# The study of oxidant-antioxidant status in type 2 diabetes mellitus

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# The study of oxidant-antioxidant status in type 2 diabetes mellitus

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**Abstract.** Hyperglycemia is considered a major initiator of oxidative stress which leads to the formation of free radicals and consequently lipid peroxidation occurs, which leads to tissue damage and diabetes mellitus development. Free radicals have been defined as intermediates of some biological redox reactions necessary for the maintenance of life. In presence of a free radical initiator and oxygen they may be oxidized this leading to lipid peroxidation, as it was suggested, might be associated with running out of hydrogen. In particular lipid peroxidation measured as levels of malondialdehyde (MDA). Glutathione (GSH), an intracellular thiol causes the eradication of free radicals or reduction in hydrogen peroxide level on state of oxidative stress. Decrease in the reduced GSH level has been reported in the erythrocyte of diabetics. Decrease in the level of GSH occurs both due to the competition between aldose reductase and glutathione reductase for NADPH, a cofactor, and increased oxidative stress (increased ratio of NADH/NAD). This study was conducted on 60 subjects. Informed consent was obtained from each person who agreed to participate in this study, while the survey was based on standardized interviews and questionnaire. Subjects were grouped as type 2-diabetics and healthy control with fasting plasma glucose (FPG) < 5.50 mmol/L. The ages ranged from 18 to 50 years. Glucose was determined by an enzymatic colorimetric test on basis of Trinder-Reaction. MDA was performed as described by Lipid Peroxidation Assay Kit (Colorimetric/Fluorometric), while the levels of GSH in all subjects were measured by the DetectX® Glutathione kit. The results show a significant elevation ($P \leq 0.05$) in levels of glucose and MDA of type 2- diabetic patients in comparison with healthy subjects, which reached to 16.30 ± 0.50 mmol/L and 0.87 ± 0.19 nmol/mL for the type 2-diabetic patients, and 4.60 ± 0.15mmol L and 0.29 ± 0.5 nmol/mL for the control group, respectively. Also, it has been found a significant decrease ($P \leq 0.05$) in GSH levels in type 2- diabetic patients, which reached to 3.43 ± 0.91 µM/mL in comparison with the control group which reached to 6.13±0.21 µM/mL. In conclusion we can observe that the increase in glucose levels leads to free radical formation by auto-oxidation and increase in lipid peroxidation (MDA levels), and inadequate antioxidant defense can occur during DM. In addition, GSH deficiency will make the present state worse by increasing the oxidative stress, since GSH is an important antioxidant.

**Keywords:** Type 2 diabetes mellitus, oxidant-antioxidant balance, MDA, GSH

## 1. Introduction.

Human body is continuously exposed to different types of agents that results in the production of reactive species called as free radicals (ROS/RNS) which by the transfer of their free unpaired electron causes the oxidation of cellular machinery. In order to encounter the deleterious effects of such species, body has got endogenous antioxidant systems or it obtains exogenous antioxidants from diet that neutralizes such species and keeps the homeostasis of body. Any imbalance between the RS and antioxidants leads to produce a condition known as "oxidative stress" that results in the development of pathological condition among which one is diabetes. Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels. Lipids, proteins, DNA damage, Glutathione, catalane and superoxide dismutase are various biomarkers of oxidative stress in diabetes mellitus. Oxidative stress induced complications of diabetes may include stroke, neuropathy, retinopathy and nephropathy (Asmat et al., 2016). Hyperglycemia is a major initiator of diabetic microvascular complications (e.g., retinopathy, neuropathy and nephropathy) results in oxidative stress by generating free radicals and causes DNA, proteins, lipids, and carbohydrate damage. Oxidative stress is caused by a relative overload of oxidants, i.e., reactive oxygen species and the complications of diabetes partially is mediated by oxidative stress (West, 2000). Reactive oxygen species (ROS) is a term which contains all highly reactive, oxygen-containing molecules, including free radicals. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. It is a highly reactive due to the presence of unpaired electron and they are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur and until subsequent free radicals are deactivated. Thousands of free radical reactions can occur within seconds of the initial reaction. However, free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. These unstable species may cause oxidative damage to DNA, carbohydrate, lipids and proteins. Furthermore, the excessive production of free radical leads to oxidation of cellular lipids, proteins and nucleic acid, which may ultimately lead to cell death (Jakus, 2000; Fiorentino et al., 2013). It is well known that a number of enzymatic systems protect cells from the damage caused by excessive production of ROS. These systems include Glutathione (GSH), an intracellular thiol causes the eradication of free radicals or reduction in hydrogen peroxide level on state of oxidative stress (convert hydrogen peroxide to water). Decrease in the reduced GSH level and impairment in GSH metabolism have been reported in the erythrocyte of diabetics. Decrease in the level of GSH occurs both due to the competition between aldose reductase and glutathione reductase for NADPH, a cofactor, and increased oxidative stress (increased ratio of NADH/NAD) (Pastore et al.,2003; Kalkan and Suher, 2013). The aim of the present article is to determine the effect of hyperglycemia (oxidative stress) on the biochemical parameters of diabetic patients compared to control.

