DOI: https://doi.org/10.54393/pbmj.v6i04.898

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 4 (April 2023)

Original Article

Determination of Physiological, Biochemical and Anti-oxidative Status in Type 1 Diabetes Mellitus Patients

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ARTICLE INFO

Key Words:
Diabetes, SOD, MDA, CAT, GSH, NO, AOPP

How to Cite:
Arsalan, H. M. ., Kousar, G. ., Akylbek Amanbekovich, A. . ., & Ashfaq, M. . (2023). Determination of Physiological, Biochemical and Anti-oxidative Status in Type 1 Diabetes Mellitus Patients : Physiological, biochemical and anti-oxidative status in T1DM patients. Pakistan BioMedical Journal, 6(04). https://doi.org/10.54393/pbmj.v6i04.898

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Received Date: 4th April 2023
Acceptance Date: 26th April, 2023
Published Date: 30th April, 2023

ABSTRACT

Type 1 Diabetes Mellitus (T1DM) disorganization of glucose equilibrium distinguishes by autoimmune disruption of the insulin producing pancreatic β-cell that constantly leads to insulin scarcity and resulting hyperglycemia **Objective:** To determine the physiological, biochemical, and anti-oxidant status in Type 1 Diabetes Mellitus Patients. **Methods:** It is a comparative study. 60 diabetic patients and 50 Samples of healthy individuals were taken from Nawaz Sharif Hospital. Blood samples (5.0 ml) were obtained and centrifuged at 4000 rpm for 10 minutes to separate the serum. Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Nitric oxide (NO), micronutrients (Vitamin A, Vitamin C and Vitamin E) and Electrolytes was determined. **Results:** MDA level is progressively higher in T1DM (14.01±0.06) as compared to control group (1.27±0.21) (P- Value 0.000). GSH status is notably reduced in diabetic patients (0.15±.05) as compared to normal (6.24±0.33). Comparable anti-oxidant catalase is reduced (2.82±.04) in affected individuals as compared to normal individuals 4.19±1.09. SOD level was remarkably marked up to (13.52±3.21) in susceptible persons as compared to normal (2.15±0.23). Vitamin A level was markedly reduced to (1.62±0.26) in patients as compared to healthy individuals (7.18±0.33). **Conclusions:** T1DM patients particularly showed reduced amounts and competency of antioxidant protections due to elevated consumption of specific anti-oxidant components such as low level of intracellular glutathione and Catalase and primarily low levels of vitamin A, vitamin E and vitamin C and exalted level of MDA, SOD and NO.

INTRODUCTION

Typically Diabetes mellitus (DM) is a conflation of multifarious derangements displaying with development of glucose intolerance and high blood glucose level, consequence deficiency of insulin and imperfect insulin functioning [1]. Complications, such as disorders in the regulatory mechanism for mobilization and storage of metabolic fuels arise in the anabolism and catabolism of lipids, proteins and carbohydrates due to insufficient insulin secretions, insulin action or both [2, 3]. Globally in 2010, approximate 285 million people in the age group 20-79 anticipated to have diabetes. By 2030, supposed this approximation is elevated to 438 million. Moreover, in adult population the magnitude of people with impaired glucose tolerance (IGT) is extrapolated to rise to 472 million by 2030. The draining impact of DM interpolates disorders of numerous organs, as a result metabolic complications such as vision impairment, nephrosis, and neuralgia [4]. Regular energy source is imperative for every cell to work in the human body. Glucose is the basic energy source for body, a mobilizable fuel source for cells which rotate in the blood [5]. Pancreatic hormone insulin is effective for blood glucose level regulation on auxiliary side of the cell membrane. The hormone coheres to its receptor sites. Across mandatory channel through glycolysis it manages entry of glucose into breathing cells and tissues. Insulin triggers catabolism, regulates lipogenesis from extreme component of cytoplasm acetyl CoA and glycogenesis from extravagant component of cytoplasm glucose. The above

