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Original Article

Relationship of thiol/disulphide homeostasis with oxidative stress parameters in non-diabetic, prediabetic and type 2 diabetic Turkish women

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ABSTRACT

Background and Aims: Deterioration of thiol/disulphide homeostasis (TDH) is found in several diseases, including diabetes. This study aimed to examine the association between TDH and oxidative stress parameters in nondiabetics, prediabetics and newly diagnosed type 2 diabetics.
Methods: A total of 26 non-diabetic, 24 prediabetic and 19 type 2 diabetic women were involved in our study. They all applied to Zonguldak Bulent Ecevit University, Health Practice and Research Center, Endocrinology and Metabolism Diseases, Diabetes Polyclinic to be tested for type 2 diabetes mellitus. The demographic and laboratory data were collected from the patient files. Oxidative stress parameters and dynamic TDH were investigated using ELISA kits.
Results: Total oxidant status (TOS), total thiol and disulphide levels were significantly higher in type 2 diabetics than the non-diabetics (24.24±14.93 versus 14.14±12.19, 646.47±75.51 versus 470.88±180.85, and 179.32±51.24 versus 91.85±40.29, respectively). In type 2 diabetics, a positive correlation between TOS and native thiol, total thiol and disulphide levels was found (P=0.000). In prediabetics, a significant positive correlation was found between total antioxidant capacity and total thiol levels (P<0.05), and also between arylesterase and native and total thiol levels (P<0.05).
Conclusion: The elevation of oxidative stress and the deterioration of TDH might cause the formation of symptoms related to high blood glucose levels in type 2 diabetics.

Keywords: Sulfhydryl compounds; Diabetes mellitus, type 2; Prediabetic state; Oxidative stress

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterised by hyperglycaemia due to insufficient insulin oscillations. Its prevalence is increasing each day. In 2020, the WHO reported that about 422 million people have diabetes worldwide and each year 1.6 million deaths result from diabetes (Lovic et al., 2020). It has been known that insufficient insulin secretion from pancreatic beta cells causes metabolic syndromes before an individual is diagnosed as having type 2 diabetes. Prediabetes is a condition of having high blood sugar levels, but the whole range of symptoms of diabetes is not yet present. Therefore, prediabetes is considered as an underlying risk factor for T2DM (Khetan & Rajagopalan, 2018; Garber et al., 2019).

Cells can be damaged by the enhancement of free radicals, which are highly reactive chemicals. The antioxidant defence system protects the body against the hazardous effects of free radicals. Oxidative stress (OS) occurs when the equilibrium between

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the amount of free radicals and the amount of antioxidants deteriorates (Sies, 1997). Reactive oxygen species induce thiol groups (-SH) to form disulphide (RSSR) bonds. This reaction is reversible (Erel & Neselioglu, 2014). Under the conditions of OS, due to its cysteine residue, glutathione (GSH) becomes oxidized to glutathione disulphide (GSSG). NADPH-dependent glutathione reductase then reduces GSSG to GSH. The cellular redox state can be determined by measuring the GSH/GSSG ratio (Wu, Fang, Yang, Lupton & Turner, 2004). In many diseases, such as diabetes mellitus, hypertension, non-small cell lung cancer, familial Mediterranean fever (FMF), inflammatory bowel diseases, occupational diseases, gestational diabetes mellitus and preeclampsia, OS occurs and the dynamic thiol/disulphide homeostasis (TDH) deteriorates (Erel & Erdogan, 2020). OS is a crucial factor in the development of complications related to diabetes such as hyperglycaemia, insulin resistance, inflammation and dyslipidaemia due to high blood glucose levels (Hamamcioglu, 2017).

