Oxidative stress and expression level of Catalase, Glutathione S Tranferase Enzyme in type 2 Diabetes Patients

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ABSTRACT

Type2 diabetes formerly known as non insulin dependent diabetes is the most common form of hyperglycemia, insulin resistance and relative insulin deficiency. The type 2 diabetes is caused due to metabolical disorder. Oxidative stress, through production of reactive oxygen species (ROS) to develop insulin resistance, beta cell dysfunction, impaired glucose tolerance there by impairing antioxidant expression level such as catalase.Type2 diabetes is develop by oxidative stress due to imbalance in antioxidant expression and activity. Peroxisome proliferators' activated receptor (PPAR) is able for catalase enzyme development. Reactive molecule species are bind with PPAR and inhibit to signalling pathways which initial for activity of catalase enzyme due in that condition decrease level of catalase activity so that catalase can not convert to lot of reactive molecule in H_2O and O_2. The data are suggested that PPAR mediated receptor signalling pathway and expression of gene may block to normal catalase expression which by reactive molecules species. In study this we have to measure Red Blood Cell catalase (CAT), Total Antioxidant Level (TAC), Glutathione S Transferase (GST) to understand weather what kind of Enzyme activity in Type2 Diabetes Patients and in Healthy person male or female. **Method and Result** We measure activity of CAT enzyme in Diabetes patients (N=60) and Healthy individual (N=30) Mean and SEM value of CAT enzyme in Diabetes & Healthy Individual respectively 0.6283 ± 0.1209 and 0.9754±0.1787. GST in Diabetes & Healthy Individual respectively 61.70 ± 9.123 and 55.52 ± 8.094.TAC level of Diabetes & Healthy Individual respectively 17.41 ± 0.8497 and 39.58 ± 2.061.

Key words

Catalase, Glutathione S Transferase Oxidative stress, PPAR, Reactive oxygen species, Total Antioxidant Level

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INTRODUCTION:

Diabetes mellitus is a heterogonous metabolic disorder. In case of type2 diabetes mellitus which generally develops by Reactive oxygen species(ROS) in kindly ageing persons the principle of type2 diabetes is defect is insulin resistance leading to relative insulin deficiency in liver and peripheral tissues could lead to hyperglycemia up to turn cause fourfold increase glucose. The principal source of type2diabetes develop by reactive oxygen species in cells which generate by metabolically reaction increase level of ROS called to oxidative stress which are main effect to cause type2diabetes in human. Increase level of ROS is equal to decrease level of antioxidant enzyme. Oxidative stress is wildly playing key role in electron for inhibit the process of reactive oxygen species. Catalase an endogenous antioxidant enzyme are initial for regulate to inhibit process of ROS. Catalase interact with reactive molecules (Free Radical) split to reactive molecule in to H_2O and O_2. Glutathione S Transferase and Catalase both are necessary enzyme which protect to cell from oxidative damage and prevent the formation of free radical. The Total antioxidant capacity (TAC) in the red blood cell is not simple sum of the various antioxidant substances. It is a dynamic equilibrium that is influenced by the interaction such as protein and cholesterol.

MATERIAL AND METHOD:

Human Blood Sample:

Human's Diabetes Blood Samples 100 and Healthy 100 were collected from south India Hyderabad. The Blood were rinsed from human body and then stored at -4^{o}c temperature.

Extraction and Purification Procedure of Red Blood Cells:

All steps were carried out at 0–4^{O}C. Human blood samples was centrifuge 3000rpm for four times with 0.9%NaCl for each 10minute.The homogenate was immediately stored at - 4^{o}c.

Activity Assay:

Activity was measured spectrophotometrically (Varian UV visible spectrophotometer, Cary 1C, Australia) at room temperature by following the decrease in absorbance at 240 nm in case of catalase resulting from the disappearance of H2O2. The molar extinction coefficient of H2O2 was 0.071/M/cm at 240 nm (Kaur et al). One unit of catalase activity is defined as the amount of activity required to convert 1m mole of hydrogen peroxide to H_2O and O_2per minute at 25C.

