



A STUDY OF PLASMA GLUCOSE AND SERUM CERULOPLASMIN IN TYPE-2 DIABETES MELLITUS (T2DM) AT A TERTIARY CARE HOSPITAL

Mukul Sharma¹, Kapil Bhatia², Pratibha Misra³, Sibin MK⁴, Vivek N Ambade⁵, Bhasker Mukherjee⁶

¹MBBS, Junior Resident, Department of Biochemistry

²MD, Associate Professor, Department of Biochemistry

³ MD, HOD, Department of Biochemistry

⁴ PhD., Department of Biochemistry

⁵ PhD., Department of Biochemistry

⁶ MD, Associate Professor, Department of Biochemistry

^{1,2,3,4,5,6} Armed Forces Medical College, Pune

Article Info: Received 18 May 2019; Accepted 20 June. 2019

DOI: <https://doi.org/10.32553/ijmbs.v3i6.334>

Address for Correspondence: Kapil Bhatia, MD, Associate Professor, Department of Biochemistry, Armed Forces Medical College, Pune

Conflict of interest: No conflict of interest.

Abstract

Introduction : Diabetes mellitus (DM) is a metabolic disorder occurring due to either defect in the secretion of insulin or defect in the action of insulin characterized by hyperglycemia. Hyperglycemia causes oxidative stress due to increased production of mitochondrial Reactive oxygen species (ROS) in T2DM. Ceruloplasmin (Cp) acts as an antioxidant through its ferroxidase activity. There is an association between the raised serum Cp levels and elevated plasma glucose levels in Type-2 Diabetes mellitus (T2DM) patients.

Aim and objectives : The aim of this study is to evaluate the correlation between the fasting plasma glucose (FPG), 2hour plasma glucose (2hPG), and serum Cp level in T2DM patients as compared to non diabetics.

Materials and methods : 165 cases of T2DM were recruited along with the 40 healthy age and sex matched controls. The blood samples were analyzed for serum Cp and FPG and 2hPG after 75-gram oral glucose.

Results : The serum Cp levels of the patient group with T2DM were significantly higher than the control group ($p = 0.000$). There was a significant positive association between serum Cp level and 2hPG level of the patient population ($r = 0.283, p = 0.000$), but there was no significant correlation found between serum Cp levels and fasting plasma glucose levels in patients ($r = 0.146, p = 0.061$). Similar findings were seen in the sub group analysis.

Conclusion : Our study concludes a significant positive correlation between serum Cp and 2hPG levels in T2DM patients. Hence Cp levels may be considered as a part of the routine diagnostic panel to assess diabetes mellitus.

Keywords : Serum Ceruloplasmin, Type-2 Diabetes Mellitus, Fasting plasma glucose, 2hour plasma glucose

Introduction

Diabetes mellitus (DM) is a metabolic disorder occurring due to either defect in the secretion of insulin or defect in the action of insulin and it is characterized by hyperglycemia (1). The insulin resistance is the prime cause of Type-2 Diabetes mellitus (T2DM) and metabolic syndrome (2).

According to World Health Organization (WHO) factsheet, the number of people with DM has

increased from 108 million in 1980 to 422 million in 2014 globally. The worldwide prevalence of DM among adults over 18 years of age has risen to 8.5% in 2014 in comparison to 4.7% in 1980 (3). More than 90% of DM cases belong to T2DM and Asia is the major global center of the T2DM epidemic (4). As per the worldwide distribution of diabetes, India is amongst the top six countries in the South East Asia region (SEA) based on prevalence according to the International Diabetes Federation (IDF). The IDF

estimates 82 million people in the SEA region; this is estimated to almost double to 151 million by 2045 (5) .

Hyperglycemia causes oxidative stress due to increased production of mitochondrial Reactive oxygen species (ROS) by nonenzymatic glycation of proteins in T2DM (6) . Ceruloplasmin (Cp) is a metalloenzyme (E.C. 1.16.3.1) (1) . Among various roles of Cp, one important role is that it acts as an antioxidant through its ferroxidase activity (7) . Cp is synthesized primarily in the liver as a single chain polypeptide and afterward, the six atoms of copper are incorporated in the biosynthetic pathway and further secreted into the plasma as an α_2 -glycoprotein (8) . Serum Cp levels tend to decrease or increase in various clinical conditions. Elevated serum level of Cp was significant during infection and tissue injury as an acute phase reactant (APR) (8,9) .