There are some studies that focus on detecting OS and dynamic TDH in diabetic patients. In a study performed by Ates et al (2015) with newly diagnosed prediabetics and healthy volunteers, a positive correlation between the disulphide and blood glucose and HbA1c levels was reported. After a year, the group published another study with type 1 diabetics and suggested that an elevation of thiol oxidation in type 1 diabetics is associated with hyperglycaemia and chronic inflammation present in these patients (Ates et al., 2016). In T2DM patients, the progression of diabetic retinopathy was found to be related with an increase in both TDH and ischemia-modified albumin (IMA) levels (Gulpamuk et al, 2018). In a study performed by Ergin et al (2020), three groups of T2DM patients were included (T2DM patients with complications, T2DM patients without complications and newly diagnosed T2DM patients). They reported a gradual increase in disulphide levels due to the severity of the disease. In children with type 1 DM, TDH was found to be deteriorated and shifted towards the disulphide direction. They proposed that this shift is due to damage in pancreatic β-cells. However, none of these studies examined newly diagnosed prediabetics together with newly diagnosed T2DM patients and healthy volunteers.

Paraoxonase 1 (PON1) is a hydrolase with a glycoprotein structure which demonstrates both paraoxonase and arylesterase (ARES) activities. It is known to be synthesised in the liver and then secreted into the bloodstream in association with HDL. It helps to protect lipoproteins from oxidation (Unal et al., 2012). It was stated previously that during diabetes mellitus, under the conditions of hyperglycaemia, PON1 is separated from HDL and therefore, it loses its protective property against the oxidation of lipoproteins. This was reported as a risk factor for the development of diabetes-induced coronary artery diseases (Rosenblat, Sapir, & Aviram, 2008).

Our study aimed to understand the balance between total oxidants and antioxidants in prediabetic and type 2 diabetic (T2D) Turkish women together with a novel OS marker, TDH, and to compare the differences between the groups. We also aimed to understand the activities of paraoxonase 1 (PON1) and arylesterase (ARES) in all three groups and to discuss the correlations between all parameters.

MATERIAL AND METHODS

Individuals who applied to Zonguldak Bulent Ecevit University, Health Practice and Research Centre, Endocrinology and Metabolism Diseases, Diabetes Polyclinic to be tested for T2DM between September 2018 and March 2019, who met the inclusion criteria and who signed an informed consent form were involved in the study. Non-diabetics (NDs) formed our control group; the individuals who were found to be prediabetic formed our prediabetic group (fasting blood glucose level was between 100-125 mg/dl, blood glucose level was between 140-199 mg/dl in the second hour of oral glucose tolerance test and the levels of HbA1c were between 5.9-6.4%) and the ones who were diagnosed as T2DM for the first time (fasting blood glucose level was above 130 mg/dl and HbA1c level was above 6.5%) formed our patient group. NDs, prediabetics and T2Ds had no other disease related to organ damage, were all above 18 years of age, were not pregnant or breastfeeding, were non-smokers and were non-alcoholic. None of the individuals was under any medication.

The study was approved by Zonguldak Bulent Ecevit University Ethics Committee adhering to the Declaration of Helsinki (approval number 2018-49-14/02) and informed consent forms were signed by all the individuals prior to the study. Out of 69 individuals, 19 were diagnosed as T2D, 24 were diagnosed as prediabetic and 26 were found to be ND. Blood samples (10 ml) were collected on their first visit to the hospital.

Biochemical data collection and laboratory analysis

Peripheral blood samples were drawn after an overnight fast. A total of 10 ml venous blood samples were collected and were centrifuged (1500 g at 4°C for 10 min) to separate sera. The samples were stored at -80°C until usage. Fasting blood glucose levels, lipid profiles (HDL, LDL, cholesterol, and triglycerides), HbA1c and creatinine levels were all measured in a standard fashion at the Zonguldak Bulent Ecevit University Hospital Biochemistry laboratory, and demographic data such as height, weight, age, etc. were collected from the patients' files.

Total antioxidant capacity (TAC)

TAC levels of the sera were determined using ELISA kits (Relassay, Turkiye). The principle of the method depends on the decolorising of a characteristic colour produced by ABTS (2, 2′- Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)). The reaction was measured spectrophotometrically at 660 nm. The data were expressed as mmol Trolox equivalent per litre (Erel, 2004).

