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Correlation of oxidative stress with 2-hour Plasma Glucose in normal subjects and diabetic families

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Abstract: Background: There is substantial proof that the hyperglycemia results in generation of reactive oxygen species (ROS), eventually lead to increased oxidative stress in various tissues that results in diabetic complications. This study evaluates the role of oxidative stress in three groups, viz. Diabetics (aged 22 to 60 years) and non-stressed controls and stressed controls (22 to 30 years).

Method: The Study population from amongst the diabetic families were divided into three groups on the basis of clinical history, 2hPG and HbA1c. The blood samples of the normal control and stressed were taken for investigation of oxidative enzymes and were estimated spectro photo metrically.

Results:

The oxidative stress in diabetics and controls (stressed and non-stressed) were correlated with 2 h-PG (two hour plasma glucose) in serum samples collected from all the subjects.

According to initial findings the correlation (at the 0.05 level, 2-tailed) was negatively correlated in the non-stressed controls. CAT, SOD, MD), VIT.C, VIT.E and positively in the GSH, NO. In stressed subjects (exercising) groups; it was negatively correlated in SOD, VIT.C, VIT. E and while it was positively correlated in CAT, GSH, MDA, NO. The correlation coefficient was significant in case of diabetics with CAT, SOD, NO, VIT.C and VIT.E.

It is observed that there is relationship between oxidative stress and 2h-PG that shows close association with the changes in antioxidant defense system resulting in the complications of diabetes mellitus type-II.

Conclusion: There is positive correlation of oxidative stress with 2h-PG normal persons, while it is negatively correlated with diabetic patients.

Keywords: Diabetes mellitus type-II, oxidative stress, reactive oxygen species.

Abbreviations: CAT = Catalase. SOD = Superoxide dismutase, MDA = Malone di aldehyde , GSH = Glutathione peroxidase. NO = Nitric oxide. VIT.C = Vitamin-C. VIT.E =Vitamin-E.

1. INTRODUCTION

Oxidative stress develops when the synthesis of free radicals exceeds the body's ability to neutralize and eliminate them. Normally reactive oxygen intermediate (ROI) and reactive nitrogen intermediate (RNI) are constantly produced physiologically (Kröncke, 2003; Nathan, 2003) It is the precarious occurrence in living organisms. The oxidative stress is actually dealing with the production of reactive oxygen intermediates such as hydroxyl and superoxide radicals, and hydrogen peroxide and oxygen anion and other molecules included into this are reactive nitrogen intermediates such as nitric oxide (NO), per-oxynitrite and *S*-nitrosothiols. (Kröncke, 2003) The proteins, carbohydrates and lipids after reacting with reactive oxygen intermediates and reactive nitrogen intermediates results in intracellular and intercellular changes and brings cell death and regeneration (Garrido, *et al.*, 2004). In order to deal with the oxidative stress, the human and animals have evolved a defense system of various enzymes and chemicals in the form of antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase, low molecular-weight antioxidants such as ascorbate, α-tocopherol and glutathione, cysteine, thioredoxin, vitamins, etc. (Fridovich, 1997).

The damage to the DNA and biological molecules due to oxidative stress lead to various diseases and aging and render impairment of normal functions of cells and tissues (Beckman and Ames, 1999).

The stable compound in the body is formed due sharing of electrons, atoms or molecules of weak compound, atoms or molecules with highly unstable compounds or atoms after pairing. To achieve the stable state, hydrogen atoms are being sliced from another molecule, in this way it interact other free radicals. During respiratory chain reaction, oxygen is playing the main role and produce free radicals. In molecular state oxygen can take up four electrons and same number of protons so that two water molecules are formed. Oxygen radicals in form of transitional products such as superoxide anion, peroxide (exists as H_2O_2 in cells) and hydroxyl radicals are generated during this process (Wu and Cederbaum, 2003).

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Approximately 2-3% of oxygen is utilized during the respiratory chain process is transformed to reactive oxygen species (Chance, Sies, and Boveris, 1979)**,** but the toxic effects exhibited by it such as lipid oxidation, enzyme inactivity, DNA mutations are fairly related with its conversion into ROS from oxygen (De Groot, 1994; Nakazawa, Genka, and Fujishima, 1996; Toyokuni, 1999).

The Reactive oxygen and Nitrogen Species (ROS and RNS) which include superoxide anion, nitric oxide, peroxynitrite, they play an essential role in the development of diabetes mellitus and cardiovascular problems. Oxygen reduction by various oxidases such as dihyronicotinamide adenine dinucleotide phyosphate NADPH results in a electron production. During oxidative phosphorylation ATP is generated (Evans, *et al.,* 2003).

The increased levels of glucose and free fatty acids may induce the production of reactive oxygen species (ROS) creating the imbalance amongst the development of radicals and the insufficient removal via antioxidant defenses, leading to oxidative stress. The experiments performed in natural and in controlled environments proved that oxidative stress has the substantial role in the development of diabetes type-2 (Ceriello, 2000, 2003; West, 2000). Oxidative stress beyond the normal limits has the damaging role in beta cell dysfunction, insulin resistance and impaired glucose tolerance and finally, diabetes type-2 (Evans, *et al.,* 2003; Shah, *et al.,* 2007).

