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LETTER TO THE EDITOR

SERUM AND URINE PROTEIN THIOLS IN TYPE 2 DIABETES MELLITUS PATIENTS

Sir,

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Oxidative stress is known to be a component of molecular and cellular tissue damage mechanism in a wide spectrum of human diseases (1). Diabetes is associated with a number of metabolic alterations, principal among these is hyperglycemia. Hyperglycemia in diabetic patients can increase the oxidative stress by several mechanisms, including glucose autooxidation, nonenzymatic protein glycation and activation of polyol pathway (1). Several studies indicated that, the increased free radical activity is suggested to play an important role in the lipid peroxidation and protein oxidation of cellular structures causing cell injury and is implicated in the pathogenesis of vascular disease in type 1 and type 2 diabetes (1).

The –SH (reduced thiol) groups that exist both intracellularly and extracellularly either in free form (reduced glutathione) or bound to proteins (protein bound thiols) play a major role in maintaining the antioxidant status of the body (2). The thiols are the major antioxidants in body fluids which are known to reduce highly reactive free radicals thus protecting the biomolecules (2). Such thiols have been studied and determined in different disease conditions and found to be decreased in different diseases compared to healthy controls (2, 3). Thiol status has also been determined in both type 1 and type 2 diabetes mellitus patients and the levels were found to be decreased (4–6). The current study was designed to know the relation between fasting plasma glucose, serum thiols and urine thiols in type 2 diabetes mellitus patients.

The study was conducted on urine and serum samples collected from healthy controls (n=25) and diabetes mellitus cases (n=50). Known type 2 diabetes mellitus who were on oral antidiabetic medication were included in the study, the duration of diabetes in these patients was 5±2 years. All the diabetic patients who were on antioxidant medications were excluded from the study. The healthy controls were not on any kind of prescribed medication or dietary restrictions. This study was approved by Institutional Review Board and informed consent was obtained from all subjects involved in the study.

Under aseptic conditions blood samples were collected in two vacuum tubes one with heparinised vacutainer and another into plain vacutainers from ante-cubital veins of controls and diabetes mellitus patients. Blood was centrifuged at 2000 g for 15 minutes at 4°C for clear separation of

plasma and serum and all assays were performed immediately. Random urine samples were collected from cases and controls and assayed for urine thiols. Special chemical 5 5′ dithio-bis (2-nitrobenzoic acid) (DTNB), was obtained from Sigma Chemicals, St Louis, MO, USA. All other reagents were of analytical grade.

Serum and urine thiols were measured by a spectrophotometric method using DTNB (7, 8). Briefly, 900 µL of 0.2 M Na_2HPO_4 containing 2 mM Na_2EDTA, 100 µL serum or urine and 20µL of 10 mM DTNB in 0.2M Na_2HPO_4 were taken in an Eppendorf tube and warmed to 37^0C. The solution was mixed in a vertex mixer and transferred to a cuvette, and the absorbance was measured at the end of 5 min at 412 nm in Genesys 10 UV spectrophotometer. Appropriate sample and reagent blanks were prepared and the corrected absorbance values [absorbance of T–(absorbance of standard blank+ absorbance of reagent blank)] were used to calculate the concentration of thiols using calibration curve. Values were expressed in µmoles/L for serum and urine thiols. FPG levels were determined by glucose oxidase-peroxidase method using automated analyzer (Hitachi 912). The results were expressed as mean±standard deviation (SD). A P value of <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-10, Chicago, USA). An independent sample t test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

As depicted in Table I, FPG levels were significantly higher (P<0.01) and serum thiols were significantly lower (P<0.01) in type 2 diabetes mellitus patients compared to healthy controls. We found significantly decreased urine thiols in diabetes mellitus patients as compared to healthy controls (P<0.01). FPG levels correlated negatively with serum thiols ($r^2 = 0.431$), P<0.01) (Fig. 1) and urine thiols ($r^2 = 0.476$, P<0.01) (Fig. 2). The results presented in this study demonstrated that the concentration of sulphydryl groups (–SH) in serum were markedly reduced in type 2 diabetes mellitus patients as compared with healthy

TABLE I: Demographic characteristics and biochemical parameters of diabetes mellitus patients and healthy controls (expressed in mean±SD).