In such conditions of elevated oxidative stress, Cp has pro-oxidant activity directed towards ferrous ion stimulated lipid peroxidation and production of hydroxyl radical in Fenton reaction (7) . Copper is toxic in its unbound form, develops redox imbalance due to its high redox activity, which further leads to activation of stress-sensitive intracellular signaling pathways through Haber-Weiss reaction (6,7,10,12) . Therefore, high plasma Cp levels could be an indicator of abnormally high oxidative stress (10) . Increased oxidative stress has also been associated with the pathogenesis of DM and diabetic microvascular complications such as diabetic nephropathy and retinopathy (7,9,10) .

Elevated serum Cp levels are associated with T2DM (8,10,11) . This study evaluated the correlation between the fasting plasma glucose (FPG), 2hour plasma glucose (2hPG), and serum Cp level in diabetes mellitus type 2 as compared to nondiabetic controls .

MATERIAL & METHOD

Study Design : Cross-sectional study

A total of 165 consecutive patients diagnosed with T2DM, who visited Medicine OPD from Aug 2018 to Oct 2018 of a tertiary care center, were included in the study. Subjects between the age of 18 to 90 years were enrolled for the study, who were not diagnosed with any complications like cardiac failure, hepatic failure, renal failure, and thyroid ailments and

not having any comorbidity like hypertension . Forty non-diabetic age and sex matched healthy subjects were also recruited as the control in this study .

The study was conducted after obtaining approval from the Institutional Ethics Committee of Armed Forces Medical College, Pune . Written informed consent was taken from the subjects and 05 ml blood samples were collected under aseptic precautions in fasting condition from cases and controls in yellow top serum gel and grey top sugar vacuum evacuated tubes . FPG samples were collected after an overnight fast for 08 hours and 2hPG samples were obtained exactly after two hours of 82 gm of glucose monohydrate load, dissolved in 250 –300 ml of water and to be taken over 5 minutes . Blood samples were immediately processed to obtain serum for the estimation of Cp by the Government of India patent (No. 192356) Somani Ambade colorimetric method (14) on semi autoanalyzer and plasma for the estimation of glucose by Hexokinase enzymatic method (15) on the fully automated analyzer (Siemens Dimension EXL 200) in our National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited laboratory . The Normal reference range for serum Cp was 500 to 1000 U/L according to the method used for estimation (14) . Normal reference ranges used for plasma glucose in the study are shown as in table-1, as per the American Diabetes Association criteria (ADA-2019) (16) .

Statistical Analysis :

The Data were analysed by using SPSS 20 . 0 statistics software for Windows . Correlation between the Cp and Plasma glucose values was evaluated by using the Pearson correlation method . Subgroup Analysis for the Cp with various levels of plasma glucose groups performed using independent samples T-test and the multiple comparison graphs were plotted . Cut off values in subgroups of glycemic control for DM as per ADA glycemic target: standard of medical care in diabetes –2019 are shown as table-2 (17) . P value<0 .05 was taken as significant .

RESULTS

In our study, the clinical and biochemical characteristics of the study population are shown in table-3. The serum Cp levels of the patient group with T2DM were significantly higher than the control group ($p = 0.000$) . There was a significant positive correlation between serum Cp level and

2hPG level of the patient population ($r = 0.283$, $p = 0.000$), as depicted in the scatter plot (Fig. 1). But there was no significant correlation found between serum Cp levels and FPG levels in patients ($r = 0.146$, $p = 0.061$), as illustrated scatter plot (Fig. 2).