Experimental studies were carried out in triplicate, and the results were expressed by statically analysis the value means and standard error of mean.

Protein Determination:

Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. In addition, during the enzyme estimation with speculation method, the protein elution profile was monitored spectrometric ally as the absorbance at 660 nm.

Catalase Estimation:

The catalase activity was estimated using method of Aebi *et al*., 1984. 0.02 ml of red blood cell (Test Sample) solution was added to 1.580 ml of 50 mM phosphate buffer (pH 7.0). 0.4 ml of 30 mM hydrogen peroxide (H2O2) was added and absorbance was followed for 60 sec intervals at 240 nm. The catalase activity was calculated using the millimolar extinction coefficient of H2O2 (0.071 mmol cm-1) and the activity was expressed as micromoles of H2O2 oxidized per minute per milligram protein.

$$\textbf{Catalase Activity:} \quad \frac{\Delta 1 - \Delta 2}{0.071 \times \text{Volume .of Sample} \times \text{Protein conc.}}$$

Glutathione S Transferase:

GST activity was spectrophotometrically measured at a wavelength of 340 nm according to the method defined by Habig *et al*.1974. Under standard conditions, the amount of enzyme conjugating one micromole of 1-chloro-2, 4-dinitrobenzene (CDNB) with Glutathione (GSH) in one minute was defined as one unit activity (mmol/L).

$$\textbf{GST Activity:} \quad \frac{\Delta 2 - \Delta 1}{0.0096 \times \text{Volume .of Sample}}$$

Total Antioxidants Measurement:

Total Antioxidant activity was spectrophotometrically identified at 593 nm according to the method defined by Benzie et al 1996. Total antioxidant activity was given as mmol/L.

Chemical:

All are chemical were analytically pure and supplied by the sigma chemical company.

Statistical Analysis:

All parametric data are expressed as the mean and standard error of mean. The statistical package for the Graph Pad Prism version 6.04 software were used for statistical analysis parametric data were evaluated

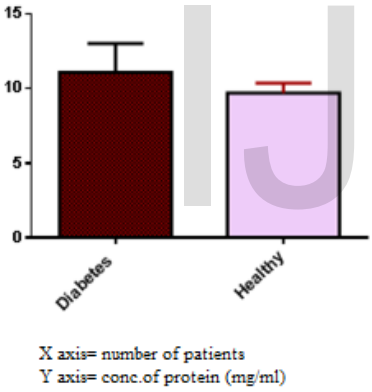
independent sample t- test value ≤ 0.05 was considered as significant.

RESULTS

Hundred patients of type2diabetes mellitus and 100 Healthy as control subjects were recruited for the study of diabetes patients.

Table1. Mean Standard error of mean, CAT, GST and TAC levels of Type2Diabetes and controls.

	Mean/SEM Patients(n=100)	Mean/SEM Healthy(n=100)	P
Total Protein Level (mg/ml)	11.09± 1.944	9.702± 0.6822	0.0002
Catalase (U/ml)	0.6283± 0.1209	0.9754±0.1787	0.0005
GST (µmol/ml)	61.70± 9.123	55.52± 8.094	0.0001
TAC (mmol/ml)	17.41± 0.8497	39.58± 2.061	0.0004



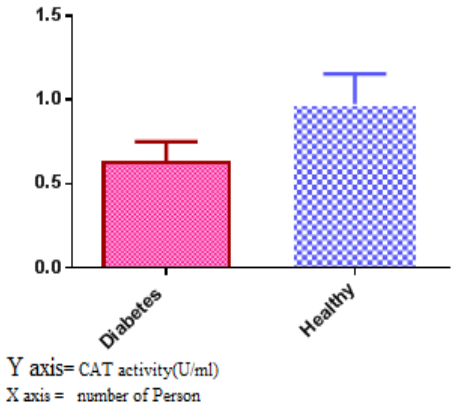
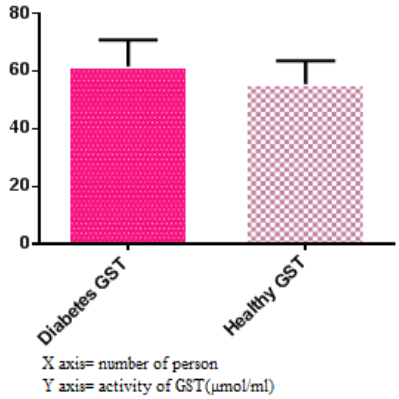


