SERUM CERULOPLASMIN LEVELS IN TYPE 2 DIABETES MELLITUS – ASSESSING THE CARDIOVASCULAR RISK.

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Abstract

Type 2 diabetes mellitus is an endocrinological disease associated with hyperglycemia characterized by both insulin resistance and defective insulin secretion. Cardiovascular diseases are the peak reason for mortality in India, highest rate seen in Tamil Nadu state, from the age of 25 to 69 years. Many studies have revealed the importance of inflammatory pathway playing a pivotal role in the development and progression of diabetic complications. Ceruloplasmin, a copper containing metallo-enzyme having antioxidant property (e.g ferroxidase activity) is an ideal marker to know the cardiovascular status, glycemic status, dyslipidemia and cancer risk. The purpose of the present study was to evaluate the effects of Body Mass Index (BMI) and Ceruloplasmin levels on the incidence of cardiovascular diseases in Type 2 Diabetes mellitus patients. Fasting Blood glucose, lipid profile which includes serum triglyceride, total cholesterol, LDL, VLDL, HDL and serum ceruloplasmin were investigated using fully automated chemistry analyzer. Diabetic patients were segregated into three groups based on their BMI, which was correlated with inflammatory marker ceruloplasmin to assess the cardiovascular risk. The mean value and standard deviation of ceruloplasmin in controls (n=60) and diabetic cases divided into normal weight, over weight and obese (n=20 each) were 38.3±8.45, 36±7.99, 33.2±3.57 and 34.2±6.24 mg/dl respectively. Ceruloplasmin among the cases and controls were not statistically significant. Serum ceruloplasmin levels and BMI showed no correlation among the group 1 diabetic cases and was not significant (p=0.29), among the group 2 diabetic cases it showed weak correlation and was not significant (p=0.85), and it also showed no correlation among the group 3 diabetic cases and was not significant (p=0.65). This study revealed no significant association between serum ceruloplasmin level and BMI in all diabetic group subjects separately.

Keywords: Type 2 Diabetes mellitus, Ceruloplasmin, Cardiovascular disease, Obesity, Body mass index, Lipid profile.

1. INTRODUCTION:

India, often called the diabetic capital stands first in the world ranking of countries with the highest number of diabetes patients. With over 70 million diabetes cases, India has a serious necessity to focus more on this killer disease.

Diabetes mellitus is a group of metabolic and endocrinological disorders with the phenotype of hyperglycemia caused by defective secretion of insulin and/or insulin resistance. Type 2 diabetes mellitus is a disease characterized by hyperglycemia associated with insulin resistance.¹

Cardiovascular disease that includes the deposition of atheromatous plaques in arteries and blood vessels

of the heart is perhaps the most perilous diabetic complication, which is a cardinal reason for sudden mortality and morbidity among diabetic patients. In India it has become a major cause for mortality, being responsible for mortality estimates of 49% in Punjab, 42% in Goa and 36% in Tamil Nadu.²

Ceruloplasmin (CP) is an alpha-2-glycoprotein containing six to eight copper atoms with a molecular weight of approximately 132 kDa. It can also bind other cations such as magnesium. Ceruloplasmin has diverse functions. It is essential for iron homeostasis, copper transportation, also involved in angiogenesis and coagulation, and acts as a pro-oxidant or an antioxidant.³ It has been revealed that ceruloplasmin catalyzes the oxidation of iron(II) to iron(III), with a

catalytic cycle that involves copper atoms and uses dioxygen as an electron acceptor without the mediation of reactive oxygen species, such as a superoxide anion or hydrogen peroxide.⁴ It is known that serum ceruloplasmin levels increase in cardiovascular diseases, like atherosclerosis, as a response to inflammation.

Oxidative modification of low density lipoprotein (LDL) by lipid peroxidation causes greater uptake of LDL by macrophages and cellular accumulation of cholesterol in the arterial walls, progressing to atherosclerosis.⁵ This effect is countered by antioxidants such as ceruloplasmin. An imbalance between oxidative damage and antioxidant protection leads to coronary artery disease.⁶ Hence, ceruloplasmin is used in clinical practice to measure the degree of inflammation.

