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The association between oxidative stress markers and thiol/disulfide homeostasis in Turkish women who are not diabetic, at risk for diabetes, or have type 2 diabetes

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ABSTRACT

Context and Objectives: Diabetes is one of the disorders characterized by a breakdown of thiol/disulfide homeostasis (TDH).

The purpose of this research was to look at those who have type 2 diabetes, those who are prediabetic, and those who have just been diagnosed with blood sugar levels to see how TDH relates to oxidative stress markers.

Methods: Our research included 26 women who did not have diabetes, 24 women who were prediabetic, and 19 women who had type 2 diabetes. Tests for type 2 diabetes mellitus were requested by all of them at the Diabetes Polyclinic at Zonguldak Bulent Ecevit University's Health Practice and Research Center, Endocrinology and Metabolism Diseases. The patient's medical records were combed through for demographic and laboratory information. In order to study oxidative stress parameters and dynamic total DH, ELISA kits were used.

Compared to non-diabetics, type 2 diabetics had considerably higher total oxidant status (TOS), total thiol, and disulphide levels (24.24±14.93 vs 14.14±12.19, 646.47±75.51 versus 470.88±180.85, and 179.32±51.24 versus 91.85±40.29, respectively). There was a significant positive connection (P=0.000) between TOS and levels of native thiol, total thiol, and disulphide in type 2 diabetics. A strong positive association was seen in prediabetics between total antioxidant capacity and total thiol levels (P<0.05), as well as between arylesterase and both native and total thiol levels (P<0.05). However, no such association was detected for total antioxidant capacity.

In conclusion, type 2 diabetics may have symptoms associated with elevated blood glucose levels due to an increase in oxidative stress and a decline in TDH.

Topics covered include sulfhydryl compounds, type 2 diabetes mellitus, prediabetes, and oxidative stress.

INTRODUCTION

Hyperglycemia, caused by inadequate insulin oscillations, is a hallmark of type 2 diabetes mellitus (T2DM). Its frequency is growing daily. The World Health Organization estimated 422 million people as having diabetes in 2020, with 1.6 million people losing their lives to the disease annually (Lovic et al., 2020). It is well-established that metabolic syndromes may develop in the absence of a definitive diagnosis of type 2 diabetes due to inadequate insulin production by pancreatic beta cells. When blood sugar levels are elevated but no other diabetic symptoms have shown, this condition is known as prediabetes. According to Khetan and Rajagopalan (2018) and Garber et al. (2019), prediabetes is therefore seen as a precursor to type 2 diabetes.

The production of more free radicals, which are very reactive substances, might harm cells. To counteract free radical damage, the body employs an antioxidant defense mechanism. When there is a disruption in the balance between declining levels of antioxidants and free radicals (Sies, 1997). Thiol groups (-SH) are able to create disulphide (RSSR) bonds when exposed to reactive oxygen species. This process can be undone (Erel & Neselioglu, 2014). The cysteine residue in glutathione (GSH) causes it to be oxidized to glutathione disulfide (GSSG) under OS

conditions. Then, GSSG is reduced to GSH by an enzyme called NADPH-dependent glutathione reductase. According to Wu, Fang, Yang, Lupton, and Turner (2004), the GSH/GSSG ratio is a good indicator of the cellular redox status. There are a number of diseases where OS occurs and the dynamic thiol/disulfide homeostasis (TDH) deteriorates. These include hypertension, non-small cell lung cancer, familial Mediterranean fever (FMF), inflammatory bowel diseases, occupational diseases, preeclampsia, gestational diabetes mellitus, and diabetes mellitus (Erel & Erdogan, 2020). High blood sugar levels, inflammation, insulin resistance, hyperglycemia, and dyslipidaemia are all consequences of diabetes, and OS is an important risk factor for these problems (Hamamcioglu, 2017).