In subgroup analysis, independent samples t-test was performed between serum Cp and subgroups of FPG & 2hPG – Within Glucose Target Value (WGT) & Not in Glucose Target Value (NGT) as considering

95% confidence Interval with 5% standard error of the mean was used. The results were significant for all the subgroups of plasma glucose, as shown in tables-4 and 5. Cp values were higher in patients, who were not in glycemic control according to FPG ($p=0.001$) and according to 2hPG ($p=0.005$). The representation of the same results for subgroup analysis was exhibited as Box & Whisker plots (Fig. 3&4).

Table1: Normal Reference Ranges for Laboratory Parameters Used in the Study

Sr. No.	Lab Parameter	Normal Reference Range		
		Normal	Prediabetes	Diabetes
1	Fasting Plasma Glucose (FPG)	< 100 mg/dL	100 – 125 mg/dL IFG	≥ 126 mg/dL
2	2-hour Plasma Glucose (2hPG) – Post Glucose Load	< 140 mg/dL	140 – 199 mg/dL IGT	≥ 200 mg/dL

Table 2: Cut off values of the glycemic control for DM as per American Diabetes Association (ADA)
Glycemic targets: Standards of medical care in diabetes-2018 in subgroup analysis:

Plasma Glucose : Cut off values for Groups		
Glycemic Control Target Values	Within Glucose Target Value (WGT)	Not in Glucose Target Value (NGT)
Fasting Plasma Glucose (FPG)	80–130 mg/dL	>130mg/dL
2-hour Plasma Glucose (2hPG)	<180 mg/dL	≥ 180 mg/dL

Table 3:

Characteristics	Patients	Controls
Age (Mean \pm SD) in years	54.63 \pm 13.92	51.70 \pm 16.40
Male (%)	94(57)	24(60)
Female (%)	71(43)	16(40)
Serum ceruloplasmin (U/L) (Mean \pm SD)	744.64 \pm 192.71	685.4 \pm 119.23
Fasting plasma glucose (mg/dL) (Mean \pm SD)	157.86 \pm 54.45	82.55 \pm 8.45
2-hour plasma glucose (mg/dL) (Mean \pm SD)	230.9 \pm 78.18	127.825 \pm 6.99

Independent Samples t-Test:
Subgroup analysis:

Table 4 :

Variables		
Glycemic Control Target Values	Within Glucose Target Value(WGT)	Not in Glucose Target Value(NGT)
Fasting Plasma Glucose(FPG)	80 - 130 mg/dL	>130 mg/dL
No of Patients (165)	53	112
Ceruloplasmin (Mean ± SD)	670.98±164.31	779.50±195.97
Significance (2-tailed)		0.001

Table 5 :

Variables		
Glycemic Control Target Values	Within Glucose Target Value(WGT)	Not in Glucose Target Value(NGT)
2-Hour Plasma Glucose (2hPG)	<180 mg/dL	≥180 mg/dL
No of Patients (165)	43	122
Ceruloplasmin (Mean ± SD)	673.95±178.41	769.55±192.06
Significance (2-tailed)		0.005