	Healthy controls (n=25)	*Diabetes mellitus patients (n=50)*
Age (years)	45±10	50±8
Male/Female	20/5	41/9
FPG (mg/dl)	78.1±8.4	324.5±53.3*
Serum thiols (µmoles/L)	250.9±42.2	173.9±51.3*
Urine thiols (µmoles/L)	310±251.3	72.42±65.8*

*P value <0.01 compared to healthy controls.

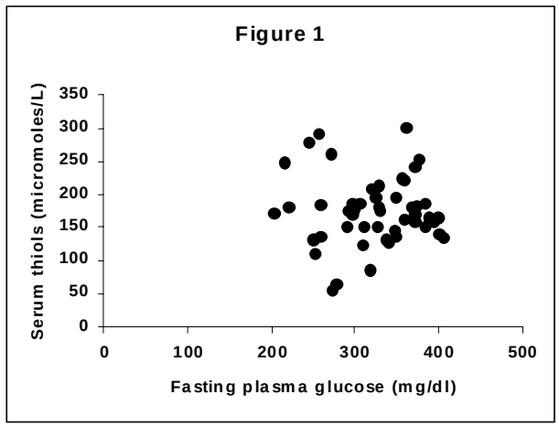

Fig. 1: Correlation between FPG and serum thiols.

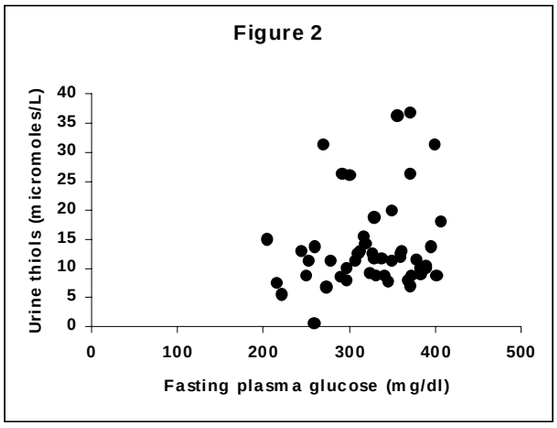

Fig. 2: Correlation between FPG and urine thiols.

controls. Existence of oxidative stress in diabetes mellitus is well proved (1). Decreased –SH levels may be due to enhanced free radical generation in diabetes mellitus. These reduced thiol groups were oxidized by electron deficient free radicals, in the process of oxidation of –SH groups present over plasma proteins occurs. Since –SH groups are the major antioxidants that contribute to the antioxidant pool of the body fluids, hence oxidation of such –SH groups can significantly contribute to the oxidative damage to biomolecules in type 2 diabetes mellitus patients.

We speculate that the decreased thiols in urine of type 2 diabetes mellitus patients could be because of increased oxidation of –SH groups in serum due to already existing oxidative stress. Depletion of –SH groups by oxidative free radicals may be the possible cause for decreased levels of urine –SH levels seen in our study in type 2 diabetes mellitus patients, although other possible causes cannot be ruled out.

Hyperglycemia in diabetic patients can increase the levels of free radicals through glucose autooxidation, nonenzymatic posttranslational modification of proteins resulting from chemical reaction between glucose and primary amino groups of proteins-glycation and also through polyol pathway and protein kinase activation (9–12). Our correlation results suggests possibly increased free radical generation in hyperglycemic state may enhance consumption of available reduced –SH groups.

In conclusion, our study indicates decreased levels of reduced –SH groups both in serum and urine of type 2 diabetes mellitus patients, and hyperglycemia is correlating negatively with such –SH groups.

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