## 2. Materials and methods

*2.1. Subjects.* This study was conducted on 60 subjects (table 1.). Informed consent was obtained from each person who agreed to participate in this study, while the survey was based on standardized interviews and questionnaire. Subjects were grouped as type 2- diabetics and healthy control with fasting plasma glucose (FPG) < 5.50 mmol/L. The ages ranged from 18 to 50 years. About (10 mL) of fasting venous sample all included subjects were taken and allowed to clot to get serum by putting it in empty disposable tube's centrifuge to separate it in the centrifuge at 3000 (rpm) for 10 min, the serum samples were separated, stored at (-20˚C) for later measurement biochemical parameters, unless used immediately.

**Table 1.**Clinical characteristics of the diabetic patients and healthy subjects

| **Parameters** | **Control (non- diabetic)** | **Type 2 diabetic patients** |
|---|---|---|
| Number of subjects | 29 | 31 |
| Gender (M:F) | (15:14) | (16:15) |
| Age (years) | 18-50 | 18-50 |

*2.2. Measurements Biochemical Parameters.*

*2.2.1. Glucose determination* is an enzymatic colorimetric test on basis of Trinder-Reaction which is important in the diagnosis and control of carbohydrate metabolism illnesses such as diabetes mellitus, The test is based on the coupling of the enzymatic oxidation of glucose by glucose oxidase resulting in production of hydrogen peroxide, which is subsequently used for the generation of a colored product by peroxidase (Trinder, 1969; Greiling et al., 1995).

*2.2.2. Lipid Peroxidation Assay Kit* (Colorimetric/Fluorometric) is a robust and sensitive kit for detection of malondialdehyde (MDA) produced as an end product of lipid peroxidation. In this assay, free MDA present in the sample reacts with Thiobarbituric Acid (TBA) and generate a MDA-TBA adduct, which can easily be quantified colorimetrically (OD 532 nm) or fluorometrically (Ex/Em = 532/553 nm).

*2.2.3. The DetectX® Glutathione kit* is designed to quantitatively measure glutathione (GSH), and oxidized glutathione (GSSG) present in a variety of samples. Thoroughly mix sample with an equal volume of cold 5% SSA. Incubate for 10 minutes at 4°C. Centrifuge at 14,000 rpm for 10 minutes at 4°C. Collect the supernatant. The supernatant must be diluted 1:2.5 with Assay Buffer by mixing one part with 1.5 parts of Assay Buffer to bring the SSA concentration to 1%. The sample will have been diluted 1:5 at this point. All final dilutions are made in Sample Diluent. The concentration of GSH can be determined either as an endpoint read of the color developed at 405 nm or by measuring the rate of color development at 405 nm.