Obesity is a public health problem that is thoroughly known to be related to chronic diseases like dyslipidemia, metabolic syndrome, type 2 diabetes, atherosclerosis, and cardiovascular diseases.⁷ Adipose tissue is known to generate proinflammatory cytokines which in turn leads to the synthesis of ceruloplasmin, an Inflammation Sensitive Plasma protein (ISP) by the liver. Hence obesity elicits a chronic, low-grade systemic inflammatory response. Ceruloplasmin is used to measure the degree of inflammation.⁸

The altered lipid parameters in type 2 diabetes mellitus possess significant cardiovascular risk. Recent studies have shown that ceruloplasmin having antioxidant property, is an ideal marker to know the cardiovascular status, degree of insulin resistance and cancer risk.⁹

The possible mechanism is explained: Type 2 Diabetes Mellitus $\Rightarrow$ Insulin deficiency causing Hyperglycemia $\Rightarrow$ Increased fat oxidation – increased BMI leads to Obesity $\Rightarrow$ Increased ISPs inflammatory sensitive plasma proteins produced by adipose tissue $\Rightarrow$ High ceruloplasmin cause vascular injury by generating free radicals and oxidizes LDL $\Rightarrow$ Atherosclerosis leading to Myocardial infarction.

Limited studies have investigated the association between serum ceruloplasmin level and obesity, and mainly studies focused on the association of this inflammatory marker to cardiovascular diseases. Considering these facts, the present study was designed to find out the changes in levels of the

acute phase reactant ceruloplasmin in normal weight, overweight and obese diabetes mellitus patients for assessing the cardiovascular risk. Type 2 diabetes mellitus patients with very high serum ceruloplasmin levels should be suspected for cardiovascular disease. Measures to decrease BMI in type 2 diabetes mellitus patients will help to prevent development of cardiovascular disease. New therapeutic strategies based on inflammatory properties with beneficial actions on diabetic complications possibly are translated into real clinical treatments in the coming years.

AIMS AND OBJECTIVES

1. To assay the serum levels of fasting blood glucose, lipid profile in type 2 diabetes mellitus patients.
2. To evaluate serum levels of ceruloplasmin in type 2 diabetes mellitus patients.
3. To examine the relation between ceruloplasmin, and BMI in type 2 diabetes mellitus patients.

MATERIALS AND METHODS

This study was conducted during March to August 2018 in the department of Internal Medicine and department of Biochemistry in Government Medical College, Omandurar Government Estate, Chennai. A requisite clearance from the institutional ethical committee was obtained. The study was explained in local language and informed consent was obtained from all the participants of the study.

60 type 2 diabetes mellitus cases without complications included in the study were divided into three groups according to the BMI, each containing 20 subjects.

Group 1: Normal weight diabetic patients, Group 2: Overweight diabetic patients and Group 3: Obese diabetic patients. Results were compared with 60 sex/ age matched normal controls.

Inclusion criteria:

- Age group of 40-60 years with known case of diabetes mellitus patients without any complications of both sexes was included in the study.

Exclusion criteria:

- Known Type 2 Diabetes mellitus patients with cardiovascular disease
- Pregnant women
- Type 1 diabetes mellitus
- Serious infections

- Patients with chronic hypertension, thyroid disease, cardiovascular disease, renal failure, chronic liver failure.

5 ml of overnight fasting venous blood sample is collected and is kept in a tube containing no anticoagulants for 30 minutes at room temperature. They are then centrifuged at 2500rpm for 10 minutes to get clear serum. From the serum, parameters such as fasting blood glucose, lipid profile and ceruloplasmin were estimated by enzymatic method using commercial kits in EM 360 fully autoanalyser.

Fasting blood sugar concentration was determined by GOD/POD method (Enzymatic end point method). Blood urea concentration was determined by UV-GLDH method. Serum creatinine concentration was determined by Modified Jaffe's Reaction. Total Cholesterol (TC) concentration was determined by CHOD-PAP (Enzymatic End point Analysis). Triglyceride (TGL) concentration was determined by GPO-PAP (Enzymatic End point analysis).

High density lipoprotein (HDL) concentration was determined by Phosphotungstate method.

Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) concentration was calculated using Friedewald formula, $VLDL = TGL/5$, $LDL = TC - (HDL + VLDL)$. Serum ceruloplasmin concentration was determined by quantitative Turbimetric method by using 6 point protein calibrator high. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

RESULTS

The study enrolled 20 type 2 diabetic patients with normal weight as Group 1, 20 overweight diabetic patients as Group 2 and 20 obese diabetic patients as Group 3. Fasting plasma glucose, lipid profile and ceruloplasmin levels were analyzed among the three groups and compared with healthy controls. Statistical analysis was performed using SPSS package v21.0 for all the variables of the study. The results were expressed as mean $\pm$ standard deviation (SD). Student's 't' test was used to determine significant difference between cases and controls. Pearson correlation coefficient was applied to correlate

between the variables. $P < 0.05$ was considered statistically significant.