Total oxidant status (TOS)

ELISA kits developed by Erel (2005) were also used to detect serum TOS levels (Relassay, Turkiye). Generally, the principle depends on the formation of ferric ions by the oxidants present in the sample. Glycerol molecules present in the reaction medium enhanced the oxidation reaction. A coloured complex formed by ferric ions and xylene was measured at 530 nm..

Hydrogen peroxide was used to calibrate the assay and, therefore, "µmol hydrogen peroxide equivalent per litre" was used to express data.

Oxidative stress index (OSI)

Oxidative stress index (OSI) was calculated by dividing TOS to TAC values and was expressed in Arbitrary Units (AU) (Harma, Harma & Erel, 2003; Kosecik, Erel, Sevinc & Selek, 2005; Yumru et al, 2009).

Thiol/disulphide homeostasis (TDH)

TDH assay was developed by Erel and Neselioglu (2014). According to the principle of the assay, free thiol groups were formed by the reduction of disulphide bonds in the presence of sodium borohydride. To determine the amount of disulphides, the amount of native thiols were subtracted from the amount of total thiols and the result was divided into two. Percentages of the ratio of disulphide to total thiol and native thiol, as well as the ratios of native thiol to total thiol, were reported.

PON1 and ARES activities

PON1 and ARES activities in the sera samples were detected using ELISA kits (Relassay, Turkiye). Paraoxon was used as a substrate for PON1 activity and phenyl acetate for arylesterase activity. The enhancement of absorbance at 412 nm and 37°C were used to determine PON1 activity in terms of international units per 1 litre of sera (U/L). ARES activity was expressed in terms of kilo units per 1 litre of sera (KU/L) and measured at 270 nm and 37°C (Eckerson, Wyte, & La Du, 1983; Aldemir et al, 2015; Kilinc et al., 2016).

Statistical analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to understand the distribution of our data. Numerical variables having normal distribution were described as mean + standard deviation and those not having normal distribution were described with mean values. Student t test was used to compare the NDs with prediabetics and T2Ds. For continuous variables with non-normal distribution, the Mann Whitney U test was used to compare the clinical features of the NDs, prediabetics and T2Ds.The relationships between numeric parameters were determined through Pearson Spearman correlation analysis. P <0.05 was considered significant.

RESULTS

Our study consisted of 26 ND, 24 prediabetic and 19 T2D women. The demographic data and laboratory findings of NDs, prediabetics and T2Ds involved in our study are included in Table 1. Body mass index, fasting blood glucose and HbA1c levels were higher in T2Ds than the other groups, as estimated. Triglyceride levels were lower in NDs than prediabetics and T2Ds (P <0.05). HDL levels were higher in the NDs than the T2Ds (P<0.05). Even though total cholesterol and LDL levels were higher in T2Ds than the other groups, this difference was not found to be significant. In addition, T2Ds were older than NDs and prediabetics (55.68 + 10.91 versus 33.00 + 10.10 and 55.68 + 10.91 versus 42.83 + 11.18, respectively).

When the OS parameters were evaluated, no significant difference in the TAC values was found between NDs and the prediabetics nor the T2Ds. However, there was a significant reduction (P<0.05) in the TOS levels of prediabetics and a significant increase (P<0.005) in the TOS levels of T2Ds compared to NDs. TOS values of prediabetics were significantly lower (P<0.001) than the TOS values of T2Ds. When the OSI values were calculated, a non-significant decrease in prediabetics compared to NDs was found. OSI values of T2Ds increased significantly compared to the NDs and prediabetics (P<0.05 and P<0.001 respectively) (Table 2).

Both total thiol and native thiol levels decreased in prediabetics and increased in T2Ds compared to NDs. An elevation 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.

total thiol levels in T2Ds was significant (P<0.001) compared to NDs. A reduction of native thiol levels in prediabetics was significant (P<0.005) compared to NDs. The level of total thiol and native thiol decreased significantly in prediabetics compared to T2Ds (P<0.001). Disulphide levels increased significantly in prediabetics and T2Ds when compared to the NDs (P<0.001) (Table 2).

