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Research Article

Comparison of oxidative stress parameters in patients with prediabetes and type 2 diabetes mellitus: A preliminary study

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Abstract

Objectives: Type 2 diabetes mellitus (T2DM), also known as adult-onset diabetes, is caused by insulin resistance or insufficient insulin production. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) indicate blood glucose levels that are higher than normal but not enough to be diagnosed as diabetes. An oral glucose tolerance test (OGTT) and the fasting blood glucose (FBG) level, respectively, are used to determine these 2 prediabetic groups. Oxidative stress is the common pathogenic factor leading to insulin resistance, β-cell dysfunction, IGT, and ultimately to T2DM. This study is an evaluation of the concentration of antioxidant and oxidant parameters of total oxidative status (TOS) and total antioxidant status (TAS) as well as paraoxonase-1 (PON1), and ischemia-modified albumin (IMA) in diabetic and prediabetic patients, and a normoglycemic control group.

Methods: Serum TAS, TOS, PON1, IMA were measured in a total of 117 prediabetic, diabetic, and normoglycemic individuals.

Results: The TAS was lower in the IGT patient group (2.41±0.2 mmol Trolox Eqv/L) than in the IFG group (2.52±0.18 mmol Trolox Eqv/L) and the normoglycemic control group (2.52±0.18 mmol Trolox Eqv/L) (p=0.03 and p=0.006, respectively). The serum IMA level was found to be significantly different between the T2DM patients (0.70±0.11 ABSU) and the IGT patients (0.77±0.14 ABSU) (p=0.045). There was also a significiant difference in the IMA level between the IGT patients (0.77±0.14 ABSU) and the IFG patients (0.68±0.13 ABSU) (p=0.037).

Conclusion: The high IMA and low TAS levels in the IGT patient group may have been a result of the high-glucose solution administered in the OGTT causing an increase in reactive oxygen species synthesis. TAS synthesis may not be sufficient to compensate for this rapid increase in blood glucose.

Keywords: Diabetes, oral glucose tolerance test, oxidative stress, prediabetes

Type 2 diabetes mellitus (T2DM), also known as adult-onset diabetes, is caused by insulin resistance or insufficient insulin production. It is one of the most common chronic illnesses worldwide, affecting more than 400 million people and causing around 1 million deaths per year [1]. Current knowledge of the nature, history, and pathogenesis of diabetes has revealed that there is a prolonged period before disease progression, which is called the prediabetic phase. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are considered indicators of the prediabetic phase of T2DM [2, 3]. According to American Diabetes Association (ADA) criteria, IFG and IGT patients have a blood glucose level greater than the upper limit of the normal range but not enough to be classified as diabetic. IGT patients have 2-hour 75-g oral glucose tolerance test (OGTT) values between 140 to 199 mg/dL (7.8 to 11.0 mmol/L), while IFG patients have fasting plasma glucose (FBG) values of 100 to 125 mg/dL [4]. Elevation in postprandial glucose levels is the primary abnormality to be detected due to a decline in first-phase insulin secretion. In time, beta-cell function continues to deteriorate and this leads to an increase in fasting glucose levels. A continuous decrease in the secretion of insulin results in T2DM [5]. Oxidative stress caused by

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the production of reactive oxygen species (ROS) has been proposed as the major cause underlying the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance, and T2DM. Both macrovascular and microvascular complications associated with T2DM have been attributed to the accumulation of ROS [3]. This is of critical importance, as therapies targeting a reduction in oxidative stress would be useful in T2DM patients as well as those in the prediabetic phase [6, 7]. Oxidative stress is essentially caused by the imbalance between antioxidants and pro-oxidants. Generally, this imbalance is due to an excess production of ROS. There may also be a problem with the elimination of ROS by antioxidants [8].

When the production of free radicals cannot be neutralized by antioxidant mechanisms, oxidative stress occurs and the harmful effects of these free radicals are seen. Paraoxonase-1 (PON1) is an antioxidant enzyme-linked to plasma high-density lipoprotein (HDL) and it has been shown to protect low-density lipoprotein (LDL) from oxidation by free radicals and to reduce oxidative stress [9]. PON1 activity and oxidative status have been seen to be altered in diabetes and cardiovascular diseases, which are related on the basis of endothelial dysfunction [10, 11]. Ischemia-modified albumin (IMA) is a systemic marker of oxidative stress [12]. When tissue ischemia occurs, circulating albumin is structurally altered and the cobalt-binding property of albumin is decreased. This non-cobalt-binding form of albumin is known as ischemia-modified albumin (IMA) [13]. The present study examined the total oxidative stress (TOS) and total antioxidant capacity (TAS) of patients with T2DM, IGT, and IFG, as well as PON1 and IMA levels and compared the oxidative stress parameters in these groups.

