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ANALYSIS OF LEVEL OF ANTIOXIDANTS AND OXIDATIVE STRESS IN DIABETES MELLITUS: A POPULATION BASED STUDY

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Abstract:

Introduction: *Oxidative stress is caused by an unfavorable balance between reactive oxygen species (ROS) and antioxidant defenses. ROS are generated during normal cellular metabolism, as a result of the influence of various environmental factors, as well as during pathological processes.* ***Objectives:*** *The main aim of our study is to find the role of antioxidants and measure the level of oxidative stress and their role in diabetic patients.* ***Methodology:*** *The study was conducted at Rai medical college Sargodha in the period of Jan 2018 to March 2018. For this purpose we selected the patients who were suffering from common disease diabetes. This study is based on the local population of Pakistan, which shows the stress level in Pakistani environment.* ***Results:*** *According to analysis of data level of antioxidant and oxidative stress is increasing in diabetic patients because cell becomes destroyed. GSH is important non-enzymatic antioxidant which helps in scavenging of free radical mechanism.* ***Conclusion:*** *It is concluded that level of antioxidants in our body plays an important role. It is obvious from the presented data that a relation exists between hyperglycaemia, oxidative stress, cellular and endothelial dysfunction.*

Key Words: *Antioxidant, Oxidative, Stress, Diabetic*

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INTRODUCTION:

Oxidative stress is caused by an unfavorable balance between reactive oxygen species (ROS) and antioxidant defenses. ROS are generated during normal cellular metabolism, as a result of the influence of various environmental factors, as well as during pathological processes. Reactive oxygen species play an important role in the pathogenesis of cancer [1]. Oxidative stress caused by increased free radical generation and/or decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis [2]. Free radicals are capable of altering all major classes of biomolecules, such as lipids, nucleic acids and proteins, with changes in their structure and function. Prime targets of free radicals are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation. The levels of free radical molecules are controlled by various cellular defense mechanisms, consisting of enzymatic (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic (vit. E, vit. C, glutathione) components [3].

ROS can be produced endogenously or exogenously. In vivo free radicals are created during normal aerobic respiration, by commencement of phagocytosing cells, in peroxisomes where fatty acids are degraded, and by auto-oxidation of various molecules. The mitochondria plays very important role and it is a major physiologic source of reactive oxygen species (ROS), which can be generated during mitochondrial respiration [4]. Super oxide radicals, formed by minor side reactions of the mitochondrial electron transport chain or by an NADH-independent enzyme can be converted to H_2O_2 and to a powerful oxidant, the hydroxyl radical. Oxidative stress in organisms leads to the peroxidation of all major biomolecules, such as DNA, proteins and lipids. The most widely used method to find oxidative stress is to determine lipid peroxidation with the Thiobarbituric acid reactive substances (TBARS) method. Among these targets, the peroxidation of lipids is basically damaging because the formation of lipid peroxidation product leads to spread of free radical reactions. The general process of lipid peroxidation consists of three stages: initiation, propagation and termination.[5].

Diabetes is a major cause of mortality globally, and it has been estimated that 400 million people worldwide will suffer from it by 2030. Despite the fact that hereditary qualities seems to assume an essential part in the advancement of diabetes, examine recommends that dietary decisions driven by natural and financial components are of critical significance. Amazing eating regimens assume an essential part in diabetes avoidance.[1] Suitable dietary adherence can enhance insulin affectability and glycemic control, and consequently add to way of life change and general personal satisfaction [6]. Nonetheless, past research recommends that dietary adherence is seemingly among the most troublesome foundations of diabetes administration [2]. Higher HEI scores demonstrate nearer adherence to current dietary rules for singular food and supplement gatherings. For the sufficiency segments, for example, vegetables and natural product, a higher score demonstrates higher utilization. Dietary proposals depend on the useful effects of devouring products of the soil and expressly stress their constructive outcomes of decreasing corpulence and certain sorts of growths. The last three segments of the HEI incorporate refined grains, sodium, and discharge (calories from strong fats, liquor, and included sugars) and a higher score demonstrates bring down utilization [3,4].

Objectives

The main aim of our study is to find the role of antioxidants and measure the level of oxidative stress and their role in diabetic patients.

MATERIAL AND METHODS:

The study was conducted at Rai medical college Sargodha in the period of Jan 2018 to March 2019. For this purpose we selected the patient who was suffering from a common disease diabetes. This study is based on the local population of Pakistan, which shows the stress level in Pakistani environment.