*2.2.4. Statistical analysis* was carried out by one way ANOVA-test which used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant. The results were expressed as mean ± standard deviations (mean ± SD).

***3. Results and discussion.***

The results obtained (table 2.) showing a significant increase in ($P \leq 0.05$) in levels of glucose and MDA of type 2- diabetic patients in comparison with healthy subjects suggestion that the Hyperglycemia is a result in increased production of the reactive oxygen species which leads to may be due to the increasing of oxidative stress in type2 diabetes because of the exposure to prolonged periods of hyperglycemia, which causes glucose to be in its highest concentrations. Also, it has been found a significant decrease ($P \leq 0.05$) in GSH levels in type 2- diabetic patients in comparison with the control group. The possible sources of oxidative stress in diabetes might include auto-oxidation of glucose, shifts in redox balances, and decreased tissue concentrations of low molecular weight antioxidants, such as reduced glutathione (GSH).

**Table 2.** Mean and Standard Deviation values for all measurement parameters in all studied groups

| | Mean ± SD | |
|---|---|---|
| Parameters | Control (non- diabetic) | Type 2 diabetic patients |
| FPG (mmol/L) | 4.60±0.15 | 16.30±0.50 |
| MDA (nmol/L) | 0.29±0.50 | 0.87±0.19 |
| GSH (μM/mL) | 6.13±0.21 | 3.43±0.91 |

Diabetes mellitus is a metabolic disorder which affects carbohydrate, fat and protein metabolism and results from a disorder either in insulin secretion or its action on target tissues or both, this causes sugar accumulate in the blood, often leading to chronic hyperglycemia. Hyperglycemia may also result in increased production of the reactive oxygen species (ROS) within numerous biochemical pathways that have potential to initiate changes in endothelial functions (Kalaivanam et al., 2006). Because of hyperglycemia is associated with the increase of the oxidative stress and free radicals production so it's effected on oxidant-antioxidant balance where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants (Bonnefont-Rousselot et al., 2000). So in this work we tried to determine the effective of this disease on the oxidation state by measurement lipid peroxidation and determined its effect on the levels of lipid peroxidation marker (MDA), and on antioxidant state by measurement the levels of antioxidant which can be shown by determining the levels of GSH, the levels of MDA were significantly elevated suggesting a positive relationship with hyperglycemia induced oxidative cellular damage, also malondialdehyde (MDA) is highly toxic by-products partly produced by oxidation and derived from lipid. MDA reacts both reversibly and irreversibly with proteins and phospholipids with profound effects. When the generation of reactive oxygen species (ROS) exceeds cellular defense mechanisms, these unstable molecules interact with biologic macromolecules such as lipids, proteins and DNA and lead to structural changes as well as functional abnormalities. Our findings strongly confirmed the evidence that diabetic patients were susceptible blood glucose level has an association with free radical mediated lipid peroxidation which acts as an indicator for oxidative stress in the body as suggested by (Bhutia et al., 2011; Nair and Nair, 2017). When ROS accumulated because of oxidative stress, they are produced faster than they can be safely neutralized by in vivo antioxidant defense mechanisms- non enzymatic antioxidant systems, such as glutathione (GSH) and this explain the low levels in diabetes patients while malondialdehyde (MDA) and other oxidation markers and /or from a weakened or impaired intrinsic antioxidant defense, with resultant formation of pathological conditions (Chuemere et al., 2018). Diabetes induces alterations in activity of enzymes glutathione reductase which can break free radical chain reactions. Any alteration in their levels will make the cells prone to oxidative stress and hence cell injury (Asmat et al., 2016; Balbi et al., 2018). Superoxide, hydroxyl radical and hydrogen peroxide are the most important free radicals that cause oxidative stress. In human, there are antioxidant enzymes like glutathione (GSH) which scavenges the action of free radicals in order to protect the body (Singh and Singh, 2017). Reduced glutathione (GSH), plays a major role by protecting cells from oxidative damage by neutralizing the free radicals. Oxidative stress is being considered as a common pathogenic factor in diabetes mellitus which results in reducing extracellular and intercellular antioxidant GSH is a non-enzymatic antioxidant delays or inhibits the oxidative process through different mechanisms. Antioxidant enzyme levels are particularly sensitive to oxidative stress and both increase and decrease these have been reported in different disease states in which an enhancement of oxygen species is a cause or a consequence of the diabetes mellitus (Lutchmansingh et al., 2018).

