Original Article

Study of Antioxidant Effect in Patients with Type 2 Diabetes

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Abstract

Background: Diabetes stands as the predominant etiological factor behind the global prevalence of chronic renal disease. Among the myriad challenges faced by diabetic individuals, oxidative stress looms prominent. The intricate antioxidant system acts as a crucial defense, shielding cells from potential harm induced by these oxidative species. **Objectives:** This study endeavors to delve into the biochemical milieu; specifically focusing on enzymatic and non-enzymatic antioxidants in subjects afflicted with type 2 diabetes mellitus. **Materials and Methods:** blood specimens were procured from a cohort of 60 participants, segregated into two cohorts: the patient group encompassing 30 individuals with diabetes, and the control group comprising 30 healthy counterparts. Measures of Random Blood Sugar, HBA1C, Catalase (CAT), Superoxide Dismutase (SOD), and Malondialdehyde (MDA) were undertaken. **Results:** The outcomes revealed a marked escalation in HBA1C, Random Blood Sugar levels, and Malondialdehyde levels within the patient group in comparison to the control cohort. However, no substantial variance was discerned in Catalase and Superoxide Dismutase levels between the diabetic and healthy groups. **Conclusions:** The findings underscore a surge in free radical production in diabetic subjects, precipitating diminished antioxidant levels of Catalase and Superoxide Dismutase. Notably, Malondialdehyde emerges as a promising indicator for assessing oxidative stress and holds potential as a discerning marker for the early detection of diabetes.

Keyword: Antioxidant, MDA, CAT, SOD, T2DM.

Introduction

Diabetes mellitus (DM) represents a prevalent endocrine disorder stemming from deficiencies in insulin action or secretion [1]. The hallmark symptom of DM manifests as hyperglycemia in the bloodstream, attributed to either inadequate pancreatic insulin secretion or diminished insulin-mediated facilitation of glucose uptake by target cells. The spectrum of DM encompasses various types, with Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) standing out as the most prevalent forms. While T1DM necessitates insulin replacement therapy as its cornerstone of management, T2DM typically requires lifestyle adjustments and adherence to a controlled diet [2]. Traditionally labeled as non-insulin dependent diabetes or adult-onset diabetes, T2DM has been characterized by insulin resistance that could progress to absolute resistance over time. However, recent insights have spotlighted decreased β-cell function as a pivotal concern in T2DM over the past decade [3].

Indeed, in the past two decades, Type 2 Diabetes Mellitus (T2DM) has emerged as a concerning and perilous health challenge affecting children [4]. The definition provided by the WHO accurately describes diabetes mellitus as a chronic metabolic disorder marked by high blood glucose levels, leading to adverse effects on various organs such as the heart, blood vessels, eyes, kidneys, and nerves over time. T2DM is specifically characterized by insufficient insulin secretion from tissue insulin resistance, pancreatic islet β-cells, and an inadequate compensatory insulin secretory response. It is noteworthy that T2DM constitutes over 90% of all diabetes cases [5]. Hyperglycemia ensues as insulin secretion falters in upholding glucose homeostasis with the progression of the disease. A hallmark feature of individuals with T2DM is obesity or an elevated percentage of body fat, predominantly concentrated in the abdominal region. The surge in global obesity rates, sedentary lifestyles, consumption of high-calorie diets, and demographic aging have been identified as primary drivers of the T2DM epidemic, having quadrupled both its incidence and prevalence. Various organs including the pancreas (β- and α-cells), small intestine, liver, adipose tissue, kidneys, skeletal muscles, and brain partake in the intricate pathogenesis of T2DM [6]. T2DM has a complicated and multifaceted pathophysiology. The formation of prooxidants as a result of glucose autoactivation, which occurs after circulation glucose levels are consistently increased, is one of the most significant factors. A disruption of prooxidants, or reactive oxygen species (ROS), and the antioxidant micro-ecosystem, which favors prooxidant overproduction in comparison to antioxidant defense, is what causes oxidative stress [7]. Reactive oxygen species (ROS) are produced through a complex interplay of various mechanisms, illustrating their biological complexity and their influence on the genetic characteristics of individuals. These mechanisms involve both enzymatic and non-enzymatic pathways, including processes such as oxidase (NOXs), oxidative phosphorylation, the activity of plasma membrane proteins like lipid metabolism in peroxisomes, nicotinamide adenine dinucleotide phosphate (NADPH), and cyclooxygenases. Perturbations in these pathways can lead to oxidative stress, thereby expediting the development of complications linked to diabetes, encompassing both microvascular and cardiovascular disorders. Free radicals, a subgroup of ROS, play a significant role in inducing oxidative stress. Cells have mechanisms in place to protect themselves from potential damage caused by oxidants, with antioxidants, present in limited concentrations, working to counteract oxidants through diverse mechanisms [8, 9]. The primary aim of this research is to investigate specific antioxidants present in the blood serum of diabetic patients as potential biochemical markers for the early detection of diabetes.

