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Total Antioxidant Capacity, Catalase and Superoxide Dismutase on Rats Before and After Diabetes

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Abstract: This study is conducted in order to investigate the correlation between Superoxide Dismutase (SOD), Total Antioxidant Capacity (TAC) and Catalase (CAT). In this context, TAC, SOD, CAT, cholesterol, triglyceride, HDL and LDL were evaluated in 10 weeks old 20 healthy rats and 20 rats with experimentally-induced diabetes. Average SOD, CAT, TAC, HDL were found to be significantly lower in diabetes group than control group ($p<0.05$). But cholesterol, triglyceride and LDL were found to be significantly lower in control group than diabetes group ($p<0.05$). A correlation between SOD, CAT activity and TAC has been established both in control and diabetes group. In the result of the study, lower SOD, CAT and TAC levels were observed in diabetes group when compared to control group. According to the data obtained in this study, it was concluded that SOD, CAT and TAC are capable of serving as a parameter to monitor diabetes of patients with type 1 DM.

Key words: Superoxide dismutase, catalase, antioxidants, diabetes mellitus, HDL, LDL

INTRODUCTION

Free radicals, are structures that easily react with biomolecules and responsible for the pathogenesis of several disease including diabetes mellitus, cancer, myocardial infarct, immune diseases.

Oxygen derivatives, which constitute a large portion of FRs, give rise to reversible or irreversible damages to nucleic acids, proteins, amino acids, lipids, carbohydrates and connective tissue macromolecules (Cross *et al*., 1987). Defensive mechanisms that minimize radical production and defuse produced radicals have evolved in the organism. These are antioxidant systems (Belce and Kokoglu, 1994).

Antioxidants are present in two distinct environments, namely intracellular and extracellular. Immediate response to oxidant damage is granted by intracellular enzymatic system which comprises of Copper-Zinc-Superoxide Dismutase (CuZnSOD), Glutathione Peroxidase (GPX), Catalase (CAT) and Glutathione Reductases (GR) (Halliwell and Gutteridge, 1990; Halliwell, 1984). A direct correlation between diabetes mellitus complications and oxidative stress is shown before by many researchers (Belce and Kokoglu, 1994; Hagglof *et al*., 1983; Dierckx *et al*., 2003).

Most of the information on the role of the oxidation in diabetes occurrence is obtained in the studies using alloxan and Streptozotocin (STZ) as experimentally inducer of diabetes. Both of these chemicals impair selectively Langerhans islets by producing oxidant substance. At the beginning of the diabetes, mellitus inflammation of pancreas islet cells is frequently observed and free radicals released from phagocytes play important role in this insulitis inflammation (Wollf, 1993; Godin *et al*., 1988; Marklund and Hagglof, 1984; Hagglof *et al*., 1983). STZ-diabetes is characterized as an experimental model for insulin dependent type 1 diabetes and provides an example for endogenous chronic stress (Scribner *et al*., 1991, 1993).

Known as a notable metabolic disorder and characterized by hyperglycemia, in diabetes cases, increase in free radical formation is observed. It was proposed that free radical production increases and radical binding systems reduce in diabetes. These progresses raise the attention towards free radicals in pathogenesis of diabetes complications (Ganong, 1995).

In this study, we aimed to investigate correlation between free radicals and antioxidants in healthy and diabetic rats. For this purpose, we assessed TAC, SOD, CAT, cholesterol, triglyceride, HDL and LDL level in healthy and diabetes-induced rats.

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MATERIALS AND METHODS

In order to constitute experimental material, 10 weeks old 40 male Wistar albino rat are obtained from FUTDAM (FU Tip Fakultesi Deneysel Arastirma Merkezi-FU Medical School Research Center).

Ten weeks old rats that are used in the study are divided into 2 groups as control (20) and diabetes group (20).

First group: It was control group and live-weights of rats were measured. Each rat was intraperitoneally (i.p.) administered single dose of 1 mL phosphate-citrate buffer (pH: 4.5).

Second group: The 20 rats were included in this group. Their live weights were measured and starvation blood glucose levels were determined by Contour TS glucometer. Afterwards, 60 mg kg^{-1} streptozotocin was dissolved in phosphate-citrate buffer (pH: 4.5) and single dose intraperitoneally administered to each rat. Starving blood glucose levels were determined again 72 h after injection. Those rats having blood glucose levels above 200 mg dL^{-1} are accepted as diabetic.