Several investigations have focused on the detection of OS and dynamic TDH in individuals with diabetes. There was a favorable association between the disulphide and blood glucose and HbA1c levels in a research conducted by Ates et al. (2015) with newly identified prediabetics and healthy volunteers. A year later, the same group published yet another research involving type 1 diabetics; this time, they posited that hyperglycemia and chronic inflammation are linked to an increase in thiol oxidation in type 1 diabetics (Ates et al., 2016). Gulpamuk et al. (2018) discovered that elevated levels of ischemia-modified albumin (IMA) and total diphtherine (TDH) were associated with the development of diabetic retinopathy in type 2 diabetes individuals. Researchers Ergin et al. (2020) divided type 2 diabetes patients into three groups: those who had complications, those who did not, and those who had just been diagnosed. They found that as the condition progressed, the levels of sulfur dioxide gradually rose. There was a decline in TDH and a shift towards disulfide in children with type 1 DM. They suggested that damage to pancreatic β-cells is the cause of this change.

Nevertheless, not a single one of these research included newly identified prediabetics in their analyses with newly diagnosed type 2 diabetes patients and healthy controls.

Hydrolase paraoxonase 1 (PON1) exhibits arylesterase (ARES) and paraoxonase (PON1) activity due to its glycoprotein structure. The liver is known to produce it and release it into the circulation with HDL. It aids in preventing the oxidation of lipoproteins (Unal et al., 2012).

As mentioned earlier, PON1 loses its protective ability against the oxidation of lipoproteins when it is detached from HDL, which occurs during hyperglycaemia in diabetes mellitus. According to Rosenblat, Sapir, and Aviram (2008), this was shown to be a risk factor for the development of coronary artery disorders caused by diabetes.

The purpose of our research was to evaluate and contrast the antioxidant and total oxidant profiles in Turkish women who were prediabetic or had type 2 diabetes (T2D), as well as to learn more about a new oxidative stress marker called TDH. Our secondary objective was to compare and contrast the three groups' paraoxonase 1 (PON1) and arylesterase (ARES) activities and to analyze the relationships between each parameter.

MATERIAL AND METHODS

Participants were individuals who met the inclusion criteria and signed an informed consent form. They were tested for type 2 diabetes mellitus at the Diabetes Polyclinic at Zonguldak Bulent Ecevit University, Health Practice and Research Centre, Endocrinology and Metabolism Diseases, between September 2018 and March 2019. Our control group consisted of people who do not have diabetes. Our prediabetic group included people whose fasting blood glucose levels were 100-125 mg/dl, blood glucose levels were 140-199 mg/dl in the second hour of the oral glucose tolerance test, and HbA1c levels were 5.9% to 6.4%. Our patient group consisted of people who were diagnosed with type 2 diabetes for the first time, with fasting blood glucose levels above 130 mg/dl and HbA1c levels above 6.5%. Everyone with ND, prediabetes, or T2D was at least 18 years old, not pregnant or nursing, a nonsmoker, and not an alcoholic. They also didn't have any additional diseases that might cause organ damage. Everyone involved was free of medicines.

All participants had to sign an informed consent form before the research could begin, and the Zonguldak Bulent Ecevit University Ethics Committee gave its stamp of approval in accordance with the Declaration of Helsinki (approval number 2018-49-14/02). Nineteen were determined to have type 2 diabetes, twenty-four to be at risk for

developing the disease, and twenty-six to have no diabetes at all. On their first visit to the clinic, we took a 10-milliliter blood sample.

Laboratory analysis and collecting of biochemical data

A fast the night before allowed for the collection of peripheral blood samples.

The sera were separated by centrifugation at 1500 g for 10 minutes at 4°C after 10 milliliters of venous blood samples were taken.

Before being used, the samples were kept at a temperature of -80°C. The biochemistry laboratory at Zonguldak Bulent Ecevit University Hospital took standard measurements of fasting blood glucose, lipid profiles (HDL, LDL, cholesterol, and triglycerides), hemoglobin A1c, and creatinine. Demographic data, including patients' height, weight, age, etc., was retrieved from their medical records.