The overall mean age of controls and case were 50.34 and 53.3 (range 35 to 65 yrs) respectively. The mean value and standard deviation of fasting blood glucose in controls ($n=60$) and diabetic cases divided into group 1, group 2, group 3 ($n=20$ each) were 98 ± 29.8 , 180 ± 42.3 , 185 ± 43.6 and 187 ± 44.7 mg/dl respectively. FBG among the cases and controls were increased and statistically significant ($p < 0.05$) as shown in table 1.

The mean value and standard deviation of BMI in controls ($n=60$) and diabetic cases divided into group 1, group 2, group 3 ($n=20$ each) were 21.94 ± 1.23 and 22.7 ± 1.45 , 27.1 ± 1.30 , 32.5 ± 1.75 kg/m^2 respectively. BMI among the diabetic groups were increased and statistically significant ($p < 0.05$) as shown in table 1.

The mean value and standard deviation of lipid profile parameters like total cholesterol, triglycerides, HDL, VLDL and LDL in controls ($n=60$) and diabetic cases divided into group 1, group 2, group 3 ($n=20$ each) were increased and statistically significant ($p < 0.05$) as shown in table 1.

The mean value and standard deviation of ceruloplasmin in controls ($n=60$) and diabetic cases divided into group 1, group 2, group 3 ($n=20$ each) were 38.3 ± 8.45 , 36 ± 7.99 , 33.2 ± 3.57 and 34.2 ± 6.24 mg/dl respectively. Ceruloplasmin among the cases and controls were not statistically significant as shown in table 1.

The comparison between serum ceruloplasmin and BMI among the cases was as shown in scatter diagram figure 1.

Serum ceruloplasmin levels and BMI show no correlation among the group 1 diabetic cases and not significant ($p=0.29$) as shown in the figure 2. Figure 3 shows weak correlation between Serum ceruloplasmin levels and BMI among the group 2 diabetic cases but was not significant ($p=0.85$). Figure 4 shows no correlation between serum ceruloplasmin levels and BMI among the group 3 diabetic cases and was not significant ($p=0.65$).

Table 1: Variables (Mean±SD) (mg/dl) among different groups of diabetic patients and controls.

S.No	Variables	CONTROL	GROUP 1 Diabetic patients	GROUP 2 Diabetic patients	GROUP 3 Diabetic patients
1	Glucose	98±29.8	180±42.3	185±43.6	187±44.7
2	TC	177±40.6	185.9±43.6	196.5±44.9	214.7±38.6
3	TGL	120.2±40	129.4±36.7	143.3±39.2	160±43.5
4	HDL	48±10.3	43.3±8.6	40.5±7.6	38.6±6.9
5	LDL	105.9±33.6	116.6±37.4	128.9±42.6	143.4±34.8
6	VLDL	23.2±7.8	25.8±7.4	27.7±9.8	32.1±10.7
7	Ceruloplasmin	38.3±8.45	36±7.99	33.2±3.57	34.2±6.24
8	BMI	21.94±1.23	22.7±1.45	27.1±1.30	32.5±1.75

The comparison between serum ceruloplasmin and lipid profile among the cases also didn't show any significance. Comparison of serum ceruloplasmin levels and fasting blood glucose ($p=0.66$), triglycerides ($p=0.82$) with total cholesterol ($p=0.24$) showed no correlation among the group 1 diabetic cases and not significant. Comparison of serum ceruloplasmin levels and fasting blood glucose ($p=0.36$), triglycerides ($p=0.85$) with total cholesterol ($p=0.66$) showed no correlation among the group 2 diabetic cases and not significant. Comparison of serum ceruloplasmin levels and fasting blood glucose ($p=0.45$), triglycerides ($p=0.23$) with total cholesterol ($p=0.69$) showed no correlation among the group 3 diabetic cases and not significant.

Figure 1: This bar diagram shows the comparison between the BMI and ceruloplasmin levels among controls and diabetic cases.

Figure 2: Correlation between ceruloplasmin and BMI in group 1 diabetic cases

Figure 3: Correlation between ceruloplasmin and BMI in group 2 diabetic cases.

Figure 4: Correlation between ceruloplasmin and BMI in group 3 diabetic cases.

DISCUSSION

In India, cardiovascular disease has become a major health issue, with deaths due to cardiovascular diseases increasing exponentially.

Increased Inflammation and oxidative stress are reported in patients with acute Myocardial infarction, diabetes mellitus and obesity. Ceruloplasmin is an inflammation sensitive marker as well as an acute phase protein which is measured clinically.¹⁰

Sarkar et al found negative correlation of fasting plasma glucose with antioxidants like ceruloplasmin in type 2 diabetes mellitus patient. It indicated increased glycation of protein that may damage antioxidant protein like ceruloplasmin.