Antioxidant enzymes, PON1 and ARES, both decreased significantly (P <0.05) in T2Ds when compared to the control group. PON1 levels showed a slight, non-significant increase in prediabetics. On the other hand, ARES levels of prediabetics decreased when compared to NDs. The levels of both PON1 and ARES reduced significantly in T2Ds compared to prediabetics (P<0.001 and P <0.05, respectively). (Table 2).

Correlation analysis

In T2Ds, a positive significant correlation was determined between the HbA1c levels and native thiol, total thiol and disulphide levels (r=0.351, P <0.05; r=0.543, P =0.000; and r=0.624, P =0.000 respectively). Similar to this, a significant strong positive correlation was also noticed between TOS and native thiol, total thiol and disulphide levels (r=0.814, P =0.000; r=0.828, P =0.000 and r=0.546, P =0.000, respectively). A positive correlation between triglyceride levels and total thiol and disulphide was also found (r=0.340, P<0.05 and r=0.310, P<0.05, respectively). No correlation was found between the antioxidant enzymes (PON1, and ARES) and TDH parameters (Table 3).

In prediabetics, both triglyceride and total cholesterol levels were found to correlate with disulphide ratio to both native thiol and total thiol, as well as the ratio of native thiol to total thiol. A significant positive correlation was found between TAC and total thiol levels (r=0.457, P<0.05). A strong positive correlation between ARES and native thiol as well as total thiol levels was also determined (r=0.706, P =0.000 and r=0.525, P <0.05, respectively) (Table 4).

DISCUSSION

Insufficient insulin effects and/or insulin release causes T2DM and is characterized by hyperglycaemia. It is a chronic disease where micro and macro complications occur due to the deterioration of carbohydrate, lipid and protein metabolisms.

OS has been defined by an imbalance between the levels of free radicals and antioxidants within the body. This causes molecular and cellular dysfunction. The effects of OS have been investigated by several researchers in diabetics, mostly in T2Ds (Sozer et al., 2014; Eljaoudi et al., 2017; Nair & Nair, 2017).

Thiols, also known as mercaptans, are organic compounds with a sulfhydryl group. They serve as the component of a mitochondrial antioxidant defence mechanism. Under OS conditions, the thiol groups of the aminoacids with sulphur groups such as cysteine and methionine form reversible disulphide bonds with the low molecular weight thiol groups. This is known as dynamic TDH and has been investigated in several diseases such as Graves' disease (Agan et al., 2019), childhood iron deficiency anemia (Topal, 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. The abnormalities in dynamic TDH may have a role in the pathogenesis of diabetes mellitus. Therefore, we aimed to detect dynamic TDH in NDs, prediabetics and T2Ds.

PON1 and ARES are calcium-dependent antioxidant enzymes. They are both encoded by the same gene. The activities of both PON1 and ARES are shown to be reduced in several diseases. In patients with renal cell carcinoma, levels of PON-1 in advanced

Table 3. Correlation analysis of type 2 diabetics (Findings related to thiol/disulphide homeostasis).