Fig1. Total Protein Level (TPL) in both groups

Fig2. Catalase activity in both groups



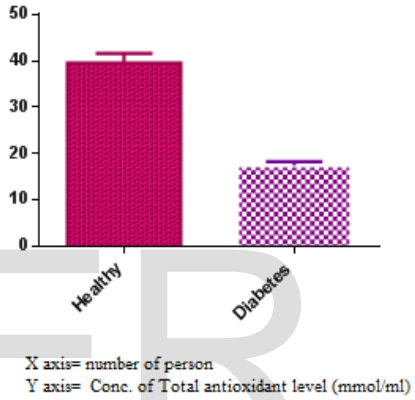


Fig3. GST activity in both groups

Fig4. Total antioxidant level in both groups

(**Figure 1**) Total protein conc. was found significantly (P = 0.0002) in the patient group (11.09± 1.944Umg/ml) compared to the control group (9.702± 0.6822) (**Figure 2**) And Catalas activities were also found significantly (P = 0.0005) and GST activities were also found significantly (0.0001) and total antioxidant level were also found significantly (P=0.0004).

DISCUSSION:

Type 2 diabetes results from an imbalance between insulin sensitivity and insulin secretion. Both longitudinal and cross-sectional studies have demonstrated that the earliest detectable abnormality in type 2 diabetes is an impairment of the body's ability for respond to insulin by free radicals. Over the last few decades, research into the role and involvement of free radicals in the pathogenesis of many of the degenerative oxygen-generated diseases such as Diabetes, CVD, Cancer and osteoarthritis has advanced (Scott, 1995; Behl, 1999).

To prevent the damage caused by oxygen-free radicals, tissues have developed an antioxidant defense system that includes enzymatic activities such as that of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione S transferase (GST). In this study we have examine the total antioxidant level as well as Catalase increase in healthy with type 2 diabetes because Catalase is one of the major enzyme components of cell defense against oxidative stress and it has been hypothesized that the polymorphism of -21 A/T CAT reduces the antioxidant capacity and may serve as a risk factor for oxidative stress associated diseases.and GST level were estimated in type 2 Diabetes increase in type 2 diabetes with healthy because increase level of glucose by insulin resistance by free radical they can inhibit to signal pathway so that synthesis of some amino acid are uncontrolled synthesized in diabetes case uncontrolled reaction of cysteine synthesis so increase level of cysteine so that increase level of GST in type 2 diabetes compare with healthy individual.

In the first series of our study we found Total protein level in Red Blood cell of diabetes patients as well as healthy person so I were getting significant result of DM and HP. Significant increase ratio of protein concentration was in Diabetes Patients and in significant decrease ratio of protein concentration in HP that was lot difference by free radical.

In the next step we have found expression level of catalase in diabetes patients where i was getting significant result whether increase level of catalase in healthy person compare with diabetes patients. As well as was getting significant result of GST

In the next step we have performed FRAP assay. FRAP assay may be considered as an easy, cost-effective method to measure the antioxidant power and it might be incorporated into risk prediction in type 2 diabetes. Our result of FRAP assay showed that increase level of TAC in Healthy compare with Diabetes Patents.

Similar results are also found in patients with type 2 diabetes, (*Firrozrai* M, Nourbakhash M, 2008) however (Rizvi SI. and Srivastava N. 2009) reported that decreased plasma antioxidant capacity in first degree relatives of T2DM patients.

CONCLUSION:

The result were obtained our study suggest that oxidative damage develop to diabetes and can develop to so many metabolically disease. Free radicals are potential for insulin resistance on peroxisome proliferators' activator receptor.

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