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Evaluation of Salivary Sialic Acid Level and Cu-Zn Superoxide Dismutase Activity in Type 1 Diabetes Mellitus

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Belce, A., Uslu, E., Kucur, M., Umut, M., Ipbüker, A. and Seymen, H.O. *Evaluation of Salivary Sialic Acid Level and Cu-Zn Superoxide Dismutase Activity in Type 1 Diabetes Mellitus.* Tohoku J. Exp. Med., 2000, **192** (3), 219-225 —— In this study, our aim was to determine whether or not type 1 diabetes mellitus affects salivary sialic acid level and SOD activity. For this purpose, unstimulated saliva specimen was collected. Saliva sialic acid level and SOD activity were measured by the methods of Warren and Sun, respectively. We found significantly decline in salivary sialic acid level and SOD activity. The decrease of salivary sialic acid level in type 1 diabetes may be due to changes in the activities of the enzymes taking part of in the synthesis and catabolism of sialic acid. The main reason for the decrease of salivary SOD activity may be increased glycation of the enzyme and/or deleterious effect of increased free oxygen radicals by glycated proteins on SOD activity in diabetes. We conclude the decline both in sialic acid and SOD in saliva may be a possible factor leading to oral complications of diabetes mellitus. ———— saliva; sialic acid; NANA; superoxide dismutase; diabetes mellitus © 2000 Tohoku University Medical Press

Saliva has been used for diagnostic purposes for a long time (Mandel and Wothan 1976). Recent advances in clinical assay systems have led to increased interest in utilizing saliva as a diagnostic testing fluid. This has in part been driven by the easy and safety with which saliva can be collected as compared to blood (Malamud 1992). There have been a lot of studies on saliva component change associated with different diseases but few specificially on changes in sialic acid level and Cu-Zn superoxide dismutase (SOD) activity (Mortimer and Parry 1988; Haeckel 1989; Johansson et al. 1994; Koc et al. 1996).

Sialic acid, which is found on the carbohydrate branches of glycoproteins exists in conjugate form on the external surface of cell membranes as a membrane

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receptor. It is involved in regulating the external and internal surface of the membrane and therefore the balance between conditions internal and external to the cell. It is a component of the receptors of insulin, interferon and serotonin, various blood hormones, transport proteins, lipoproteins, mucopolysaccharides and it is also found in various exudates. Sialic acid is known to affect amino acid transport on the cell surface and to retard synaptic transmission (Koc et al. 1996).

It has been established that aerobic metabolism produces potentially toxic oxygen derivatives such as the superoxide anion radical, hydrogen peroxide and hydroxyl radical. These oxygen species have been implicated in the pathogenesis of multiple disease states (Fridovich 1974; Cross et al. 1987; Halliwell 1987). SOD is believed to play a key role in the enzymatic defence of the cell against oxygen toxicity, the belief being substantiated by the ubiquitous presence of this enzyme in aerobic organism and by an impressive body of evidence for a protective role of SOD both in vitro and in vivo (Fridovich 1974, 1975). In this study, our aim was to determine whether or not type 1 diabetes mellitus affect salivary sialic acid level and SOD activity.

Materials and Methods

This study was performed in volunteer patients with type 1 diabetes mellitus (mean age: 53.04 ± 19.97) and the healthy volunteers of the laboratory and hospital personnel (mean age: 43.67 ± 9.36) as the control group. The ethical evaluation was performed before study.

Specimen collection

Unstimulated mixed saliva was collected by expectoration. The saliva was taken after an overnight fasting and after the mouth had been rinsed with distilled water. As saliva moves anteriorly in the mouth after an initial swallow, the saliva was drained continuously from the lower lip via a funnel into a test tube. Saliva samples were centrifuged at 2500 g for 10 minutes at room temperature. Supernatants were taken and stored $-20^{\circ}C$.

Biochemical analyses

Protein levels were measured by using Lowry's method (Lowry et al. 1951). Fasting blood glucose and HbA_{1c} measurements made by using GL 2623 (Randox Laboratories Ltd., Antrim, UK) and DCA 2000 kits (Bayer, Leverkusen, Germany), respectively.

Assay of sialic acid level

Saliva sialic acid level was determined by the thiobarbituric acid method described by Warren (1959).