We used enzyme-linked immunosorbent assay (ELISA) kits from Restay, Turkey, to measure the serum's total antioxidant capacity (TAC). An essential component of the process is the decolorization of a certain color that is generated by ABTS (2, 2′- Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)). Spectrophotometry was used to measure the reaction at 660 nm. Per liter, in millimoles of Trolox equivalent, the results were presented (Erel, 2004).

Erel (2005) produced total oxidant status (TOS) ELISA kits (Relassay, Turkiye) that were also used to test serum TOS levels. In most cases, the oxidants in the sample are responsible for producing ferric ions, which are crucial to the principle. There was an improvement in the oxidation process when glycerol molecules were included in the reaction media. At 530 nm, a combination of colored ferric ions and xylene was detected.

Data was expressed in "μmol hydrogen peroxide equivalent per litre" because hydrogen peroxide was used to calibrate the test.

The oxidative stress index (OSI) was determined by dividing the total oxygen consumption (TOS) by the total available carbon (TAC) and was represented in Arbitrary Units (AU) (Harma, Harma & Erel, 2003; Kosecik, Erel, Sevinc & Selek, 2005; Yumru et al, 2009).

Erel and Neselioglu (2014) created the thiol/disulfide homeostasis (TDH) test. Free thiol groups were produced by reducing disulphide bonds in the presence of sodium borohydride, as per the assay principle. The number of disulphides was calculated by subtracting the amount of native thiols from the total amount of thiols and then dividing the result in half.

The provided percentages include the native thiol to total thiol ratio, the disulphide to total thiol ratio, and the other ratios.

ARES and PON1 functions

ELISA kits (Relassay, Turkiye) were used to detect PON1 and ARES activity in the sera samples. The PON1 activity was supported by paraoxon, whereas the arylesterase activity was supported by phenyl acetate. To measure PON1 activity in international units per 1 liter of sera (U/L), the increase of absorbance at 412 nm at 37°C was used. The activity of ARES was measured at 270 nm and 37°C and quantified in kilo units per 1 liter of sera (KU/L) (Eckerson, Wyte, & La Du, 1983; Aldemir et al, 2015; Kilinc et al., 2016).

Data analysis using statistical software

This study used SPSS 22.0 for Windows, developed by SPSS Inc. of Chicago, Illinois, USA, to analyze the data. To learn about the data's distribution, we used the Kolmogorov-Smirnov test. For numerical variables that did not follow a normal distribution, we used mean values; for those that did, we used mean plus standard deviation. The

comparison between the NDs, prediabetics, and T2Ds was done using a student t test. When comparing the clinical characteristics of non-diabetics, prediabetics, and type 2 diabetics, the Mann Whitney U test was used since continuous variables with non-normal distributions were included.Pearson Spearman correlation analysis was used to find the correlations between numerical parameters. A significance level of P <0.05 was used.

RESULTS

Our research comprised of 26 ND, 24 prediabetic and 19 T2D women.

The participants in our research were classified as NDs, prediabetics, or T2Ds, and their demographic information and laboratory results are shown in Table 1. Estimates showed that T2Ds had greater levels of body mass index, fasting blood glucose, and hemoglobin A1c compared to the other groups. There was a significant difference in triglyceride levels between NDs, prediabetics, and T2Ds (P <0.05). Compared to the T2Ds, the NDs had greater HDL levels (P<0.05). The results did not show a statistically significant difference between the groups, despite the fact that T2Ds had higher total cholesterol and LDL levels. Furthermore, type 2 diabetics were older than non-diabetics (nds) and prediabetics (42.83 + 11.18 vs. 33.00 + 10.10).

Neither the prediabetics nor the T2Ds showed a statistically significant difference in their TAC levels when the OS parameters were examined. In contrast to non-diabetics, prediabetics had significantly lower TOS levels (P<0.05), whereas type 2 diabetics had significantly higher TOS levels (P<0.005).