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mentioned functions virulent to digestion events stimulate the hormone Glucagon, rather by entering the cells. Glucose remains in the blood when glucose level at below verge [6]. Signaling of DM are high blood glucose levels resulting insufficient or defective discharge of insulin from pancreas. Insulin conducted flow of glucose through target cells. Metabolic complications linked with DM that can eventually lead to premature death. At the time of prognosis 25% T2DM patients possess micro-vascular convolution & recommended that instant of prognosis they possessed disease for above than 5 years [7]. In 2006 World Health Organization (WHO) recommended that symptoms for a single elevated glucose level are: excessive quantity of urine, excessive thirst, excessive desire to eat, and weight loss. Furthermore, elevated volume on following incidents like fasting plasma glucose (FPG) ≥7.0mmol/L (126mg/dl), oral glucose tolerance test (OGTT), a plasma glucose ≥11.1mmol/L (200mg/dl) after two hours of the oral dose. For DM, International expert committee in July 2009 proposed the modifier prognosis ethic HbA1C raise ≥6.5%. Particular committee recommended word 'pre-diabetes' may be eliminated but describes the extent, HbA1c values ≥ 6.0% and < 6.5% to confirm those individuals that at high extent of progressing DM [8]. It is a general truth that oxygen is the vital component of life. Anyhow in some situations, when it produces reactive species that generates necrosis, this oxygen may be a killer of cells and eventually the cell death. By the production of particular mechanism Reactive Nitrogen Species (RNS) and Reactive Carbonyl Species (RCS) also stimulate oxidation that intervenes with the normal physiological process inside the cell [9]. Almost 0.1% - 0.5 % of oxygen that fall into the electron transport chain is transferred to superoxide Reactive Oxygen Species (ROS) and the remains are used in metabolic procedures under normal physiological conditions. Other than electron transport chain of mitochondria ROS can also be originated from other sources, like cytochrome P450 [10]. Immoderate levels of molecular oxygen or ROS could result by ineffectual removal of ROS or ROS arising from endogenous or exogenous sources, thus eventual elevated oxidative stress. Oxygen is extremely reactive specie that has the competency to become part of basically dangerous and detrimental molecules. Glutathione, vitamin C and E, cysteine etc. are the different types of biological antioxidants [11]. Acclivity of ROS level due to decrease in demolition or inflation in the generation of catalase, superoxide dismutase and glutathione peroxide antioxidants. The inequality in the levels of above mentioned enzymes make the tissues vulnerable to oxidative stress proceeding progress of diabetic ramification [12]. Mitochondria is key source of oxidative stress in diabetes. Utilized oxygen factor is converted to water and the rest of oxygen is converted to oxygen free radical which is a major ROS that transforms into other RS for instance, ONOO, OH, and H_2O_2 during oxidative metabolism in mitochondria [13]. On insulin alarming ROS and RNS have got negative codification, particularly a risk factor for T2DM [14].

METHODS

It is a comparative, cross-sectional study. The whole experimental work was conducted in the Biochemistry Lab, School of Biochemistry and Medical Lab Technology, Minhaj University, Lahore after the acceptance of ethical and Research committee, Minhaj University Lahore. 5.0 ml blood samples of 60 diabetic patients and 50 samples of healthy individuals were taken in clotted gel vials from Nawaz Sharif Hospital. For the estimation of Reduce Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Estimation of Nitric oxide (NO), Estimation of micronutrients (Vitamin A, Vitamin C and Vitamin E) and Electrolytes concentration by flame photometer (Na^+ and K^+) blood samples were further processed. Centrifugation of blood samples was conducted at 4000 rpm for 10 minutes and serum was separated. Blood samples were collected in EDTA tubes. Superoxide Dismutase (SOD) was determined by spectrophotometric method [15]. Determination of Thiobarbituric Acid Reactive Substances (TBARS) in Tissues was conducted for the measurement of MDA by spectrophotometric method [16]. Estimation of Catalase (CAT) was estimated by spectrophotometric method [17]. Estimation of Glutathione (GSH) was estimated by the mechanism of Moron [18]. Determination of Nitric Oxide (NO) was done by a well-recognized method of colorimetric Griess assay [19]. Estimation of Vitamin C (VIT C) or ascorbic acid was analyzed by the method of Roe and Keuther [20]. Estimation of Vitamin A (VIT A) or Tocopherol was analyzed in the plant samples by the Emmutir-Engel reaction as reported by Rosenberg *et al.*, 1992 [21]. Statistical analysis was done by using SPSS (Version 17).

RESULTS

The MDA level is progressively higher in T1DM patients 14±.06 as compared to control group 1.27±0.21 (Table 1). GSH status is notably reduced to 0.15±.05 from normal value 6.24±0.33 in contrast to healthy individuals. Comparable anti-oxidant catalase is reduced to 2.82±.04 in affected individuals as compared to normal individuals 4.19±1.09. SOD level is remarkably marked up to13.52±3.21 in patients as compared to normal group 2.15±0.23 (Table 1).

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Table 1: Anti-oxidative status profile of Type 1 diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
MDA	1.27±0.21	14.0±.06
GSH	6.24±0.33	0.15±.05
Catalase	4.19±1.09	2.82±.04
SOD	2.15±0.23	13.52±3.21

Table 2 demonstrates that vitamin A level is markedly reduced to 1.62±.26 in patients as compared to control group 7.18±0.33. Vitamin C value is also reduced in patient's 0.45±.07 in comparison to control group 6.23±1.08. Vitamin E status is also higher 4.44±0.82 in control group and considerably reduced 2.04±0.54 in patients.

