DIABETES WITH COMPLICATION- SCOPE OF SERUM CERULOPLASMIN AS A BIOMARKER

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ABSTRACT
Diabetes mellitus, a chronic non communicable disease is one of the most common burden in India. Complications associated with diabetes are coronary artery disease, nephropathy and neuropathy. Angiogenesis and coagulation are involved in the pathogenesis of diabetic complications. Ceruloplasmin, a ferroxidase enzyme in human encoded by Ceruloplasmin gene. The one of the main function of ceruloplasmin is its involvement in angiogenesis and coagulation. The use of serum ceruloplasmin as a marker of diabetic complication is less known. The objective of the study was to understand the usefulness of serum ceruloplasmin in individuals with diabetes as well as individuals with complications of diabetes. Ethical clearance was obtained from institutional ethical committee prior to the blood collection. Totally 163 individuals of 40-60 years of age were included in the study. Serum ceruloplasmin levels were measured by O-Dianisidine Method. Statistical analysis was done using SPSS 16.0 version. P value > 0.05 is said to be significant. Serum ceruloplasmin levels were lesser in those who did not have the complications of diabetes. There is no statistically significant difference in the serum ceruloplasmin levels in individuals with diabetes and those with complications of diabetes. Loss of glomerular charge selectivity and impairment of glomerular pore size selectivity may be the reason for increased excretion of urinary ceruloplasmin and decreased serum ceruloplasmin levels. Studies with large number of subjects might give better results and may through light on the pathogenesis of complications of diabetes.

KEYWORDS: Diabetes Mellitus, Ceruloplasmin, Nephropathy
INTRODUCTION

Type 2 diabetes mellitus (DM) is an endocrinological disease associated with hyperglycemia characterized by both insulin resistance and defective insulin secretion (1). Ceruloplasmin is a ferroxidase enzyme that in humans is encoded by the CP gene. Ceruloplasmin is the major copper-carrying protein in the blood, and in addition plays a role in iron metabolism (2). It is an enzyme synthesized in the liver containing 6 atoms of Copper in its structure. Ceruloplasmin carries about 70% of the total copper in human plasma while albumin carries about 15%. The rest is accounted for by macroglobulin. Ceruloplasmin exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of ferrous ion (Fe$^{2+}$) into ferric ion (Fe$^{3+}$), therefore assisting in its transport in the plasma in association with transferrin, which can carry iron only in the ferrie state. The molecular weight of human ceruloplasmin is reported to be 151kDa. The known functions of ceruloplasmin include copper transportation, iron metabolism, antioxidant defense, and involvement in angiogenesis and coagulation. It uses dioxide as an electron acceptor without the mediation of an incompletely reduced reactive oxygen species, such as a superoxide anion or hydrogen peroxide. (3)

Mutations in the ceruloplasmin gene (CP), which are very rare, can lead to the genetic disease aceruloplasminemia, characterized by hyperferritinemia with iron overload. In the brain, this iron overload may lead to characteristic neurologic signs and symptoms, such as cerebellar ataxia, progressive dementia, and extrapyramidal signs. Excess iron may also deposit in the liver, pancreas, and retina, leading to Cirrhosis, endocrine abnormalities such as diabetes mellitus and loss of vision respectively. Few studies have shown that glucose accelerates the ceruloplasmin and ceruloplasmin induced lipid peroxidation. (4, 5) Thus this study was designed to establish a relationship between the levels of serum ceruloplasmin and the complications of Diabetes mellitus.

Materials and Methods:

Study Center:

The study was conducted in Kasturba Medical College Hospital, Manipal University, Manipal, Karnataka, India.
Sample size:
Study group consists of 163 patients reporting to Department of Medicine being diagnosed as suffering from Diabetes Mellitus.

Ethical statement:
Ethical clearance was obtained from institutional ethics committee Kasturba Hospital, Manipal University, Manipal.

Study groups:
The study group comprised of diabetic individuals with complications of diabetes such as Nephropathy, Neuropathy and Retinopathy and the co-morbidities associated with diabetes such as Hypertension, Ischemic Heart Disease, cholecystitis, Benign Prostatic Hyperplasia, Dilated cardiomyopathy, Bronchial Asthma, Urinary tract infections.

Estimation of serum ceruloplasmin:
Serum ceruloplasmin levels were measured by O-Dianisidine Method.

Procedure:
4ml of venous blood was collected from the patient after obtaining the written consent. Serum was separated and used for estimation of ceruloplasmin. Ortho Dianisidine dihydrochloride is oxidized by ceruloplasmin at pH 5.0 in the presence of oxygen. The product formed is solubilised in sulphuric acid and color developed was measured at 540nm using spectrophotometer (Spectronic 20).