Pre-diabetes had TOS levels that were significantly lower (P<0.001) compared to type 2 diabetics. Prediabetics showed a non-significant reduction in OSI values as compared to normal controls. In comparison to non-diabetics (NDs) and prediabetics (P<0.05 and P<0.001, respectively), T2Ds had substantially higher OSI values (Table 2). In prediabetics, both total and native thiol levels were lower, while in T2Ds, they were higher than in NDs. A stepping up of

Table 1. Comparison of the demographic data and laboratory findings among the groups.

Variables	Non diabetics (n=26)	Prediabetics (n=24)	Type 2 diabetics (n=19)	p value
n (%)	26 (37.7)	24 (34.8)	19 (27.5)	
Age (years)	33.00±10.10	42.83±11.18	55.68±10.91	<0.005†, <0.001‡, =0.001§
BMI (kg/m2)	25.92±4.63	29.83±3.69	31.63±2.34	<0.005†, <0.001‡
FBG (mg/dL)	93.62±4.68	108.04±6.85	141.95±65.12	<0.001†, <0.001‡, <0.005§
HbA1c (%)	5.24±0.28	5.59±0.29	7.51±1.84	<0.001†, <0.001‡, <0.001§
Creatinine (mg/dL)	0.70±0.092	0.76±0.15	0.76±0.13	
Triglyceride (mg/dL)	125.81±60.92	149.67±69.49	200.89±67.70	<0.001‡, <0.05§
Total cholesterol (mg/dL)	193.19±46.55	204.96±38.89	214.26±46.40	
HDL (mg/dL)	54.23±13.22	49.25±10.36	45.58±6.95	<0.05‡
LDL (mg/dL)	117.27±36.68	125.79±32.43	127.95±36.41	

BMI: Body Mass Index, FBG: Fasting Blood Glucose, HbA1c: haemoglobinA1c, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein. The variables are expressed as mean±standard deviation. † shows a statistically significant difference between non-diabetics and prediabetics. ‡ shows a statistically significant difference between non-diabetics and type 2 diabetics. § shows a statistically significant difference between prediabetics and type 2 diabetics.

Table 2. Comparison of oxidative stress parameters, thiol/disulphide homeostasis parameters, and antioxidant enzymes (PON1 and ARES) among the groups.

Variables	Non diabetics (n=26)	Prediabetics (n=24)	Type 2 diabetics (n=19)	p value
Total thiol (mmol/L)	470.88±180.85	413.95±56.58	646.47±75.51	<0.001‡,<0.001§
Native thiol (mmol/L)	379.04±157.46	241.71±30.23	467.16±56.86	<0.005†, <0.001§
Disulphide (mmol/L)	91.85±40.29	172.25±43.26	179.32±51.24	<0.001†, <0.001‡,
Disulphide/ Total Thiol (%)	20.69±8.50	41.25±6.15	27.51±6.39	<0.001†, <0.005‡, <0.001§
Disulphide/ Native Thiol (%)	27.65±15.30	72.01±18.12	38.94±11.97	<0.001†, <0.005‡, <0.001§
Native Thiol/ Total Thiol (%)	79.31±8.50	58.75±6.15	72.49±6.39	<0.001†, <0.005‡, <0.001§
TAC (mmolTrolox equivalent/L)	2.41±0.41	2.38±0.49	2.60±0.57	
TOS (µmol H2O2 equivalent/L)	14.14±12.19	6.92±1.65	24.24±14.93	<0.05†, <0.005‡<0.001§
OSI	0.65±0.67	0.31±0.11	1.04±0.76	<0.05‡, <0.001§
PON1	303.88±212.93	368.75±186.74	170.21±123.85	<0.05‡, <0.001§
ARES	264.92±140.81	228.54±47.44	175.42±92.31	<0.05‡,<0.05§

TAC: Total Antioxidant Capacity, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, PON: Paraoxonase, ARES: Arylesterase.
The variables are expressed as mean±standard deviation. † shows a statistically significant difference between non-diabetics and prediabetics. ‡ shows a statistically significant difference between non-diabetics and type 2 diabetics.§ shows a statistically significant difference between prediabetics and type 2 diabetics.