Seyyed et al. found no significant correlation between serum ceruloplasmin and BMI and also waist circumference in all subjects, and even the association was not significant between case and control group. They also found no significant correlation for serum ceruloplasmin and other variables like fasting blood glucose, Total cholesterol, Low density lipoprotein, High density lipoprotein in their study.

The mean fasting blood sugar, lipid profile and BMI were increased in diabetic group compared to controls, which also showed positive correlation with serum ceruloplasmin levels in many studies like Tan KC et al, Wright E et al, Vishakaha V Mahajan et al, Nayak BS et al, Liao L et al, Panichi V et al etc. But none of these studies correlated ceruloplasmin with different BMI groups of diabetic patients.

In our study we found no significant correlation between serum ceruloplasmin and BMI among the

normal weight, overweight, obese of diabetes mellitus patients. We also investigated this association in case and control groups separately; this association was again not significant. However, in case group, there was negative association close to significant level.

We found significant correlation between the fasting blood glucose, lipid profile and BMI among the cases and control groups.

Large multi centric trial with higher sample size may be needed to confirm the association between ceruloplasmin and different BMI groups in diabetic patients to assess the future cardiovascular risk.

CONCLUSION

In the last few years studies have revealed the importance of the inflammatory pathway playing a pivotal role in the development and progression of diabetes mellitus. This new pathogenic vision of diabetes mellitus can lead new therapeutic approaches which is nowadays a matter of great interest.

We found no significant association between serum ceruloplasmin level and BMI in all diabetic group subjects and in control groups separately.

We propose for future studies with higher sample size, to consider other inflammatory markers, details of dietary intakes like intake of micronutrients, duration of hyperglycemia, and to compare the difference between diabetic males and females as the response of inflammatory status differs among these, can throw some light on the relationship between ceruloplasmin and obesity. This may change our approach and priorities during treatment and also for understanding the development of risk factors and complications of diabetes in the future.

Hence the present study concluded that, serum ceruloplasmin levels are affected by genetic and environmental factors like diet, lifestyle and diseases. We may find different results in different populations. On the other hand, because the obesity pattern in Tamil Nadu is different from those in other states, conducting such research studies appears to be necessary in their own population.

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REFERENCE

1. Dr. SajalKamat, Dr. DnyaneshMorkar,Dr. SiddharthMadnani. Serum Magnesium Levels in Type 2 Diabetes Mellitus and its Association with the Microvascular Complications. *Journal of Medical Science and Clinical Research*. 2017; 5(2):18083-18089.
2. VinayakRengan.S, Meera. V, Komala.G. Serum Ceruloplasmin Levels in Acute Myocardial Infarction. *National Journal of Basic Medical Sciences*.2017; 7(4): 220-225.
3. Macintyre. G, Gutfreund. K S, Martin. W R, Camicioli. R, Cox. DW. Value of an Enzymatic Assay for the Determination of Serum Ceruloplasmin. *Journal of Laboratory Clinical Medicine*.2004; 144(6): 294-301.
4. Giurgea. N, Constantinescu.MI,Stanciu R, Suciu.S, Muresan. A. Ceruloplasmin – Acute-Phase Reactant or Endogenous Antioxidant? The Case of Cardiovascular Disease. *Medical Science Monitor*.2005;11(2): 48-51.
5. Raja B Singh, Sushma AMengi, Yan-Jun Xu. Pathogenesis of Atherosclerosis: A Multifactorial Process. *Experimental & Clinical Cardiology*.2002;7(1): 40-53.
6. Zhang. PY, Xu. X, Li. X C. Cardiovascular Diseases: Oxidative Damage and Antioxidant Protection. *European Review for Medical and Pharmacological Sciences*. 2014;18(20): 3091-3096.
7. MM. Mello, DM.Studdert, and TA. Brennan.Obesity-The New Frontier of Public Health Law. *The New England Journal of Medicine*. 2006; 354(24):2601–2610.
8. OY. Kim, MJ. Shin, J. Moon, JH. Chung. Plasma Ceruloplasmin as a Biomarker for Obesity: A Proteomic Approach.*Clinical Biochemistry*. 2011; 44(5):351–356.
9. Vijay Kumar Sharma, ArjunTumbapo, Vivek Pant.Ceruloplasmin, A Potential Marker for Glycemic status and its Relationship with Lipid profile in Type II Diabetes mellitus. *Asian journal of medical science*. 2018;9(2): 13-18.
10. Rajendran. K, Devarajan. N, Ganesan. M, Ragunathan. M. Obesity, Inflammation and Acute Myocardial Infarction – Expression of Leptin,IL-6 and High Sensitivity-CRP in Chennai Based Population. *Journal of Thrombosis and Haemostasis*. 2012;10(1):10-13.