	Native Thiol		Total Thiol		Disulphide		Disulphide/ Native Thiol		Disulphide/ Total Thiol		Native Thiol/ Total Thiol	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
BMI	0.256	0.089	0.375	0.011	0.367	0.013	0.190	0.211	0.190	0.211	-0.190	0.211
FBG	0.040	0.792	0.207	0.173	**0.376**	**0.011***	**0.394**	**0.007****	**0.394**	**0.007****	**0.394**	**0.007****
HbA1c	**0.351**	**0.018***	**0.543**	**0.000****	**0.624**	**0.000****	**0.322**	**0.031***	**0.322**	**0.031***	**-0.322**	**0.031***
Creatinine	-0.067	0.660	0.015	0.920	0.046	0.765	0.062	0.684	0.062	0.684	-0.062	0.684
Triglyceride	0.275	0.068	**0.340**	**0.022***	**0.310**	**0.038***	0.167	0.272	0.167	0.272	-0.167	0.272
Total cholesterol	0.110	0.471	0.160	0.293	0.199	0.189	0.160	0.292	0.160	0.292	-0.160	0.292
HDL	-0.174	0.253	-0.129	0.399	-0.144	0.344	-0.040	0.793	-0.040	0.793	0.040	0.793
LDL	0.032	0.836	0.069	0.651	0.141	0.356	0.129	0.399	0.129	0.399	-0.129	0.399
TAC	-0.265	0.078	-0.197	0.194	-0.016	0.917	0.056	0.716	0.056	0.716	-0.056	0.716
TOS	**0.814**	**0.000****	**0.828**	**0.000****	**0.546**	**0.000****	-0.039	0.799	-0.039	0.799	0.039	0.799
ARES	-0.181	0.459	-0.321	0.180	-0.169	0.488	-0.123	0.616	-0.123	0.616	-0.123	0.616
PON1	-0.133	0.589	-0.196	0.422	-0.035	0.886	0.004	0.986	0.004	0.986	0.004	0.986

BMI: Body Mass Index, FBG: Fasting Blood Glucose, HbA1c: Haemoglobin A1c, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein, TAC: Total Antioxidant Capacity, TOS: Total Oxidant Status, ARES: Arylesterase, PON: Paraoxonase.* means p<0.05, ** means p<0.01.

Table 4. Correlation analysis of prediabetics (Findings related to thiol/disulphide homeostasis).

	Native Thiol		Total Thiol		Disulphide		Disulphide/Native Thiol		Disulphide/Total Thiol		Native Thiol/Total Thiol	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
BMI	-0.386	0.062	0.095	0.659	**0.461**	**0.024***	**0.548**	**0.006****	**0.548**	**0.006****	**0.548**	**0.006****
FBG	-0.236	0.268	-0.003	0.987	0.168	0.434	0.234	0.271	0.234	0.271	0.234	0.271
HbA1c	-0.201	0.346	0.204	0.340	0.267	0.207	0.281	0.184	0.281	0.184	0.281	0.184
Creatinine	0.354	0.090	**0.423**	**0.040***	0.323	0.123	0.210	0.325	0.210	0.325	0.210	0.325
Triglyceride	0.110	0.608	**0.471**	**0.020***	**0.566**	**0.004****	**0.452**	**0.027***	**0.452**	**0.027***	**0.452**	**0.027***
Total cholesterol	-0.172	0.423	0.157	0.464	0.371	0.074	**0.408**	**0.048***	**0.408**	**0.048***	**0.408**	**0.048***
HDL	-0.243	0.252	-0.315	0.134	-0.182	0.393	-0.047	0.826	-0.047	0.826	-0.047	0.826
LDL	-0.189	0.377	0.094	0.663	0.265	0.211	0.310	0.140	0.310	0.140	0.310	0.140
TAC	0.370	0.075	**0.457**	**0.025***	0.403	0.051	0.147	0.494	0.147	0.494	0.147	0.494
TOS	-0.025	0.908	-0.172	0.421	-0.303	0.149	-0.294	0.163	-0.294	0.163	-0.294	0.163
ARES	**0.706**	**0.000****	**0.525**	**0.008****	0.048	0.823	0.066	0.759	-0.279	0.187	0.279	0.187
PON1	-0.362	0.082	-0.261	0.218	-0.110	0.609	-0.279	0.187	0.066	0.759	-0.066	0.759

BMI: Body Mass Index, FBG: Fasting Blood Glucose, HbA1c: Haemoglobin A1c, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein, TAC: Total Antioxidant Capacity, TOS: Total Oxidant Status, ARES: Arylesterase, PON: Paraoxonase.* means p<0.05, ** means p<0.