After 4 weeks and starvation for 12 h, abdominal cavities of rats are opened under ether anesthesia and blood samples are obtained from vena cava caudalises according to the analyses to be conducted. For SOD and CAT analyses blood samples were collected in vessels with EDTA. For total antioxidant capacity and lipid parameters like total cholestrol, triglyceride, HDL, LDL blood samples were collected into the vessels without anticoagulant.

Catalase determination: Enzyme activities were determined by measuring either decreasing substrate amount or forming product amount. Aebi (1984) method was used to determine erythrocyte catalase activity. Absorbance of test tubes at 240 nm were measured by spectrophotomety (Shimadzu OPI-2) and recorded.

Superoxide dismutase determination: Superoxide dismutase activity was determined using a modification from the method of Sun *et al.* (1988). In this method, SOD activity is based on the reducing of Nitro Blue Tetrazolium (NBT) by superoxide produced by xanthine/xanthine oxidase system. Produced superoxide radicals reduce NBT and produces colored formazon. This complex gives maximum absorbance at 560 nm (Shimadzu OPI-2). In the absence of the enzyme, this reaction takes place and a blue-purple color is observed. In the presence of SOD in the environment, NBT reducing does not occur, blue-purple color is not observed and depending on enzyme amount and activity, a light color is produced.

Total antioxidant capacity determination: Measurements were conducted using a kit produced by Erel (2004). Antioxidants in the sample reduce dark blue-green color of ABTS until it becomes colorless. This change depending on total antioxidant level of the sample was measured at 660 nm absorbance (Shimadzu OPI-2). Calibration was done using a standard solution, which is a vitamin E analog and internationally name Trolox Equivalent.

Cholesterol, Triglyceride, HDL, LDL determination

Triglyceride determination: Triglyceride determination was performed on OLYMPUS AU-640 auto analyzer using the kit produced by Olympus. According to this method, triglycerides are enzymatically hydrolyzed by lipase enzyme. With the catalytic effect of peroxidase enzyme kinonimin (red), the indicator is produced from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol. Concentration of this red colored substance is determined at 520 nm wavelength (Jacobs and Van Denmark, 1980; Koditschek and Umbreit, 1969; Trinder, 1969).

Cholesterol determination: Cholesterol determination was performed on Olympus AU-640 auto analyzer using the kit produced by Olympus. According to this method, cholesterol is determined after enzymatic hydrolysis and oxidation reactions. Indicator substance kinonimin (red) is produced from 4-aminoantipyrin and hydrogen peroxide in the presence of phenol and peroxidase (Allain *et al*., 1974).

HDL-cholesterol determination: HDL-cholesterol determinations were performed on OLYMPUS AU-640 auto analyzer using the kit produced by Olympus. According to this method, anti human lipoprotein antibody in R1 (reagent in the test), binds to apolipoproteins except HDL (LDL, VLDL, chylomicron). Formed antibody-antigen complexes, blocks enzymatic reactions when added to R^2 (reagent in the test). HDL cholesterol amount is determined in the presence of an enzyme-chromogen system (Ehret *et al*., 1999).

LDL-cholesterol determination: LDL-cholesterol determinations were performed on OLYMPUS AU-640 auto analyzer using the kit produced by Olympus. According to this method, a protective agent in R1, protect LDL from enzymatic reaction. All the lipoproteins

except LDL (HDL, VLDL, CM) are degraded by reactions of Cholesterol Esterase (CHE) and Cholesterol Oxidase (CHO). Hydrogen peroxide produced in the result of this reaction is broken up by catalase. When R^2 is added, preventive reagent dissociates from sodium azide inactivated LDL and catalase. Then LDL amount is determined using CHO/PAP system (Mikl, 1999).

Data were evaluated in the SPSS 16 statistics program. Parametric test assumptions in comparison analysis were evaluated by levene test. In the binary comparisons in which parametric assumption were satisfied independent samples t-test was used. In the cases, when parametric test assumptions are not satisfied Mann Whitney U-test was used.

Primarily, parametric test assumptions were intended to be determined according to levene test results and accordingly, the results were interpreted by Mann Whitney U-test, for the analyses when there were significant differences and by another parametric test, independent samples t-test in other analyses.