When comparing T2Ds to NDs, the total thiol levels were significantly higher (P<0.001). When compared to non-diabetics, prediabetics had significantly lower levels of native thiol (P<0.005). In prediabetics, there was a substantial drop in both total and native thiol levels compared to T2Ds (P<0.001). Compared to the NDs, prediabetics and T2Ds had considerably higher disulphide levels (P<0.001). In Table 2.

T2Ds showed a substantial reduction (P <0.05) in antioxidant enzymes PON1 and ARES compared to the control group. Prediabetics exhibited a small but statistically insignificant rise in PON1 levels. In contrast, NDs had higher ARES levels than prediabetics. In comparison to prediabetics, T2Ds had substantially lower levels of PON1 and ARES (P<0.001 and P<0.05, respectively). In Table 2.

Analysis of correlations

A strong positive connection was found in type 2 diabetics between the levels of native thiol (r=0.351, P <0.05), total thiol (r=0.543, P =0.000), and disulfide (r=0.624, P =0.000) with regard to HbA1c levels. Similarly, there was a very significant positive connection (r=0.814, P =0.000), r=0.828, P =0.000, and r=0.546, P =0.000) between TOS and levels of native thiol, total thiol, and disulfide, respectively. There was also a positive relationship (r=0.340, P<0.05) between total thiol and disulphide levels and triglyceride levels (r=0.310, P<0.05).

The antioxidant enzymes PON1 and ARES did not show any association with the TDH parameters (Table 3).

The disulphide ratio to total thiol and native thiol, as well as the ratio of native thiol to total thiol, were discovered to correlate with triglyceride and total cholesterol levels in prediabetics. There was a strong positive relationship (r=0.457, P<0.05) discovered between total thiol levels and TAC. Both native thiol and total thiol levels were shown to have a substantial positive connection with ARES (r=0.706, P =0.000 and r=0.525, P <0.05, respectively) according to Table 4.

DISCUSSION

Type 2 diabetes is defined by persistently high blood sugar levels and is caused by an inadequate response to insulin. The degradation of glucose, lipid, and protein metabolisms leads to micro and macro problems in this chronic illness.

An OS defining feature is an intracellular imbalance between free radical and antioxidant levels. As a result, cellular and molecular processes become dysfunctional. A number of studies have looked at the effects of OS on diabetics, mostly type 2 diabetics (Sozer et al., 2014; Eljaoudi et al., 2017; Nair & Nair, 2017).

Organic substances containing a sulfhydryl group are called thiols or mercaptans. One of their functions is to protect mitochondria from free radicals. In organic solvents, reversible disulfide bonds are formed between the low molecular weight thiol groups and the thiol groups of sulfur-containing amino acids like cysteine and methionine. This has been studied in various diseases, including Graves' disease (Agan et al., 2019), Welders' lung disease (Karatas et al., 2019), gestational diabetes (Aktun, Aykanat, Erel, Neselioglu, & Olmuscelik, 2018), neonatal sepsis (Aydogan et al., 2021), urolithiasis (Sonmez et al., 2019), etc. It is possible that the pathophysiology of diabetes mellitus is influenced by the anomalies in dynamic TDH. Thus, we set out to identify NDs, prediabetics, and T2Ds using dynamic TDH.

There are two antioxidant enzymes that rely on calcium: PON1 and ARES.