Acetate Buffer (pH 5.0) was prepared with 13.608 g of sodium acetate and 2.6 ml of Glacial acetic acid dissolved in 75 ml of double distilled water and made up to 100 ml. This is preserved in refrigerator and diluted 1 ml to 10ml before use, with double distilled water. Orthodianisidine (substrate), 25 mg was ground with 0.2 ml of 1N HCl in glass pestle and mortar till it dissolves and 9.8 ml of water was added to make up 10 ml. H₂SO₄ diluted with water (1:1) acts as stopping reagent. Pink color developed as a result of reaction was measured at 540 nm. From this, the concentration of ceruloplasmin was calculated. (6)
Statistical analysis:
The levels of Ceruloplasmin were analysed separately based on the complications the diabetics and with associated co-morbidities. Statistical analysis was done using SPSS 16.0 version. Because Standard deviation of serum ceruloplasmin was 50% more than the mean, we could not use the ordinary 2-sample independent t-test. Instead we used a non-parametric - Mann-Whitney test. P value more than 0.05 was said to be significant.

Results:
From the results obtained, we could only come to a conclusion that in the present study on the 163 patients suffering from Diabetes mellitus, the change in the levels of ceruloplasmin has not been significant.

With Ischemic Heart disease (IHD) as comorbidity, the ceruloplasmin levels were found to be lesser in those who have IHD than those did not. In patients who have developed neuropathy and retinopathy too, there was a fall in ceruloplasmin level than those who did not have the same complications. All the complications put together, again the ceruloplasmin levels were lesser in those who did not have the complications. To be noted is that the fall in ceruloplasmin levels in those with the above mentioned complications was statistically not significant (P>0.05).

Exceptions to all the complications were Hypertension and Nephropathy where the ceruloplasmin levels were higher in those who had complications than those who had not. Out of these two, the rise in the ceruloplasmin levels in nephropathy showed a P value of 0.092 which was very less compared to that of other complications and was relatively nearer to the P value of 0.05.

Table 1: Shows serum ceruloplasmin levels in the presence and absence of complications.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Ceruloplasmin levels when the complication/comorbidity is absent (mg/dL)</th>
<th>Ceruloplasmin levels when the complication/comorbidity is present (mg/dL)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic Heart Disease (comorbidity)</td>
<td>35.31 (22.39,44.37)</td>
<td>34.60 (19.84,54.74)</td>
<td>0.957</td>
</tr>
<tr>
<td>Hypertension</td>
<td>34.36 (24.64,44.49)</td>
<td>35.88 (20.44,45.03)</td>
<td>0.872</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>34.83 (21.33,44.31)</td>
<td>61.85 (32.23,97.17)</td>
<td>0.092</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>35.19 (21.50,44.67)</td>
<td>35.07 (26.89,56.88)</td>
<td>0.654</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>35.31 (22.54,44.55)</td>
<td>29.15 (20.14,49.53)</td>
<td>0.547</td>
</tr>
<tr>
<td>All complications</td>
<td>35.31(23.10,45.6)</td>
<td>35.07 (21.33,44.31)</td>
<td>0.757</td>
</tr>
</tbody>
</table>
Discussion:
Ceruloplasmin apparently has no significant role in determining the frequency or the occurrence of complications in Diabetic nephropathy.

Ceruloplasmin is a copper containing protein (α2 globulin) with a molecular weight of 160000. It has oxidative property and is capable of converting Fe$^{2+}$ to Fe$^{3+}$ and also has been shown to decrease the free radical induced brain damage. (7, 8)

Studies have shown a fall in the ceruloplasmin level in patients suffering from type 2 Diabetes. The reason could be that possible increase in copper mediated generation of reactive oxygen species leading to increased consumption of available antioxidants in the body, including ceruloplasmin. Increased glycation of proteins may damage antioxidant proteins like ceruloplasmin and SH containing albumin. (9) The reason for rise in ceruloplasmin may be figured out with the pathogenesis behind nephropathy. In diabetic patients with advanced nephropathy, urinary copper excretion may be due to dissociations from both copper-albumin and ceruloplasmin-copper complexes filtered through the damaged glomerulus. Overloading of urinary copper to damaged renal tubules may play some roles in the progression of nephropathy in patients with advanced nephropathy. Free ceruloplasmin levels may go higher up in cases of advanced nephropathy. Contrasting studies have also been found regarding this condition where increased excretions of ceruloplasmin have been seen in patients with nephropathy, with or without rise in the serum ceruloplasmin levels. [10, 11, 12, 13]

The reasons for increased excretion of ceruloplasmin in those studies might have been because of any of the two reasons.

(i) loss of glomerular charge selectivity
(ii) impairment of glomerular pore size selectivity

This study was done with a lesser number of subjects and further study of ceruloplasmin in more subjects might throw more light into the pathogenesis of nephropathy and other complications of diabetes.

References