Materials and Methods

Design and study group

This study was conducted in April 2018 in the Ankara Numune Training and Research Hospital Clinical Biochemistry Department with a total of 117 total participants. The patients were divided into 3 groups of 30 according to their 75-g OGTT results: IFG, IGT, and T2DM. A control group of 27 healthy individuals was also included.

Study parameters

Venous blood samples were collected in serum separator gel tubes from all of the study participants. All of the samples were centrifuged at 1200xg for 15 minutes and the glucose level was detected using a 120-minute OGTT. The aliquoted sera were stored at -80°C until the time of analysis of other parameters. Serum TOS levels were determined spectrophotometrically using the method described by Erel et al. [14], and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H2O2 Eqv/L). Serum TAS levels were also determined using the method described by Erel et al. [15], and the results were expressed as mili-molar Trolox equivalent per liter (mmol Trolox Eqv/L). The oxidative stress index (OSI) is the ratio of TAS to TOS. Paraoxonase activity was measured by photometric assay with a paraoxonase kit (Rel Assay Diagnostics, Gaziantep, Turkey) and expressed as units per liter (U/L) [9, 10]. All of the photometric measurements were performed using an AU 680 analyzer (Beckmann Coulter, Inc., Brea, CA, USA). IMA was measured with a colorimetric assay method developed by Bar-Or et al. [16] based on the measurement of unbound cobalt after incubation with patient serum. IMA was measured spectrophotometrically (Metertech Inc., Nangang, Taipei, Taiwan). The results were reported in absorbance units (ABSU). All of the tests were conducted on the same day. The IMA values adjusted for albumin were calculated with the following formula: (Individual serum albumin concentration/median albumin concentration of the population) ¥ IMA value [17].

The study protocol was approved by the Ethics Committee of Ankara Numune Training and Research Hospital (E-17-1288). Informed consent was obtained from all of the participants and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

The data were analyzed with PASW Statistics for Windows, Version 18.0 (SPSS Inc., Chicago, IL, USA). Numerical variables were assessed for normality of distribution with the Kolmogorov-Smirnov test and expressed as mean±SD or median (minimum-maximum). Analysis of variance was used to perform multiple comparisons of categorical variables, and pair comparisons were expressed with post-hoc values. A p value of <0.05 was considered statistically significant.

Results

The age and gender distribution of study and control groups are provided in Table 1. There was no significant difference in the age and gender of the study groups (p>0.05). Serum TAS, TOS, OSI, PON1, and IMA levels of the patient and control groups are listed in Table 2.

Only the TAS level was statistically different in group comparisons (p=0.038). Paired comparisons revealed significant differences in the TAS values between the IGT patients (2.41±0.2 mmol Trolox Eqv/L), the IFG patients (2.52±0.18 mmol Trolox Eqv/L), and the normoglycemic control group (2.52±0.18 mmol Trolox Eqv/L) (p=0.03 and p=0.006, respectively). There was also a significant difference in the serum IMA level between the T2DM patients (0.70±0.11 ABSU) and the IGT patients (0.77±0.14 ABSU) (p=0.045) and between IGT patients (0.77±0.14 ABSU) and IFG patients (0.68±0.13 ABSU) (p=0.037). No statistically significant difference was found in any other comparisons (Table 2).

Table 1. Age and gender distribution of the patient and control groups

Gender	T2DM (Group 1)	IGT (Group 2)	IFG (Group 3)	Control (Group 4)	Total
Age (years)	41.4±10.7	44.1±14.1	40.2±10.9	39.2±10.7	41.3±11.7
Female, n (%)	18 (60)	17 (57)	19 (63)	18 (66)	72 (57)
Male, n (%)	12 (40)	13 (43)	11 (27)	9 (33)	45 (43)

IFG: Impaired fasting glucose, IGT: Impaired glucose tolerance, T2DM: Type 2 diabetes mellitus

Discussion

Diabetes is a major chronic health problem affecting millions of people each year all over the world [2]. T2DM is known as maturity-onset diabetes associated with insulin resistance. Diagnosis of diabetes is made by several methods, including measurement of glycosylated hemoglobin (HbA1c), Fasting Plasma Glucose (FPG), random plasma glucose and, Oral Glucose Tolerance Test (OGTT) [18]. Diabetes has various complications, mostly associated with micro and macro-vascular damage. Oxidative stress, with strong evidence is thought to be a common pathogenic factor leading to insulin resistance, β-cell dysfunction, IGT, and ultimately to T2DM with it's single unifying mechanism for production of excess superoxide [6].