stage were found to be significantly lower than the lower stage patients. In contrast, ARES levels were found to correlate with nuclear grade in renal cell carcinoma patients (Aldemir et al., 2015). In localized scleroderma patients, decreased ARES levels were reported (Kilinc et al., 2016). In *pityriasis rosea* patients, TAC levels and ARES activities were found to be significantly lower in T2Ds than the NDs (Emre et al., 2016). In our study, ARES levels were also found to be lower than NDs when compared to prediabetics and T2Ds ($p<0.05$, Table 2). In prediabetics, TAC levels were also lower than both the NDs and T2Ds, but this was not significant. This may suggest a relationship between TAC and ARES values. Similar to our findings, ARES activity in the heart and liver homogenates was found to be significantly lower in the diabetic control group than the normal control group in rats ($P<0.01$) (Zarei et al., 2016). In another study, it was also stated that PON1 and ARES activities were decreased in streptozocin-induced diabetic rats and that vitamin B6 supplementation improved PON1 and ARES activities (Tas, Sarandol, & Dirican, 2014). In our study, PON1 levels of T2Ds significantly decreased compared to the NDs ($P<0.05$, Table 2). PON-1 levels were also found to be decreased in diabetics with periodontitis, but in contrast to our high levels of PON1 in prediabetics, they did not find any impaired PON1 status in prediabetics (Noack et al., 2013).

To our knowledge, this study firstly investigates TDH together with TAC, TOS, OSI and enzymatic parameters (PON1 and ARES) in NDs as well as in newly diagnosed prediabetics and T2Ds. There are not many studies investigating OS in prediabetics. In one study, OS biomarkers were detected in elderly (> 65 years of age) prediabetics (Dziegielewska-Gesiak et al., 2014) and in another study, only nine prediabetics were involved and an elevation in lipid peroxidation and superoxide dismutase activity in T2Ds were evaluated (Bandeira et al., 2012). Prediabetics were involved in this study to understand whether the OS conditions also occur in individuals with slightly elevated blood glucose levels without diabetic complications and whether TDH was also affected. A shift towards oxidised thiols is found to be a lot higher in prediabetics than T2Ds (Table 2). In a study performed by Ates et al. (2016), they also found dynamic TDH shifted towards disulphide form in type 1 diabetic patients. Also, for the first time in our study, a positive and significant ($P<0.005$) correlation was found between ARES levels and both native thiol and total thiol levels in prediabetics. In T2Ds no correlation was found between enzymatic antioxidants (PON1 and ARES) and thiol groups.

A strong positive correlation between TOS and native thiol, total thiol as well as disulphide levels in T2Ds was also found ($P<0.001$, Table 3). However, in prediabetics this correlation was negative and insignificant (Table 4).

The main limitation of our study was its cross-sectional design. The individuals' blood was taken at the time they came to the hospital to be checked for T2DM. For our newly diagnosed T2Ds, the onset of the disease is not known. In addition, our study is designed as a pilot study with a small sample size. Another limitation was not being able to evaluate the other enzymatic and non-enzymatic parameters of OS and, therefore, not being able to make comparisons with TDH. During the study period (between September 2018 and March 2019), mostly women (69 women and only 5 men) applied to the diabetes polyclinic to be tested for T2DM. Due to the low number, men were excluded from the study in order to provide a homogenous group in terms of gender.

In conclusion, the present study demonstrated that TDH is weakened in newly diagnosed T2Ds when compared to NDs and the balance shifted towards disulphide formation. In addition, related to an increase in TOS levels, oxidized thiols were also found to be increased significantly in T2Ds. Therefore, we believe that the occurrence of diabetic symptoms due to high blood sugar levels is related to the increase in TOS levels and, consequently, to TDH. This study revealed that TDH is an independent risk factor for T2Ds. OS has major effects in type 2 diabetes ethiopathogenesis. We believe that our study will enlighten the current literature as well as extended further studies that will help with the prognosis and cure of T2Ds.

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