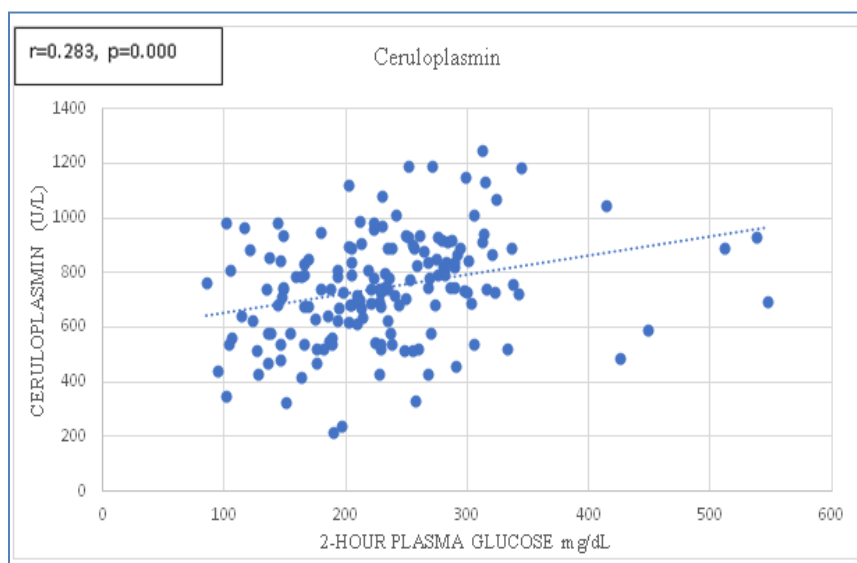


Figure 1 : Correlation between Serum Ceruloplasmin and 2–hr Plasma Glucose

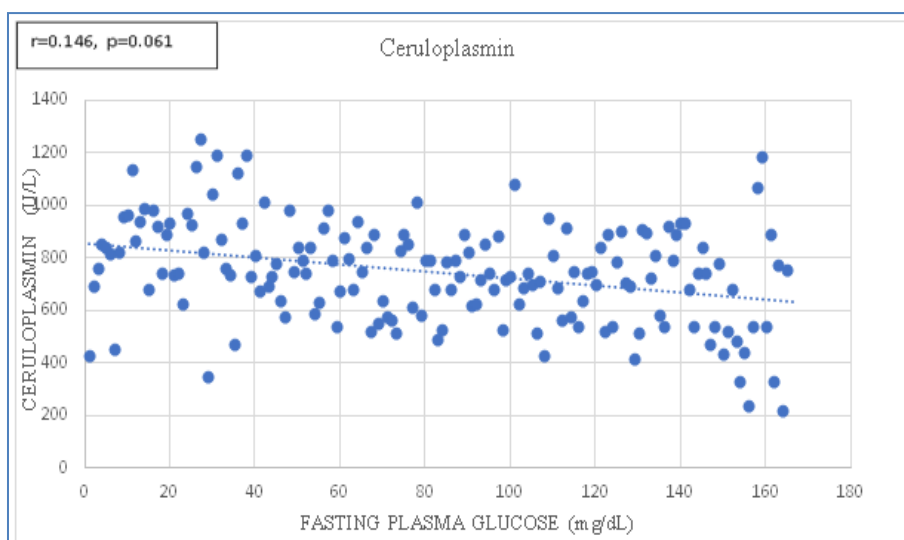


Figure 2 : Correlation between Serum Ceruloplasmin and Fasting Plasma Glucose

Box & Whisker Plots

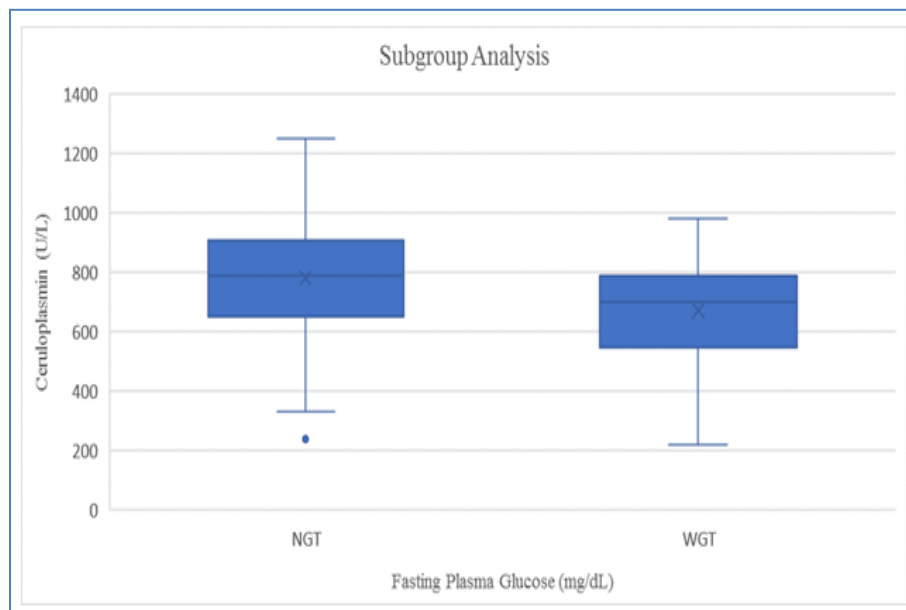


Figure 3 : Serum Ceruloplasmin in NGT and WGT subgroups with respect to Fasting Plasma Glucose