RESULTS AND DISCUSSION

In this study for control group average SOD value was found to be 3.92±0.61 (U mL^{-1}), average TAC value was found to be 2.36±0.47 (mmol Trolox equiv L^{-1}), average CAT value was found to be 9.52±1.88 (k gHb^{-1}). For diabetes group average SOD value was found to be 1.48±0.66 (U mL^{-1}), average TAC value was found to be 1.34±0.13 (mmol Trolox equiv L^{-1}), average CAT value was found to be 7.20±1.04 (k g Hb^{-1}). When this vales are compared, average SOD, CAT, TAC levels were found to be significantly lower in diabetes group when compared to control group ($p<0.05$). Additionally, cholesterol, triglyceride, HDL, LDL levels were also studied. For control group average cholesterol level was found to be 42.50±8.55 (mg dL^{-1}), average triglyceride level was found to be 60.90±10.11 (mg dL^{-1}) and average LDL level was found to be 7.30±2.00 (mg dL^{-1}). For diabetes group, average cholesterol level was found to be 69.80±8.92 (mg dL^{-1}), average triglyceride level was found to be 91.90±10.11 (mg dL^{-1}) and average LDL level was found to be 11.70±4.97 (mg dL^{-1}). Average HDL level was 42.20±7.97 (mg dL^{-1}) in control group, whereas, it was 23.60±4.60 (mg dL^{-1}) in diabetic group. When these results were compared, HDL was found to be significantly low in diabetes group when compared to control group ($p<0.05$), but cholesterol, triglyceride and LDL found to be significantly low in control group when compared to diabetic group ($p<0.05$) (Table 1-4).

In this study, for diabetic group average SOD, CAT and TAC enzyme activities were found to be significantly low when compared to control group ($p<0.05$) (Fig. 1-3). In this study, SOD activity in diabetic patients has been shown to increase, decrease or not change (Maritim *et al*., 2003). Saxena *et al*. (1993) found low CuZnSOD activity in diabetic rats. Nath *et al*. (1987) stated that SOD levels of diabetic group and control group were not different. It is known that during insulin insufficiency in Type 1 diabetes patients, hydrogen peroxide production increases due to increasing oxidation (Wohaieb and Godin, 1987). Because of the induction of hydrogen peroxide tissue SOD activity, in this study, higher SOD activity was detected in early type 1 diabetics compared to the control group (Subrahmanyam *et al*., 1993). Guzel *et al*. (2001) asserted that reduction of SOD activity observed in late diabetics

Table 1: The results of Mann Whitney U-test for catalase (k gHb^{-1}) activity of control and diabetes groups

Groups	N	$\bar{X}$	SD	df	Levene	Sig.	MVU	Asymp (sig. 2-t)	t	Sig. 2-t
Control	20	9.52	1.88	38	5.34*	0.03	61.00	0.000*	-	-
Diabetes	20	7.20	1.04	-	-	-	-	-	-	-

*p<0.05

Table 2: The results of Mann Whitney U-test for TAC (mmol Trolox equiv L^{-1}) activity of control and diabetes groups

Groups	N	$\bar{X}$	SD	df	Levene	Sig.	MVU	Asymp (sig. 2-t)	t	Sig. 2-t
Control	16	2.36	0.47	30	7.91*	0.009	16.00	0.000*	-	-
Diabetes	16	1.34	0.13	-	-	-	-	-	-	-

*p<0.05

Table 3: The results of independent sampels t-test for cholesterol (mg dL^{-1}) activity of control and diabetes groups

Groups	N	$\bar{X}$	SD	df	Levene	Sig.	MVU	Asymp (sig. 2-t)	t	Sig. 2-t
Control	10	42.50	8.55	18	0.191	0.667	-	-	-6.98	0.000*
Diabetes	10	69.80	8.92	-	-	-	-	-	-	-

*p<0.05

Table 4: The results of independent sampels t-test for triglyceride (mg dL^{-1}) activity of control and diabetes groups

Groups	N	$\bar{X}$	SD	df	Levene	Sig.	MVU	Asymp (sig. 2-t)	t	Sig. 2-t
Control	10	60.90	10.11	18	0.643	0.433	-	-	-6.69	.000*
Diabetes	10	91.90	10.57	-	-	-	-	-	-	-

*p<0.05

Table 5: The results of independent sampels t-test for HDL (mg dL^{-1}) activity of control and diabetes groups

Groups	N	$\bar{X}$	SD	df	Levene	Sig.	MVU	Asymp (sig. 2-t)	t	Sig. 2-t
Control	10	42.20	7.97	18	3.37	0.083	-	-	6.39	0.000*
Diabetes	10	23.60	4.60	-	-	-	-	-	-	-