The hyperglycemia and elevated free fatty acids (FFA) may increase the synthesis of reactive oxygen species (ROS) (Paolisso and Giugliano, 1996; Rösen , *et al.,* 2001).

Reactive oxygen species are necessary for the homeostatic mechanism of the body and its lower levels not only alter the physiological mechanisms but defense mechanisms of the body also. The increased levels of the ROS are also harmful to the cells in increasing the aging process and many diseases including diabetes mellitus (Organization, 2006)

For the identification of diabetic families the fasting blood sugar level ≥7.0 mmol/l (126mg/dl) or 2–h plasma glucose ≥ 11.1mmol/l (200mg/dl) were used. (Finkeland , *et al.,* 2003)

2. METHOD

A cross-sectional/ experimental work has been carried out for the period of one year. As per study requirement, the diabetes mellitus type-2 subjects were selected from the study areas with the help of questionnaire. A questionnaire was applied on known families of diabetes mellitus for the purpose of identification of diabetes mellitus type-2 patients. The data were obtained on the basis of disease running at least in three generations. Out of 50 families, 15 families were selected and 90 subjects with average 6 members from each family were finalized for further investigations.

The Participants of this study were selected from Hyderabad city, Qasimabad, Jamshoro, Kotri and Larkana city of Sindh (Pakistan).The subjects between 22 to 65 years of age, with history of diabetes in their families were included in this study on the basis of 2h-PG status. The subjects with smoking habits, drug abuse, and with acute/chronic infection were excluded from this study.

The Study population of the diabetic families was divided into three groups on the basis of clinical history, 2hPG and HbA1c. The "Control Group" comprised of ninty (90) persons with normal glucose level, but are not engaged in any kind of exercise and termed as non-stressed group (non-exercise). The same participants were told to go for jogging for 40 minutes daily for about 40 days and were labelled as control exercise. The third group of ninety (90) persons with known diabetes type-2 was taken as "Diabetic Group".

2.1. Anti-oxidant Analyses.

The blood samples of the normal control that were not on exercise were taken at zero time and again after 40 days of continuous 40 minutes of exercise. Blood samples were again taken from same individuals for investigation of oxidative enzymes. Spectronic 21 was used for the estimations.

Malondialdehyde in plasma was estimated by the method (Albro, *et al.,* 1986) (Das *et al.,* 1990) Reduced glutathione (GSH) was determined by the method (Owens and Belcher, 1965) Catalase activity was measured in plasma; (Johansson and Borg, 1988; Wheeler, *et al.,* 1990).

The procedure for the measurement of total nitric oxide (NO) in blood plasma is based on conversion of nitrate to nitrite by nitrate reductase enzymatic ally by using Griess reagent (Nims *et al.,* 1996).

2.2. Non-enzymic Antioxidant Analysis

Vitamin E (α-tocopherol) determination was carried out by the method of (Desai, 1984) Vitamin C (ascorbic acid) Concentration was measured by method of (Omaye, *et al.,* 1979). All the statistical calculation were performed on SPSS statistics 17.0.

3. RESULTS

3.1. Oxidative Stress Analysis

The data for diabetic group indicated that the majority of patients that had oxidative stress were between the ages of 25-60 years. It was observed in this study that the Diabetic subjects with body mass index (BMI) $\geq$ 24 are suffering more from oxidative stress as compared to subjects with body mass index (BMI) < 24. Similarly the control group comprising of exercise and non-exercise group shows that the group performing exercise have better values as compared to non-exercise group. Significant deficiency is observed in the oxidative enzymes in diabetic cases as compared to control group. The difference in oxidative enzymes was also observed in control exercising and control non-exercising group **(Table-1)**. Correlation of 2 h-PG with oxidative enzymes was observed in diabetes type-2 patients **(Table-2).**

Table-1. Mean and St. Dev. of oxidative enzymes in control (exercising and non-exercising) and Diabetes type-2 subjects.

Oxidative enzymes	2-Hour Plasma Glucose (2h-PG) (mg/dl)	CAT (MU/L)	SOD (u/ml)	GSH (ng/ml)	MDA (nmol/ml)	NO (µmol/ml)
Diabetes group	264.19±31.86	48.90±5.80	3.88±0.68	17.01±1.39	5.30±0.56	28.38±3.12
control (non-exercise)	106.79±14.52	72.08±8.90	4.91±0.36	24.67±1.52	3.75±0.38	33.67±4.27
control (exercise)	76.95±13.66	100.67±6.51	6.11±0.39	27.12±2.33	3.13±0.44	54.22±2.16

Table 2. Correlation of 2 h-PG with oxidative enzymes.