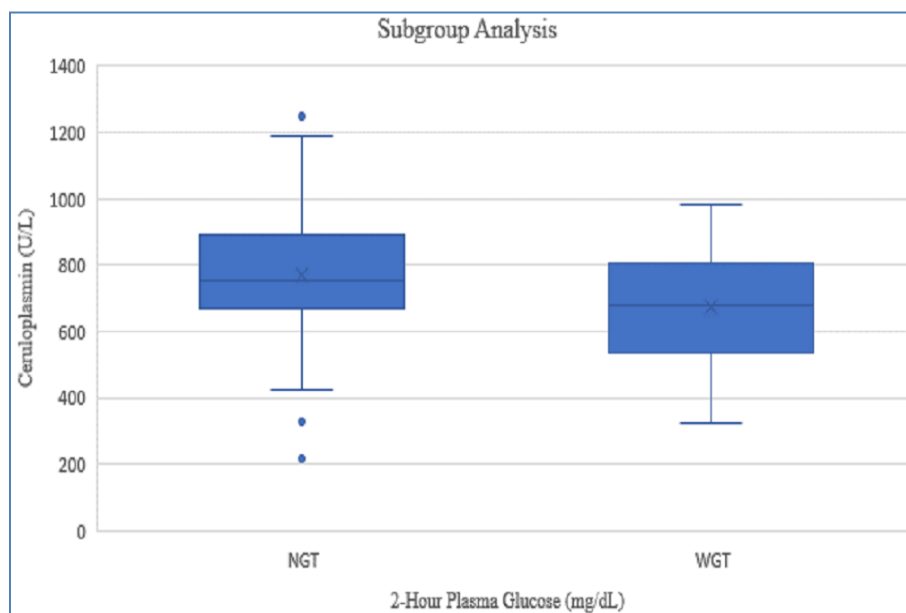


Figure 4 : Serum Ceruloplasmin levels in NGT and WGT subgroups with respect to 2-hour Plasma Glucose

WGT-Within Glucose Target Value

NGT- Not in Glucose Target Value

DISCUSSION

T2DM is an endocrinological disease that is associated with hyperglycemia and it develops due to a reduction in both insulin resistance and defective insulin secretion (12,18). Hyperglycemia consequences in the production of ROS, which eventually leading to increased oxidative stress in a

variety of tissues (6). On the other hand that serum Cp has antioxidant properties due to its ferroxidase activity and acts as a scavenger during oxidative stress in type 2 diabetes (7-9). Hence, increases in Cp levels in patients with type 2 DM may be a protective mechanism (9,10) rather than a part of the pathophysiology of DM.

In this study, there is a correlation between serum Cp levels and T2DM and we found that serum Cp levels were higher in diabetic patients in comparison to non diabetic normal healthy control subjects. Increased level of Serum Cp level has been reported in numerous diseases. The Cp values are increased in several inflammatory diseases, such as tuberculosis and acute upper respiratory tract infection; various collagen diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and rheumatic fever; malignant tumors; obstruction biliary disease; anemia; and drug administration of estrogen etc (21). The simple reason for Cp elevation is that Cp increase as an acute-phase protein, although the details of the mechanism are still under research and are not clear (8,21).

Our results strongly point toward that high plasma glucose is associated with elevated levels of serum Cp in T2DM especially with 2hPG of patients. There was no significant correlation of FPG with Cp in this study. Our results were consistent with earlier studies done by Daimon M et al (1998), Sanjeevi N et al (2018) and Sharma VK et al (2018) (8,19,20). Few studies showed a statistically significant decrease in the serum Cp in metabolic syndrome and T2DM when compared to the control group. Negative correlations were found in the studies conducted by Sarkar et al (2010) and Ashok Kumar J et al (2016) (1,12). However, in subgroup analysis we found that Cp values were higher in patients, who are in NGT as compare to WGT for fasting plasma glucose ($p=0.001$).