One gene codes for both of them. There are a number of disorders that are associated with decreased PON1 and ARES activity. Plasma oncostatin-1 (PON-1) levels in metastatic renal cell carcinoma patients in the lower stage were found to have considerably lower stages. On the other hand, Aldemir et al. (2015) discovered that in individuals with renal cell carcinoma, ARES levels were correlated with nuclear grade. Kilinc et al. (2016) found that ARES levels were lower in patients with localized scleroderma. According to Emre et al. (2016), T2Ds compared to NDs in pityriasis rosea patients had substantially lower levels of TAC and ARES activity. When comparing prediabetics and T2Ds, our research likewise discovered that ARES levels were lower than NDs ($p<0.05$, Table 2). Even though it wasn't statistically significant, prediabetics had lower TAC levels than NDs and T2Ds. There could be a connection between TAC and ARES values if this holds. The results of Zarei et al. (2016), who also discovered that ARES activity in rat heart and liver homogenates was considerably lower in the diabetic control group compared to the normal control group ($P<0.01$), are consistent with our own findings. Vitamin B6 supplementation increased PON1 and ARES activities, which were shown to be lowered in streptozocin-induced diabetic rats, according to another research (Tas, Sarandol, & Dirican, 2014).

According to Table 2, there was a substantial drop in PON1 levels of T2Ds when compared to NDs ($P<0.05$). Similarly, diabetics with periodontitis had lower PON-1 levels; however, unlike our high PON1 levels in prediabetics, they did not observe any altered PON1 status in prediabetics (Noack et al., 2013).

To the best of our knowledge, this is the first research to examine TDH in conjunction with TAC, TOS, OSI, and enzymatic parameters (PON1 and ARES) in recently diagnosed prediabetics and type 2 diabetics, in addition to NCs. Few studies have examined OS in those who are at risk of developing diabetes. Dziegielewska-Gesiak et al. (2014) found OS biomarkers in prediabetics older than 65 years old, and Bandeira et al. (2012) examined an increase in lipid peroxidation and superoxide dismutase activity in type 2 diabetics in a smaller sample of prediabetics. In order to learn whether the OS conditions also impact TDH and if those with slightly raised blood glucose levels without diabetes symptoms, this research included prediabetics. Table 2 shows that the shift towards oxidized thiols is much larger in prediabetics compared to T2Ds. Ates et al. (2016) also discovered that individuals with type 1 diabetes had a change in dynamic TDH towards the disulfide form. Moreover, our research demonstrated a positive and statistically significant connection ($P <0.005$) between ARES levels and both total and native thiol levels in prediabetics, which is a first. Thiol groups and enzymatic antioxidants (PON1 and ARES) did not correlate in type 2 diabetics.

There was also a substantial positive connection (P<0.001, Table 3) between TOS and levels of native thiol, total thiol, and disulphide in T2Ds. Table 4 shows that this connection was negative and not statistically significant among prediabetics.

Our research was primarily limited by its cross-sectional nature.

Patients were tested for type 2 diabetes by having blood samples collected when they checked in at the hospital. We do not yet know when type 2 diabetes first manifests in our newly diagnosed patients. Also, we only surveyed a tiny subset of the population since this is a pilot research. The inability to compare OS with TDH and assess its additional enzymatic and non-enzymatic properties was another drawback. Among the participants screened for type 2 diabetes mellitus at the diabetic polyclinic between September 2018 and March 2019, 69 were female and 5 were male. The research aimed to establish a gender-neutral group by excluding males due to the small sample size.

Finally, this research showed that newly diagnosed type 2 diabetes is associated with decreased total dihydrogenase (TDH) and an increase in disulfide formation, in contrast to non-diabetic controls (NDs). Additionally, there was a notable rise in oxidized thiols in T2Ds, which is associated with an increase in TOS levels. We conclude that elevated TOS and, by extension, TDH, levels are associated with the development of diabetic symptoms brought on by persistently high blood sugar. This research confirmed that TDH stands on its own as a potential cause of type 2 diabetes. The ethiopathogenesis of type 2 diabetes is significantly impacted by OS. We hope that our study will shed light on the existing literature and inspire further research that will aid in the diagnosis and treatment of type 2 diabetes.

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