## 4. Conclusion.

Our results suggested that oxidative stress increases in diabetic patients which measured by the end product of lipid peroxidation, MDA, also levels of fasting blood glucose were higher in a group of diabetic patients compare the control group, the increase of fasting blood glucose affected the levels of reduced glutathione (GSH) which was significantly higher in control group in comparison with diabetic patients.

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## 6. References

[1] Asmat U, Abad K and Ismail K 2016 Diabetes mellitus and oxidative stress- A concise review. *Saudi Pharmaceutical Journal* **24** 547–553.
[2] Balbi M E , Tonin F S, Mendes A M, Borba H H, Wiens A, Fernandez-Llimos F and Pontarolo R 2018 Antioxidant effects of vitamins in type 2 diabetes: a meta-analysis of randomized controlled trials. *Diabetol Metab Syndr,* **10** (18) 1-12.
[3] Bhutia Y, Ghosh A, Sherpa M L, Pal R and Mohanta P K 2011 Serum malondialdehyde level: surrogate stress marker in the sikkimese diabetics. *J Nat Sc Biol Med* **2** 107-112.

[4] Bonnefont-Rousselot D, Bastard J P, Jaudon M C and Delattre J 2000 Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab* **26** 163-176.
[5] Chuemere A N, Ilochi O N and Olorunfemi O J 2018 Evaluation of anti-hyperglycemic potential of allium cepa, coffee and oxidative stress. *Int J Biochem Physio* **3** (1) 1-9.
[6] Fiorentino T V, Prioletta A, Zuo P and Folli F 2013 Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. *Curr Pharm Des* **19** (32) 5695-703.
[7] Greiling H, Gressner A M, Lehrbuch D and Klinischen 1995 Chemie und Pathobiochemie, 3rd, Stuttgart/ New York Schattauer Verlag.
[8] Jakus V 2000 The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. *Bratisl Lek Listy* **101**(10) 541-51.
[9] Kalaivanam K N, Dharmalingam M and Marcus S R 2006 Lipid peroxidation in type 2 diabetes mellitus. *Int J Diab Dev Ctries* **26** 30-32.
[10] Kalkan I H and Suher M 2013 The relationship between the level of glutathione, impairment of glucose metabolism and complications of diabetes mellitus. *Pak J Med Sci* **29** (4) 938-942.
[11] Lutchmansingh F K, Hsu J W, Bennett F I, Badaloo A V, McFarlane-Anderson N, Gordon-Strachan G M, Wright-Pascoe R A, Jahoor F and Boyne M S 2018 Glutathione metabolism in type 2 diabetes and its relationship with microvascular complications and glycemia. *PLoS ONE* **13** (6) e0198626.
[12] Nair A and Nair B J 2017 Comparative analysis of the oxidative stress and antioxidant status in type II diabetics and nondiabetics: A biochemical study. *J Oral Maxillofac Pathol* **21** 394‑401.
[13] Pastore A, Federici G, Bertini E and Piemonte F 2003 Analysis of glutathione: implication in redox and detoxification. *Clin Chim Acta* **333** 19-39.
[14] Singh K and Singh G 2017 Alterations in some oxidative stress markers in diabetic nephropathy. *J Cardiovasc Disease Res* **8** (1) 24-27.
[15] Trinder P Ann 1969 *Clin Biochem* **6** 24-33.
[16] West C I 2000 Radicals and oxidative stress in diabetes. *Diabet. Med* **71** 171-180.