*p<0.05

Table 6: The results of Mann Whitney U-test for LDL (mg dL^{-1}) activity of control and diabetes groups

Groups	N	$\bar{X}$	SD	df	Levene	Sig.	MVU	Asymp (sig. 2-t)	t	Sig. 2-t
Control	10	7.30	2.002	18	6.34*	0.021	22.50	0.036*	-	-
Diabetes	10	11.70	4.97	-	-	-	-	-	-	-

*p<0.05

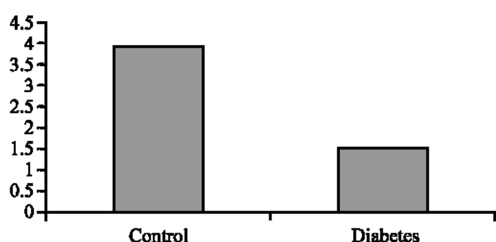

Fig. 1: The SOD average of control and diabetes case

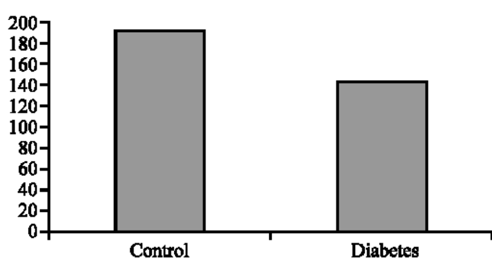

Fig. 2: The CAT average of control and diabetes case

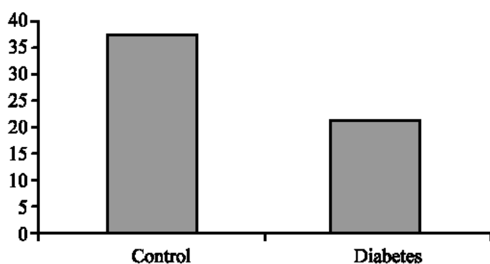

Fig. 3: The TAC average of control and diabetes case

may be due to the excessive enzyme consumption related to hyperglycemia. Kaji *et al*. (1985) did not detect any difference in SOD activity between 60 type 2 diabetes women and 71 control women. Merzouk *et al*. (2003) stated that they found SOD activity significantly low in type 2 diabetics compared to control, whereas they did not confirm this case for type 1 diabetics. Wu *et al*. (1999) found in the experimental study with diabetic rats that in diabetic rat kidney tissue, SOD, POD (Peroxidase) and CAT activities significantly decline at 8th and 16th weeks. Researchers suggested that these differences may be related to the high glucose induced antioxidation differences in the kidney. In the study, they conducted on type 2 diabetes patients, Sozmen *et al*. (2001) did not identify any significant difference in SOD level compared to controls. Turkalp *et al*. (2003) observed that there was not a significant difference between diabetic group and control group in the context of TAC level.

Cholesterol, triglyceride and LDL was found to be significantly lower in control group when compared to diabetic group (p<0.05) (Table 3-5), whereas, HDL found to be significantly lower in diabetic group when compared to control group (p<0.05) (Table 6). Jos *et al*. (1990) found no correlation between diabetes mellitus and lipids in the study conducted with diabetic patients. Godin *et al*. (1988), asserted that in experimental diabetes rats cholesterol and triglycerides increased significantly. In another study, they detected that triglyceride level in HDL increase 83%, suggesting that the reason is lipid peroxides in the membranes of the peripheral tissue cells which are product of lipoprotein metabolism as a result of diabetic damage (Maritim *et al*., 2003).

Tiedge *et al*. (1997, 1998) stated that expression of antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase and antioxidant capacity is lowest in pancreas islet cells when compared to other tissues like liver, kidney, skeletal muscle and adipose tissue. Robertson *et al*. (2004) asserted that damage observed in beta cells- known to be among most oxidative stress sensitive structures-stem from the toxic effects of hyperglycemia.

Godin *et al*. (1988) shown in their studies in rats having chemically induced diabetes with STZ and ALX that antioxidant enzyme alterations may result in a poorly controlled diabetic condition and subsequently, this may lead to secondary complications of diabetes mellitus.

CONCLUSION

When diabetes and its complications possibility to alter antioxidant protection system in relation with reactive oxygen species taken into consideration,

decrease of SOD and CAT in monitored diabetes demonstrates that protective system against oxidative stress is impaired and TAC level may be functional for monitoring diabetes.

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