Table 2: Vitamin profile of Type 1 Diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
Vitamin A	7.18±0.33	1.62±.26
Vitamin C	6.23±1.08	0.45±.07
Vitamin E	4.44±0.82	2.04±.54

Results illustrated in Table 3 depicts that the control group has reduced quantity of advanced oxidation protein products (AOPPs) 3.28±0.49 and T1DM patients have eminent quantity of advanced oxidation protein products (AOPPs) 77.29±2.41 . Nitric Oxide value is also noticeably elevated in patients 9.14±0.77 and low level of parameter in control group 2.05±0.35.

Table 3: Different biomarkers of Type 1 diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
AOPPs	3.28±0.49	77.29±2.41
Nitric Oxide	2.05±0.35	9.14±0.77

Table 4 depicts a higher quantity of sodium in T1DM patients 161.19±18.09 as compared to control group (132.23±11.26). Potassium levels are significantly raised in T1DM group (12.63±1.33) as compared to control group (6.29±0.11).

Table 4: Electrolyte profile of Type 1 Diabetes mellitus patients

Variables	Control (n=50)	Subjects (n=60)
Sodium	132.23±11.26	161.19±18.09
Potassium	6.29±0.11	12.63±1.33

DISCUSSION

Diabetes is multifarious metabolic disarray indicated by hyperglycemia developing from inadequate insulin discharge, insulin reluctance activity [22]. T1DM results in an immune-mediated deterioration of β-cells of pancreas, governing to insulin insufficiency. Insulin is required for survival. T2DM commonly develops in obese persons and is linked with high blood pressure and elevation of lipids. Nutrients ability to provoke insulin discharge from β-cell of pancreas, revert their ability progress oxidative fluctuation in islet cells [23]. Oxidation stress is also linked to insulin reluctance, furthermore, participates to poor insulin activity [24]. Hence, the medication goals to decrease insulin reluctance and to activate insulin discharge. T1DM reports 5-10% analyzed cases of diabetes and illustrates hyperglycemia as its indication. Type 1 Diabetes autoimmune disarray causes auto- reactive T cells including immune-mediated recurrence of β-cells [25]. It consequently precedes release of pro-inflammatory cytokines with reactive oxygen components. Marked demolition of pancreatic β-cells in islets of Langerhans along with deficiency of insulin discharge [26]. Free radicals are produced by glucose oxidation, non-enzymatic glycation of proteins moreover, from consequent oxidative deterioration of glycated proteins. Adversely high level of free radicals coetaneous drop of antioxidant protection system leads to degradation of cellular organelles and enzymes, enhanced lipid peroxidation, and progress of insulin reluctance [27]. Destruction in the antioxidant balance develops oxidative stress state. Here complicated association between antioxidant and oxidants for instance ROS, regulates production of oxidative stress. When production of reactive species enhances oxidative stress arises in cellular system, body's antioxidant ability and protection devastates. If free radicals are not ejected by cellular antioxidants, particularly irrupt and destroy lipids, carbohydrates, proteins and nucleic acids. There is increasing confirmation that have linked with pathological diversity of oxidative stress states, involving cancer, cardiovascular diseases, inflammatory incisive disease , deficient supply of blood to a body part and joints pain [28]. Elevated MDA level of plasma, serum and other tissues are particularly documented in diabetic patients. AOPPs that accumulate in aging patient with diabetes known as pro-inflammatory and pro-oxidative compounds may play a significant part in elevating incidence of endothelial impairment and consequent cardiovascular diseases. Various records indicate decreased GSH level in diabetes. Aberrant GSH condition included β-cell disarray moreover pathogenesis of inexhaustible aggravations of diabetes. The irregulation extensively involved during disease states. Catalase is an anti-oxidative enzyme approximately exists in all living organisms [29]. The defalcation enzyme proceeds in β-cell, cumulating in oxidative stress and approximately breakdown cell. Beta cell full in mitochondria, this organelle thought a cause of ROS. By hydrogen peroxide catalase secures pancreatic β-cells from impairment. Poor catalase capacities may induce blood disorder with hemolytic anemia which is associated either to defalcation of glucose-6-phosphate dehydrogenase or obscure conditions and also deteriorate heme proteins, induce cell death also combine with redox active metal ions, generate notably harmful hydroxyl

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radicals[30].

CONCLUSIONS

T1DM patients particularly exhibited reduced amounts and competency of antioxidant protections due to elevated consumption of specific anti-oxidant components e.g. low level of intracellular glutathione and Catalase and primarily low levels of vitamin A, vitamin E and vitamin C and exalted level of MDA, SOD and NO. Hence, prolonged exploration of correlation between ROS, T1DM and its complications in direction to interpret molecular mechanisms by which elevated oxidative stress stimulates progress of diabetes problems may be explored.

Authors Contribution

Conceptualization: HMA
Methodology: GK
Formal analysis: AAA, MA
Writing-review and editing: HMA, MA, GK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

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