A further objective of this study was to find factors in relation to an increase in serum Cp levels of patients with T2DM. The presence of complication of DM has been reported as such a factor for an increase in serum Cp levels (7–9). The specific mechanism underlying this positive correlation between Cp levels and of T2DM disease is unknown till date but few mechanisms have been hypothesized. It is proved in the pathophysiology of DM and its major complications (like nephropathy, retinopathy, neuropathy, and macro-and microvascular damage) that disproportionate and sustained ROS production in hyperglycemia can directly or indirectly affect the physiological function of cellular macromolecules and their integrity (1,7,8,23). There is also the formation of advanced glycation end products (AGE) through non-enzymatic glycation and auto-oxidation of intracellular glucose (23,24).

Even though Cp might act as a pro-oxidant under increased oxidative stress conditions, such as in T2DM (6). Therefore disruption of binding of copper from Cp by increased generation of ROS which further prompts the formation of ROS and low density lipoprotein (LDL) oxidation (7,25). Increased oxidative stress and oxidized LDL are as known to be associated with the progression of diabetic nephropathy (7, 26) and atherosclerosis (8,27).

A negative correlation of Cp with FPG may possibly show increased glycation of proteins that may damage antioxidant proteins like the Cp and free thiol (SH) group containing albumin (12).

Our study found some significant correlation between FPG and Cp in subgroup analysis, however more studies with the larger sample size are further required in this regard. The significant positive relationship between Cp and 2hPG in the subgroup analysis throughout the comparison of NGT & WGT support the hypothesis of elevated serum Cp levels are associated with level of hyperglycemia in patients diagnosed with T2DM.

CONCLUSION

Cp is a copper containing enzyme which has both antioxidant and pro oxidant activities. DM is an increased oxidative stress state.

Our study concludes a significant positive correlation between serum Cp and 2hPG levels in T2DM patients. In subgroup analysis, similar results are found which is required to be confirmed by further studies with FPG levels.

Hence Cp levels may be considered as a part of the routine diagnostic panel to assess disease progression of diabetes mellitus. Since the test is cost effective and is easily available, it may be included in the routine diagnostic panel for T2DM.

REFERENCES :

1. Jeppu AK, Kumar KA, Augusthy A. Plasma glucose and serum ceruloplasmin in metabolic syndrome and diabetes mellitus type 2. Recent Advances in Biology and Medicine. 2016 Apr 7;2 (2016) : 651.
2. Kaur J. A comprehensive review on metabolic syndrome. Cardiology research and practice. 2014;2014.