Table 2. Comparison of study parameters by group

	T2DM (Group 1)	IGT (Group 2)	IFG (Group 3)	Control (Group 4)	p	Comparison groups	Post hoc p
TAS (mmol Trolox Eqv/L)	2.48±0.23	2.41±0.2	2.52±0.18	2.55±0.18	0.038*	1 vs 2	0.144
						1 vs 3	0.465
						1 vs 4	0.181
						2 vs 3	0.03*
						2 vs 4	0.006*
						3 vs 4	0.529
TOS (μmol H2O2 Eqv/L)	5.26±0.69	5.25±0.93	5.29±0.98	5.31±0.88	0.794	1 vs 2	0.958
						1 vs 3	0.923
						1 vs 4	0.391
						2 vs 3	0.885
						2 vs 4	0.383
						3 vs 4	0.484
PON1 (U/L)	113.93±71.03	124.87±74.91	134.37±89.96	110.15±71.3	0.634	1 vs 2	0.596
						1 vs 3	0.322
						1 vs 4	0.858
						2 vs 3	0.636
						2 vs 4	0.476
						3 vs 4	0.241
IMA (ABSU)	0.70±0.11	0.77±0.14	0.68±0.13	0.75±0.23	0.191	1 vs 2	0.045*
						1 vs 3	0.691
						1 vs 4	0.287
						2 vs 3	0.037*
						2 vs 4	0.606
						3 vs 4	0.155
OSI	0.048±0.008	0.046±0.007	0.049±0.009	0.047±0.008	0.700	1 vs 2	0.482
						1 vs 3	0.611
						1 vs 4	0.891
						2 vs 3	0.238
						2 vs 4	0.588
						3 vs 4	0.535

* Statistical significance ($p<0.05$). IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; IMA: Ischemia-modified albumin; OSI: Oxidative stres index; PON1: Paraoxanase-1; T2DM: Type 2 diabetes mellitus; TAS: Total antioxidant status; TOS: Total oxidative status.

Serum oxidative stress markers (i.e. TOS, advanced oxidation protein products (AOPP), IMA, etc.) measurement have been good diagnostic parameters of oxidative stress in different diseases [19]. According to oxidative stress parameters, antioxidant parameters (i.e. TAS, ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC)) provide oxidative balance against oxidative stress [15, 20].

Korkmaz et al have studied oxidant and antioxidant parameters in diabetic and prediabetic patients and showed that oxidative and antioxidant parameters are increased in both prediabetic and diabetic groups. They evaluated IMA as an oxidative stress marker and IMA found that IMA levels were higher in the IGT patient group compared to the normoglycemic controls [21]. In the present study, IMA is higher in the IGT group than IFG and T2DM patient group. As the reason for high IMA in the IGT patient group; it is thought that the high dose glucose solution administered to patients during OGTT causes short term oxidative stress and an elevation in oxidative stress markers. In the same study, FRAP was measured as an antioxidant parameter and FRAP was found to be higher in the IGT patient group compared to the normoglycemic group and IFG group. Our results differ from the results of that study. In our study, TAS measured as an antioxidant parameter was lower in the IGT patient group compared to the IFG and the normoglycemic control group. Low TAS level in the IGT patient group may be an expected result. Because of the high dose glucose solution given during OGTT application may have caused an increase in ROS synthesis by increasing blood glucose levels suddenly. TAS synthesis may not be sufficient to compensate against this rapid increase.

In several other studies, TAS was found to be lower in T2DM patient group compared to healthy controls [22, 23]. In our study, there was no statistical difference between the TAS levels of control group and patients with T2DM group. When the diabetic patient group is evaluated in terms of TAS, some studies are supported by our results and some are in the opposite direction. The level of TAS may vary depending on the age, gender, medications used, etc. of the patients [23, 24]. Therefore, there are publications in the literature that contain various TAS results.

The limitation of the present study may be listed as lack of information about body mass index (BMI), insulin, homeostatic model assessment for insulin resistance (HOMA-IR) and the lipid profile of patient groups. But this can be considered as a preliminary study for planning more detailed studies including more detailed physical and demographical characteristics of patients and more detailed laboratory analyses to provide more information about the oxidative status of the current patient groups.

Conflict of Interest: The authors do not have any conflict of interest in the manuscript.

Ethics Committee Approval: This study was approved by the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (approval number and date: E-17-1288-08.03.2017) and the 1964 Helsinki Declaration and its later amendments.

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