)	0.313	0.098	0.134	0.172	0.331	0.003**	0.197	0.022*
SBP (mmHg)	0.211	0.274	-0.180	0.069	0.016	0.888	0.142	0.102
DBP (mmHg)	0.097	0.616	-0.200	0.044*	-0.022	0.847	0.148	0.087
HbA1c (%)	-0.092	0.637	0.319	0.001**	0.126	0.272	0.223	0.009**
Total CHOL (mg/dl)	0.051	0.793	0.366	<0.001***	0.043	0.708	0.220	0.011*
TGs (mg/dl)	0.179	0.352	0.338	<0.001***	0.188	0.099	0.487	<0.001***
LDL (mg/dl)	0.056	0.773	0.200	0.040*	0.057	0.617	0.083	0.343
HDL (mg/dl)	-0.151	0.434	0.099	0.313	0.126	0.273	0.000	-

p-value calculated using Spearman correlation analysis

Significant (p-value≤0.05); **Very significant (p-value≤0.01);Highly significant (p-value≤0.001)**

A.3 Table 3: Correlation analysis of Cu compared study groups(n=348)

DISCUSSION:

The current study evaluated the serum levels of Cp and Cu in T1D, T2D and healthy subjects and found significant elevated Cp levels in T2D group in comparison with T1D and control group. Since oxidative stress causes T2DM and Cp is known to have a protective anti-oxidant property, the non-enzymatic glycation and oxidative adaptation of plasma proteins like Cp are known to occur under hyperglycemic and oxidative stress conditions. Therefore, the significant increase in serum Cp level in T2D subjects of current study is the revelation of increased oxidative stress in type 2 diabetes. The results are consistent with the study of Golizeh *et al* (2017). However, contrary to this finding Squitti *et al* (2019) found that the ceruloplasmin levels were higher in persons with T1D compared to healthy controls. It was also found that the serum Cu levels were significantly low in T2D group and high in T1D and control group. This agrees with Sobczak *et al* (2019) who found that the mean Cu levels were significantly low in T2D in comparison to age-matched controls. However, a meta-analysis including 20,183 T2D subjects shown that plasma Cu is increased with the patients of T2DM. Other studies on evaluation of metal concentration in T1D subjects are in the line of our findings and revealed that serum Cu levels were either normal or low in subjects with T1D as compared to control group (Ozenc *et al*, 2015; Alghobashy *et al*, 2018) However, contrary to the previous studies, the present results have observed significantly higher serum Cu concentrations in subjects with T1D in comparison with healthy subjects. The current results are supported by the findings of Squitti *et al* (2019) and Qiu *et al* (2017) that Cu levels were higher in patients of T1D compared to healthy control. Moreover, Cu was independently correlated with T1D in their study subjects (Squitti *et al*, 2019). The discrepancy of the results in the concentration of Cu in various studies may be due to the variation in the ethnicity, the surrounding environment, age of the participants, dietary intake and nutritional status, glycemic control and duration of the disease of the study participants.

The present study investigated possible association of serum Cp and Cu levels with other variables and lipid profile in T1D and T2D subjects. The data indicated that serum Cp levels were positively associated with Cu levels in all groups. It was known that Cp contains 85-90 % bounded Cu (Orzheshkovskyi and Trishchynska, 2019) therefore; the association of Cp with Cu is obvious in all groups of the study. The results of our study showed incidence of positive correlation between serum Cu and Cp levels with HbA1c in T1D however, Jeppu *et al*, 2016) found contrary results and suggested that serum Cp levels were not associated with glycemic index in diabetes. Whereas Golizeh *et al* (2017) found strong association of Cp levels with HbA1c in diabetic subjects. Yet these associations of Cp with glycemic measures were observed in subjects with type 2 diabetes. The literature on the association of Cp levels and HbA1c in type 1 diabetes is scarce and needs to be explained in future studies. The association of serum Cu levels with HbA1c was also observed previously. However, the mechanisms are not completely figured out. Inflammation may be a contributing factor. Atari-Hajipirloo *et al* (2016) further suggested that serum Cu levels increases linearly with poor glycemic control i.e. high HbA1c

level in T2D. However, conflicting results found by Ruiz *et al* (1998) indicated that Cu concentration do not correlate with HbA1c level in T1D (Atari-Hajipirloo *et al*, 2016).

The abnormal lipid profile is the hallmark of oxidative stress and consequently diabetic chronic complications. In the current study we found a positive association of serum Cu and Cp level with serum triglycerides and total cholesterol in both T1D and T2D, which indicated that these molecules may be regarded as antioxidant or prooxidant depending on their concentration. These findings are in the agreement of previous report by Safavi *et al*, (2012). It is not certain that increased lipid oxidation by Cu and Cp contributes to the manifestation of diabetic complications in individuals with type 1 or type 2 diabetes. Several studies have found different associations between serum or plasma Cu and serum lipids, demonstrating great metabolomics variation among individuals (Tasic *et al*, 2015; Rai *et al*, 2013). It was observed that circulating lipids are controlled by genetic factor, metabolism, diet of habitat, way of living, and their communications (Zheng and Qi, 2014). Furthermore, oxidative stress disturbs the Cu metabolism that affects serum lipids (Gutierrez-Garcia *et al*, 2013; Liu *et al*, 2014; Morrell *et al*, 2017).

The study is not without limitations. The state of oxidative stress in patients with diabetes would be clear with the assessment of other markers like Malondialdehyde (MDA) along with copper and ceruloplasmin. The role of metals and metalloid in the pathology of diabetes needs to be more certain in the future studies with the evaluation of other metals along with copper and the genetic basis of the alteration in the concentration of the metals.

CONCLUSION:

It can conclude from the observation of the current study that perturbation in the copper and copper-containing protein may have direct or indirect effect on lipid profile of patients with T1D and T2D. The present study showed that alteration in the copper concentration in diabetics may alter the anti-oxidation equilibrium of an individual therefore, creating more oxidative stress and lipid peroxidation in patients with either T1D or T2D.

CONFLICT OF INTEREST:

Authors have no conflicts of interest among themselves.

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ABBREVIATIONS: Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), glycated hemoglobin (HbA1c), copper (Cu), Ceruloplasmin (Cp), reactive oxygen species (ROS), hydroxyl (OH^-), superoxide (O_2^-), hydrogen peroxide (H_2O_2), oxygen (O_2), cardiovascular system (CVS), Baqai Medical University (BMU), Baqai Institute of Diabetology and Endocrinology (BIDE), Total cholesterol (CHOL), High density lipoprotein (HDL), Triglycerides (TGs), Low density lipoprotein (LDL), Enzyme-Linked Immunosorbent Assay (ELISA) and blood pressure (BP).

DECLERATION OF INTEREST: None**.**