ORIGINAL ARTICLE

COPPER AND CERULOPLASMIN LEVELS IN RELATION TO TOTAL THIOLS AND GST IN TYPE 2 DIABETES MELLITUS PATIENTS

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ABSTRACT

Presence of oxidative stress in type 2 diabetes mellitus (DM) is well proved. Current study was undertaken to know the relation between fasting plasma glucose (FPG) and copper along with antioxidants like total thiols and ceruloplasmin, and antioxidant enzyme glutathione S transferase (GST). The study group consisted of a total of 201 subjects which included nondiabetic healthy control subjects ($n = 78$) and diabetic patients ($n = 123$). Plasma total thiols, GST, copper and ceruloplasmin levels were measured all the subjects using spectrophotometric methods and FPG levels were determined in clinical chemistry analyzer Hitachi 912. There was significant increase in FPG ($P < 0.001$) and copper ($P < 0.001$) and decrease in ceruloplasmin ($P < 0.001$) and protein thiols ($P < 0.001$) in type 2 DM cases compared to healthy controls. There was no significant change in GST between type 2 DM cases and controls. There was significant negative correlation of FPG with antioxidants like ceruloplasmin ($r = -0.420$, $P < 0.001$) and total thiols ($r = -0.565$, $P < 0.001$). Protein thiols correlated positively with ceruloplasmin ($r = 0.364$, $P < 0.001$). Our study indicates possible increase in copper mediated generation of ROS leading to increased consumption of available antioxidants in the body.

KEY WORDS

Copper, Ceruloplasmin, Glutathione S transferase, Oxidative stress, Type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus (DM) is an endocrinological disease associated with hyperglycemia characterized by both insulin resistance and defective insulin secretion (1). It is proved that excessive and/or sustained reactive oxygen species (ROS) production in hyperglycemia can directly or indirectly disturb the integrity and physiological function of cellular macromolecules and also by forming advanced glycation end products (AGE) through non-enzymatic advanced glycation and intracellular glucose auto-oxidation (2). Apart from being toxic substance, ROS also act as a physiological mediator of many cellular responses including glucose dependent

metabolic signal that contributes to glucose stimulated insulin secretion (GSIS) in β -cells (3, 4).

There is considerable current interest in role of transition metal ions in inducing ROS generation. Previous studies proved involvement of transition metal ions like iron (5) and copper to cause oxidative stress (6). Majority of plasma copper is transported bound to ceruloplasmin (>95%); rest is bound to albumin, transcuprein and copper-amino acid complexes (6). Ceruloplasmin (EC 1.16.3.1) is an acute phase reactant, has ferro- O_2 -oxidoreductase (pro-oxidant) activity directed towards ferrous ion stimulated lipid peroxidation and formation of hydroxyl radical in Fenton reaction (6). Copper is toxic in its unbound form, causes redox imbalance due to its highly redox active nature, which leads to activation of stress sensitive intracellular signaling pathways through Haber-Weiss reaction (6, 7).

Total thiol pool contributes majority of antioxidant activity of the body. Major contribution to total thiol pool is from reduced glutathione (GSH) and free thiol (SH) group present on protein

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mainly on albumin (8). Glutathione S-transferase (GST; EC 2.5.1.18) is an anti-oxidant, comprises a multigene family of proteins involved in the metabolism of many disease-causing electrophilic substrates and protects the cells against oxidative stress, and is also useful in monitoring of cellular induction (9). The current study was designed to establish the relationship between fasting plasma glucose (FPG) and copper with ceruloplasmin, total thiols and GST in type 2 DM patients.

MATERIALS AND METHODS

The study group consisted of a total 201 subjects which included non-diabetic healthy control subjects ($n = 78$) and diabetic patients ($n = 123$). Informed consent was taken from all subjects involved in the study and study was approved by institutional ethics committee. Type 2 diabetic patients were free from any clinical evidence of retinopathy, nephropathy, or neuropathy and patients with coronary heart disease, or hypertension were excluded from the study. A total of 21 subjects were treated with insulin therapy (after failure of oral hypoglycaemic agents), 66 subjects with oral hypoglycaemic agents (sulfonylureas or sulfonylureas plus biguanides), and 15 with the combination of insulin and oral hypoglycaemic agents, and 21 with lifestyle modification alone. None of the patient groups received any form of antioxidant medication. The healthy controls were not on any kind of prescribed medication or dietary restrictions.

Special chemicals like 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), 1-chloro-2,4-dinitro benzene (CDNB), reduced glutathione (GSH), bathocuprine disulphonate disodium salt (BCDS), p-phenyl diammonium dichloride (PPD) were obtained from sigma chemicals, St Louis, MO, USA. All other reagents were chemical grade. FPG was determined in patients with type 2 DM and healthy controls using modified glucose oxidase-peroxidase method in clinical chemistry auto-analyzer (Hitachi 912). Serum copper using BCDS (10), ceruloplasmin using PPD (11), GST kinetic activity using GSH (12, 13), total thiols using DTNB (14) were assayed by spectrophotometric methods.