	CAT	SOD	GSH	MDA	NO
Control (exercise) correlation coefficient	0.088*	-0.034	0.474*	0.153	0.445*
Control (non-exercise) correlation coefficient	-0.12	-0.168	0.06	-.007	0.23*
Diabetes type-2 Correlationcoefficient	-0.24	-0.073	0.298*	0.047	-0.038

*Correlation is significant at the 0.05 level (2-tailed)

3.2. Vitamin C and E Analysis

Significant deficiency of vitamin C and E was also observed in case of diabetes type-2 subjects and non-exercising group as compared to control exercising group **(Table-3).** Correlation of 2 h-PG with vitamins was also seen in diabetic patients **(Table-4).**

Table-3.Mean and St. Dev. of vitamin-C and E in Diabetes type-2 subjects with control (exercising and non-exercising) group.

	Vit.C(mg/dl)	Vit.E (µg/ml)
Diabetes group	0.88 ±0.30	0.86 ±0.25
control (non-exercise)	1.38 ±0.15	1.83 ±0.36
control (exercise)	1.33 ±0.10	2.15 ±0.33

Table 4. Correlation of 2 h-PG with Vitamin-C and E in Diabetes type-2 subjects with control (exercising and non-exercising) group.

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	VIT.C	VIT.E
Control (exercise) correlation coefficient	-0.133	-0.066
Control (non-exercise) correlation coefficient	-0.059	-0.62
Diabetes type-2 Correlationcoefficient	-0.092	-0.092

4. DISCUSSION

The aim of this study is also to ascertain the association of oxidative stress vs exercise status in normal and type 2 diabetic subjects, and to establish the correlation with oxidative enzymes and type 2 diabetic subjects **(Table. 1 and 2).** Exercise is also a strong forecaster of oxidative enzymes in type 2 diabetes along with some of the trace elements(McCord and Aizenman, 2014), hence excessively released during exercise. In

fact oxidative stress induced due to lack of exercise can cause the diabetes type-2 at earlier stages of life, thus rendering it from the protective effects of oxidative enzymes as (Ristow *et al.,* 2009)has reported that due to exercise, the free radical production promote insulin production hence helps in prevention of diabetes type-2.

Catalase: The mean values of catalase in diabetes type-2 subjects are considerably decreased as compared to control non-exercising and control exercising. Catalase is negatively correlated with 2h-PG in diabetes type-2 subjects. The reason being cited is that the catalase activity decreases in the diabetes type-2 subjects as compared to the control group and this is in agreement as reported earlier (Gönenç *et al.,* 2006; Yeh *et al.,* 2005)

Super Oxide Dismutase: The SOD is also decreased in diabetic subjects compared to control non-exercising and control exercising subjects (Table 1). This is in confirmation with the earlier reports (Kesavulu *et al.,* 2001). These significantly reduced result are in agreement as also reported by different researchers (Brownlee 1984; Ceriello and Testa, 2009; Pasupathi 2009)

Reduced Glutathione (GSH): GSH keep the cells from the damaging effects of reactive oxygen species (ROS), and 90% of this antioxidant is in reduced form. The increased state of GSH is the indication of its activity against the free radicals which is especially significant in case of control-exercising group. The studies indicated a link between non-enzymatic glycosylation and oxidative stress, (Sasaki and Inoguchi, 2012; Sharma and Sharma, 2007) and also due to bonding of glucose or fructose molecule with proteins so as to lead to the formation of free radicals; hence persistent higher levels of glucose results in diabetes mellitus type-2. The research carried by(Avogaro, Fadini, Gallo, Pagnin, and de Kreutzenberg, 2006; Griesmacher *et al.,* 1995; Khanna, *et al.,* 2008; Waggiallah and Alzohairy, 2011) supports our results.

Malonedialdehyde (MDA): MDA produced partially by oxidation and free lipid radicals. The diabetic subjects have significantly higher levels of MDA as compared to control and control exercising subjects with reduced anti-oxidative activity. The higher levels of MDA in diabetes type-2 patients indicate the lipid peroxidation because of oxidative stress. The levels of MDA in blood serum are in accordance with others that the MDA increases in diabetes mellitus type-2 as well as in hypertension (Griesmacher *et al.,* 1995; Khanna *et al.,* 2008). It has been shown that hyperlipidemia plays a key role in the formation of lipid peroxides thus mediating oxidative stress, as previously described (Okoduwa, *et al.,* 2013).

Nitric Oxide (NO): Oxidative stress because of increased sugar levels results in decreased production of NO not only affects the endothelial dependent vasorelaxants such as acetylcholine, bradykinin, stress etc. (Avogaro *et al.,* 2006). This rapid swing of blood sugar level in diabetics are injurious to endothelium functioning in diabetes type-2 patients (Ceriello *et al.,* 2008) and shows conformation of the nitrosative stress in diabetic patients in this study; where the mean values in diabetic subjects are comparatively lower than control exercising and non-exercising normal subjects. The correlation is negatively significant in diabetes type-2 subjects and positively correlated in control exercising subjects.

5. CONCLUSION

The ROS tests may be taken as significant biomarker for type-2 diabetes mellitus in future studies. We hope that the study of free radicals will highlight the major steps where we should act to treat and prevent the disease.

Exercise is positive stress, that enhance the anti-oxidant activity.

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