Materials and Methods

Study Population

Samples collection and practical work of this study were done from January 2024 to June 2024. The study subjects comprised 30 patients with Diabetes selected from Merjan Teaching Hospital in Babylon-Iraq.

Blood samples of 30 healthy individuals were collected to compare with case patients.

A questionnaire was taken from the subjects included in the study. It included age, sex, weight, family history, and duration of disease.

Blood Samples

Five millilitres of venous blood were drawn from the patients and the control group. The blood

was centrifuged at 5000 rpm for 10 minutes to clot it, and the serum was then separated and stored in Eppendorf tubes at -20 ºC until it was needed.

Biochemical Analysis

Antioxidants Enzymes

Superoxide dismutase (SOD)

The method described by Marklund and Marklund [10] was employed for assessing the activity of superoxide dismutase (SOD) in the study. A robust and sensitive assay for SOD activity was devised, utilizing its ability to inhibit the auto-oxidation of epinephrine at a pH of 10.2.

Catalase (CAT)

The Goth et al. technique was used to estimate catalase [11]. The procedure is based on the finding that heated dichromate in acetic acid reduces to chromic acetate in the presence of hydrogen peroxide (H2O2), with perchromic acid developing as an unstable intermediate. Colorimetry is used to measure the chromic acetate that results.

Malondialdehyde (MDA)

The Buege & Aust procedure [12] was utilized to estimate plasma MDA after thiobarbituric acid and malondialdehyde interacted. The reaction product was extracted in butanol and measured.

Statistical analysis

Statistical analysis was done using SPSS version 26 and a p-value <0.05 was set as a cutoff value of statistical significance.

Ethical Approval

The College of Pharmacy at the University of Babylon ethical committee approved this study's ethical approval, obtaining verbal consent from each patient and control. A local ethics committee reviewed and approved the subject information and consent form.

Results

The results in Table (1) summarize the demographic characteristics of the two studied groups of 30 patients with DM and 30 healthy individuals. Among all the participants in the different groups, females were more represented than males. Body weight did not significantly differ between the two groups. While the HBA1C and RBS levels in DM patients were more significant ($P \leq 0.05$) than in controls.

Table 1: Comparison between according to demographic data.

Demographic Data	Patients	Control	P value
Age	50.03±12.95	33.63±10.51	P ≤ 0.05 *
Males	(%30) 13	(%40)11	P <0.05
sFemale	(%70) 17	(%60) 19	
BMI	82.89±16.62	82.9±16.43	P <0.05
RBS mm/l	9.49±3.88	5.49±0.92	P ≤ 0.05 *
HBA1C	9.27±2.82	5.67±0.98	P ≤ 0.05 *

Based on their family history of the condition, the distribution of diabetes prevalence among research participants is shown in Figure 1. The data clearly shows that people with a family history of diabetes had a higher prevalence of the condition than people without a history.

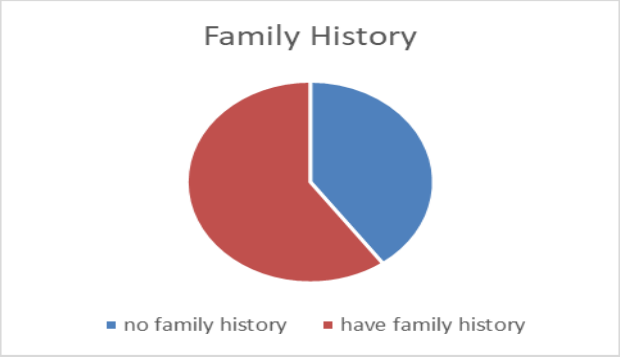

Figure 1: The distribution of DM in patients according to family history

Figure. 2 shows the percentages of complications in individuals who had diabetes in the study. The eye complications prevalence for individuals with DM was (75%), followed by heart disease (45%) and foot complications (35%).