Collection of data

5.0 ml blood sample was taken from vein. Blood was further processed for the estimation of GSH, Catalases, SOD, MDA, Neuraminidase and Sialic acid. Commercially available enzymatic kits of Randox were used. Blood was centrifuged at 4000 rpm for 10 minutes and serum was separated. Blood samples will be collected into EDTA tubes from fasting proteins. The blood will be centrifuged and indomethacin and butylated hydroxytoluene will be added into the plasma samples before they will be stored at -80°C until analysis**.** The sample were processed and analyzed for the estimation of SOD, GSH, CATALASES, MDA, NO, neuraminidase and sialic acid levels.

Tissue homogenate preparation

Blood was drawn and immediately placed it on ice cold normal saline. Then 2ml 0.15M Tris HCl and 2ml phosphate buffer was added and grind in micro tube by micro pestle. The mixture was centrifuged and stored in cold place.

Statistical analysis

The collected data were analyzed using SPSS software (version 17). The results are presented as a mean with 95% confidence interval limits or standard deviations. The significant value for P <.05 was accepted as statistically significant.

RESULTS:

According to analysis of data level of antioxidant and oxidative stress is increasing in diabetic patients because cell become destroyed. GSH is important non-enzymatic antioxidant which helps in scavenging of free radical mechanism. According to data the levels of GSH become decreases in diabetic patients. The data pertaining in the table shows that levels of sialic acid become increases in patients. The level becomes increases in all cases. As the value in this case is 3.48±0.65. According to our data MDA is considered to be an important antioxidant and serum stress biomarker in case of diabetic patients.

Table 01: Level of anti-oxidants in control and diabetic patients

Variable	CONTROL (moles/ml)	(moles/ml) (n=100) Diabetic patients
SOD	0.32	3.5±0.74
MDA	2.35	3.6±0.82
Catalases	4.16	0.00±0.00
SOD	0.326	3.27±0.16
Sialic acid	0.37	1.05±0.08
GSH	8.26	3.48±0.65

Means±SD

DISCUSSION:

Reactive oxygen species (ROS) cause oxidation of DNA, proteins and lipids, and induce carcinogenesis. Some studies have reported high lipid peroxidation levels become high in human colorectal cancer tissue and gastric cancer tissue. The major aldehyde products of lipid peroxidation are malondial-dehyde (MDA) and 4-hydroxynonenal. MDA is mutagenic in mammalian cells and carcinogenic [7].

Peroxidation of lipids can disturb the assembly of the membrane, causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes. Injure to mitochondria induced by lipid peroxidation can direct to further ROS generation. Catalase is a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second [8].

Superoxide is one of the main reactive oxygen species in the cell and as such, super oxide dismutase (SOD) serves a key antioxidant role. The physiological importance of SODs is explained by the severe pathologies evident in mice genetically engineered to lack these enzymes. In mammals there are several types of SODs, which differ with respect to their location in the cell and the metal ion they require for their function. For example, a copper-zinc SOD is present in the fluid filling the cell (i.e., the cytosol) and in the space between two membranes surrounding the mitochondria [9]. Furthermore, a manganese-containing SOD is present in the mitochondrial interior. Both of these enzymes are critical for prevention of ROS-induced toxicity.

Glutathione (GSH) is a molecule which contains three peptide linkages. It is an antioxidant, and it helps to protect the cells from ROSs and free radical damages. It contains three amino acids; cysteine, glutamic acid and glycine [10]. It is the most important antioxidant which produced within the cell and directly participates in the neutralization of ROSs and free radicals. From the

studies we revealed that, it also reduces the cancer development by changing the level of Ross. Mitochondria cannot synthesize GSH but import it from the cytosol using a carrier protein embedded in the membrane surrounding the mitochondria. Alcohol appears to interfere with the function of this carrier protein, thereby leading to the depletion of mitochondrial GSH [11].

Diabetes Mellitus is a widespread disease and affects all nationalities and ages. The number of patients in 2003 has reached an epidemic proportion totaling a whopping 194 million with patients of 20 to 79 years of age affected (5.1 % of the population in this age group). A rise to 50% more is expected in 2010, mainly from new cases in Africa, Asia and South America. A projection of this figure shows that in 2025 diabetes patients will be 333 million or 6.3% of the total population on Earth [12-15].

To this alarming trend must be added the fact that chronic complications of diabetes micro and microangiopathic, are the causes of 4 times higher mortality in patients with diabetes mellitus in comparison with healthy individuals. Therefore the great social importance of the disease is determined not only by the millions of patients, but also by the high mortality. This explains the intensive studies done on this disease[13]. Diabetes Mellitus is a heterogeneous disease characterized by broken synthesis and/or secretion of insulin, as well as by resistance of the peripheral tissues to the hormone activity [16].

CONCLUSION:

It is concluded that level of antioxidants in our body plays an important role. It is obvious from the presented data that a relation exists between hyperglycaemia, oxidative stress, cellular and endothelial dysfunction.

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