3. Apps.who.int. (2019). [online] Available at: http://apps.who.int/iris/bitstream/10665/204871/1/9789241_565257_eng.pdf (Access Online)
4. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology*. 2018 Feb;14 (2) : 88.
5. Federation ID. IDF Diabetes Atlas Eighth Edition 2017.
6. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocrine reviews*. 2002 Oct 1;23 (5) : 599–622.
7. Lee MJ, Jung CH, Kang YM, Jang JE, Leem J, Park JY, Lee WJ. Serum ceruloplasmin level as a predictor for the progression of diabetic nephropathy in Korean men with type 2 diabetes mellitus. *Diabetes & metabolism journal*. 2015 Jun 1;39 (3) : 230–9.
8. Daimon M, Susa S, Yamatani K, Manaka H, Hama K, Kimura M, Ohnuma H, Kato T. Hyperglycemia is a factor for an increase in serum ceruloplasmin in type 2 diabetes. *Diabetes Care*. 1998 Sep 1;21 (9) : 1525–8.
9. Jung CH, Lee WJ, Yu JH, Hwang JY, Shin MS, Koh EH, Kim MS, Park JY. Elevated serum ceruloplasmin levels are associated with albuminuria in Korean men with type 2 diabetes mellitus. *Diabetes research and clinical practice*. 2011 Oct 1;94 (1) : e3–7.
10. Cunningham J, Leffell M, Mearkle P, Harmatz P. Elevated plasma ceruloplasmin in insulin-dependent diabetes mellitus: evidence for increased oxidative stress as a variable complication. *Metabolism*. 1995 Aug 1;44 (8) : 996–9.
11. Walter RM, Uriu-Hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, Keen CL. Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. *Diabetes care*. 1991 Nov 1;14 (11) : 1050–6.
12. Sarkar A, Dash S, Barik BK, Muttigi MS, Kedage V, Shetty JK, Prakash M. Copper and ceruloplasmin levels in relation to total thiols and GST in type 2 diabetes mellitus patients. *Indian journal of clinical biochemistry*. 2010 Jan 1;25 (1) : 74–6.
13. Rangaswamy R, Santosh RG. A study on relationship between fasting plasma glucose, copper and ceruloplasmin levels in type 2 diabetes mellitus. *J Evol Med Dent Sci*. 2015 Aug 20;4 (67) : 11674–6.
14. Somani BL, Ambade V. A kinetic method amenable to automation for ceruloplasmin estimation with inexpensive and stable reagents. *Clinical biochemistry*. 2007 May 1;40 (8) : 571–4.
15. AMBADE VN, Sharma YV, Somani BL. Methods for estimation of blood glucose: a comparative evaluation. *Medical Journal Armed Forces India*. 1998 Apr 1;54 (2) : 131–3.
16. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes–2019. *Diabetes Care*. 2019 Jan;42 (Suppl 1) : S13.
17. American Diabetes Association. 6. Glycemic Targets: Standards of Medical Care in Diabetes–2019. *Diabetes Care*. 2019 Jan;42 (Suppl 1) : S61.
18. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*. 2003 Jan 1;46 (1) : 3–19.
19. Sanjeevi N, Freeland-Graves J, Beretvas SN, Sachdev PK. Trace element status in type 2 diabetes: A meta-analysis. *Journal of clinical and diagnostic research: JCDR*. 2018 May;12 (5) : OE01.
20. Sharma VK, Tumbapo A, Pant V, Aryal B, Shrestha S, Yadav BK, Tuladhar ET, Bhattarai A, Raut M. Ceruloplasmin, a potential marker for glycemic status and its relationship with lipid profile in Type II diabetes mellitus. *Asian Journal of Medical Sciences*. 2018 Mar 1;9 (2) : 13–8.
21. Danks DM: Disorder of copper transport. In *The Metabolic and Molecular Basis of Inherited Disease*. 7th ed. Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. New York, McGraw-Hill, 1995, p. 2211–2236.
22. Ferreira CR, Gahl WA. Disorders of metal metabolism. *Translational science of rare diseases*. 2017 Jan 1;2 (3–4) : 101–39.
23. Thallas-Bonke V, Thorpe SR, Coughlan MT, Fukami K, Yap FY, Sourris KC, Penfold SA, Bach LA, Cooper ME, Forbes JM. Inhibition of

- NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C- α -dependent pathway. *Diabetes*. 2008 Feb 1;57 (2) :460-9.
24. Thallas-Bonke V, Lindschau C, Rizkalla B, Bach LA, Boner G, Meier M, Haller H, Cooper ME, Forbes JM. Attenuation of extracellular matrix accumulation in diabetic nephropathy by the advanced glycation end product cross-link breaker ALT-711 via a protein kinase C- α -dependent pathway. *Diabetes*. 2004 Nov 1;53 (11) :2921-30.
25. Shukla N, Maher J, Masters J, Angelini GD, Jeremy JY. Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? . *Atherosclerosis*. 2006 Aug 1;187 (2) :238-50.
26. Ujihara N, Sakka Y, Takeda M, Hirayama M, Ishii A, Tomonaga O, Babazono T, Takahashi C, Yamashita K, Iwamoto Y. Association between plasma oxidized low-density lipoprotein and diabetic nephropathy. *Diabetes research and clinical practice*. 2002 Nov 1;58 (2) :109-14.33.
27. Memişoğulları R, Bakan E. Levels of ceruloplasmin, transferrin, and lipid peroxidation in the serum of patients with Type 2 diabetes mellitus. *Journal of diabetes and its complications*. 2004 Jul 1;18 (4) :193-7.