The results were expressed as mean $\pm$ standard deviation (SD). $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-16, Chicago, USA). Independent sample student's 't' test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

Table 1: Plasma oxidative markers & antioxidants in controls & patients with diabetes mellitus type 2 (mean $\pm$ SD)

	Controls	Type 2 DM
Age (years)	55.0 $\pm$ 9.0	54.0 $\pm$ 10.0
Sex (M/F)	48.0 $\pm$ 30.0	77.0 $\pm$ 44.0
FPG (mg/dL)	73.34 $\pm$ 13.18	189.55 $\pm$ 54.72 *
Ceruloplasmin (mg/dL)	29.58 $\pm$ 11.91	16.9 $\pm$ 7.96 *
Copper (μ g/dL)	103.48 $\pm$ 28.58	191.63 $\pm$ 30.72 *
PSH (μ M)	331.32 $\pm$ 55.04	234.15 $\pm$ 67.44 *
GST (IU)	1.43 $\pm$ 0.25	1.47 $\pm$ 0.28

* $P < 0.001$ compared to normal controls

RESULTS AND DISCUSSION

We have found significant increase in levels of copper ($P < 0.001$) and FPG ($P < 0.001$), and decrease in ceruloplasmin ($P < 0.001$) and protein thiols ($P < 0.001$) in type 2 DM patients compared to healthy controls. There was negative correlation of FPG with antioxidants like ceruloplasmin ($r = -0.420$, $P < 0.001$) and total thiols ($r = -0.565$, $P < 0.001$). Protein thiols correlated positively with ceruloplasmin ($r = 0.364$, $P < 0.001$).

Our study indicates possible increase in copper mediated generation of ROS leading to increased consumption of available antioxidants in the body. Negative correlation of FPG with ceruloplasmin may possibly indicate increased glycation of proteins that may damage antioxidant proteins like ceruloplasmin and SH containing albumin. It appears that increased oxidative stress in presence of hyperglycemia may lead to increased availability of transition metals like copper released from its storage site. Copper in its free form is a potent cytotoxic element because of its redox chemistry it readily participates in Fenton and Heiber-Weiss reactions to generate ROS. This elevated ROS could have consumed considerable amount of total thiol, which may explain the decreased level of total thiols in patient with hyperglycemia. Previous studies on animal model suggest that, a combination of diabetes and copper deficiency resulted in increased level of hepatic GST (15). However in our study there was no significant change in GST between type 2 DM patients and healthy controls. In conclusion our study suggests that there is increased serum copper and decreased ceruloplasmin and total thiol levels in type 2 DM patients.

REFERENCES

1. Kahn SE. The relative contributions of insulin resistance and beta cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetol* 2003; 46: 3-19.
2. Thallas-Bonke V, Thrope SR, Coughlan MT, Fukami K, Yap FYT, Sourris KC, Penfold SA, Bach LA, Cooper ME, Forbes JM. Inhibition of NAD(P)H oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C- α -dependent pathway. *Diabetes* 2008; 57: 460-9.
3. Hancock JT, Desican R, Neill SJ. Role of reactive oxygen species in cell signaling pathways. *Biochem Soc Trans* 2001; 29: 345-50.
4. Pi J, Bai Y, Zhang O, Wong V, Floering LM, Daniel K, Reece JM, Deeney JT, Anderson ME, Corkey BE, Collins S. Reactive oxygen species as a signal in glucose stimulated insulin secretion. *Diabetes* 2007; 56: 1783-91.
5. Shetty JK, Prakash M, Ibrahim MS. Relationship between free iron and glycated hemoglobin in uncontrolled type 2 diabetes patients associated with complications. *Ind J Clin Biochem* 2008; 23(1): 67-70.
6. 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; 44(8): 996-9.
7. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type 2 diabetes. *Endo Rev* 2002; 23: 599-622.
8. Prakash M, Upadhya S, Prabhu R. Protein thiol oxidation and lipid peroxidation in patients with uremia. *Scand J Clin Lab Invest* 2004; 64: 599-604.
9. Hayes JD, Wolf CR. Role of glutathione-S-transferase in drug-resistance. In: *Glutathione Conjugation*, Editors Cap X, Sies H, Ketterer B, Academic Press, London, 315-55.
10. Prakash M, Shetty JK. A modified spectrophotometric micromethod to determine serum copper. *Asian J Biochem* 2008; 3(1): 38-42.
11. Ravin HA. An improved colorimetric enzymatic assay of Ceruloplasmin. *J Lab Clin Med* 1961; 58: 161-8.
12. Beutler E. Red cell metabolism. In: *A manual of biochemical method* 3rd Ed., Editors Grune and Startron, London. Page 8-78; 1984.
13. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-Transferases: The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; 249: 7130-9.
14. Motchnik AP, Frei B, Ames NB. Measurement of Antioxidants in Human blood plasma: Protein Thiols. In: *Oxygen radicals in biological systems; Methods in Enzymology*, Editor Packer. L, California: Academic Press, volume 234, Part D, Page 273-4, 1994.
15. McDermott BM, Flatt PR, Strain JJ. Effects of copper deficiency and experimental diabetes on tissue antioxidant enzyme levels in rats. *Ann Nutr Metab* 1994; 38(5): 263-9.