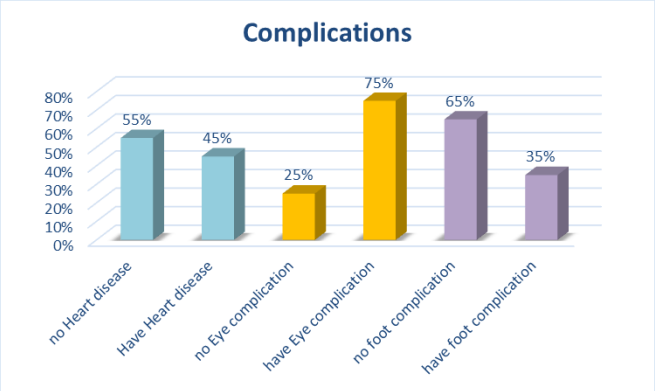

Figure 2: The distribution of DM complications among patients

The findings delineated in Table 2 present the serum levels of distinct measures in the two investigated cohorts. Noteworthy is that the superoxide dismutase (SOD) activities exhibited no substantial variance ($P > 0.05$) between diabetic participants (45.91±7.51 U/ml) and the control group (47.19±12.73 U/ml). Similarly, there were insignificant distinctions in serum catalase (CAT) activity between individuals with diabetes (31.71±10.87 KU/l) and the control cohort (33.81±8.49 KU/l) ($P > 0.05$). Conversely, the malondialdehyde (MDA) levels were markedly higher ($p < 0.05$) in diabetic individuals (30.84±6.10 µmol/l) compared to the control participants (20.09±2.03 µmol/l).

Table 2: Level of Superoxide dismutase, Catalase, Malondialdehyde among sera from participant groups

Variables	Patient	Control	P value
SOD	45.91±7.51	47.19±12.73	P< 0.05
CAT	31.71±10.87	33.81±8.49	P< 0.05
MDA	30.84±6.10	20.09±2.03	P≤ 0.05

Discussion

Multiple metabolic disturbances linked to type II diabetes mellitus may result in secondary pathophysiological alterations in various organ systems. Consequently, there may be a significant increase in morbidity and death due to micro- and macrovascular problems [13]. Twenty patients with type 2 diabetes and twenty healthy participants served as the control group in our study. Numerous research have looked into the role of oxidative stress in metabolic diseases. Evidence exists that connects oxidative stress to diabetes mellitus (DM), including pre- and post-clinical stages of the illness. Oxidative stress is a key player in the pathophysiology of type 2 diabetes and the development of its complications, according to several studies [14]. Even though diabetes is linked to higher ROS generation, there is conflicting evidence about diabetes's antioxidant defense. In contrast to the control group, our investigation showed that patients with DM2 had lower levels of CAT and SOD activity. The correlation between elevated blood glucose levels and the increased generation of reactive species through oxidative stress leads to a decrease in the levels of enzyme antioxidants like Superoxide Dismutase (SOD) and Catalase (CAT). The decreased activity of non-enzymatic antioxidants SOD and CAT as observed by Gilani et al. [15] underscores the impact of oxidative stress in the context of elevated blood glucose levels. Kumawat et al. [16] demonstrated that patients with DM2 have reduced activity of SOD levels in serum. However, Bandeira, et al. [17] found that diabetics have higher total SOD activity than non-diabetics do. The reduction in serum Superoxide Dismutase (SOD) activity levels could stem from heightened excretion in the inflamed kidney during nephropathy and excessive consumption during the autoxidation

process. Additionally, low SOD activity can be attributed to about 50% of SOD becoming glycated within the erythrocytes of diabetic patients [18]. In diabetic individuals, the levels of malondialdehyde (MDA) in serum were found to be significantly higher compared to control individuals, as shown in Table 2. This observation aligns with the findings of Bikkad et al. [19], who suggested that the elevated MDA levels may be attributed to increased free radical formation and prolonged exposure to hyperglycemia, resulting in heightened oxidative stress. Similar conclusions were reached by Al-Rawi, Kumawat et al., Mahadevan and Velavan, and Padalkar et al. [20, 16, 21, 22]. Diabetic problems may be related to peroxidative damage, as indicated by elevated MDA levels in diabetics. A decrease in the protective mechanisms of both enzymatic and non-enzymatic antioxidants is also indicated by an increase in lipid peroxidation [23, 24].

Conclusion

The association of age and gender with diabetes risk, coupled with the imbalanced oxidative status observed in T2DM patients, highlights the importance of addressing oxidative stress in the management and understanding of diabetes, particularly in older individuals and females. Further research into targeted antioxidant therapies and lifestyle interventions may be beneficial in mitigating oxidative damage and potentially reducing the risk of T2DM complications.

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