TABLE 1. *Values of the analysed parameters and statistical comparison in the groups (mean ± S.D.)*

	Diabetic group	Control group	p
n	27	25	
Glucose (mg/100 ml)	180.66±78.70	83.40±8.45	<0.001
HbA_{1c} %	8.11±1.82	4.18±0.60	<0.001
Sialic acid (mgNANA/100 mg protein)	5.35±1.99	8.42±2.03	<0.001
SOD (U/mg protein)	4.79±1.31	6.19±2.40	<0.05

Assay of Cu-Zn SOD activity

Saliva Cu-Zn SOD activity was determined by the method of Sun et al. (1988). The assay involves inhibition of nitroblue tetrazolium reduction with xanthine-xanthine oxidase used as a superoxide generator (Sun et al. 1988).

Statistical analysis

The data are presented as mean±S.D. Statistical significance between the groups was determined by Levene's test and $p<0.05$ was considered significant.

RESULTS

Table 1 shows fasting blood glucose, Hb A_{1c} levels, saliva sialic acid level and Cu-Zn SOD activity in the diabetic and control groups. In the diabetic group, saliva sialic acid level and SOD activity were significantly lower than in the control group ($p<0.001$, $p<0.05$, respectively).

DISCUSSION

Sialic acids are widely distributed component of many sialomucoids. In the organism they form a considerably important part of carbohydrate units; especially in the structure of glycoprotein and lipoprotein. Alterations in the contents of sialic acid in certain tissues and in the serum of diabetics were reported by a few investigators (Maley et al. 1971; Chakrabarti et al. 1972; Kokoglu et al. 1988). In diabetes mellitus, the serum sialic asid level was found to be significantly increased but erythrocyte sialic acid level was found to be decreased (Chakrabarti et al. 1972; Gandhi and Roychoudhary 1979; Chari and Nath 1984; Gavalla and Lipovac 1985). Also a declined sialic asid level was reported in the polymorphonuclear leucocyte of diabetics (Chari and Nath 1984). In streptozotocin diabetic rats sialic acid level of hepatocytes was found decreased and insulin therapy restored simultaneously normal blood glucose and hepatic membrane sialic acid levels (Durand et al. 1980). Diabetic patients have been reported to be more susceptible to gingivitis and periodontitis than healthy subjects and these diseases are commonly considered to be oral complications of diabetes.

Recent observational epidemiological studies suggest that diabetes should not be considered as the direct cause of periodontal disease but rather as a systemic promoting factor, able to produce conditions suitable for local agents producing gingivitis and periodontitis (Gensini et al. 1992).

Sialic acid is a part of the membrane glycoproteins and mucoproteins. The permeability of the membrane is closely related to its viability and integrity. Significant changes of the sialic acid content of membrane may cause to increased destruction of membrane integrity and to arise oral complications of diabetes. The decrease of salivary sialic acid level in type 1 diabetes may be due to changes in the activities of the enzymes taking part of in the synthesis and catabolism of sialic acid.

Evidence is accumulating that most of the degenerative diseases that afflict humanity have their origin in deleterious free radical reactions (Carone et al. 1993; Florence 1995). Reactive oxygen species are related to both the arrest of growth and the start of cell differantiation (Johansson et al. 1994). Human diabetes is accompanied by a strong oxidative predominance (oxidative stress) in blood (Matcovics et al. 1998). In diabetes, the persistance of hyperglycemia has been reported to cause increased production of oxygen free radicals through glucose autooxidation and nonenzymatic glycation (Dominguez et al. 1998). Generation of oxygen free radicals by glycated proteins is widely believed to be one of the main causes of oxidative stress in diabetes (Mossine et al. 1999).

Superoxide dismutase scavenges superoxide anion and participates in an essential role as a defence system against oxidative stress in body (Fujii et al. 1995; Mates and Sanches-Jimenez 1999). Many investigators generally found a decline in SOD activities of various tissues in both Type 1 and Type 2 diabetes mellitus (Litvinenko 1991; Zbronska et al. 1995; Makar et al. 1995; Parthiban et al. 1995; Reddi and Bollineni 1997; Vucic et al. 1997; Gupta and Baquer 1998; Kotake et al. 1998).

Purified bovine SOD was nonenzymatically glycosylated in vitro at a rate proportional to incubation time and glucose concentration. Inverse correlation between glycosylation and enzyme activity showed that increased glycosylation was accompanied with inactivation of the enzyme (Oda et al. 1994; Yan and Harding 1997). Erythrocyte SOD undergoes glycation and inactivation in vivo and in vitro. Glycated and less active SOD is increased in erythrocytes of patients with insulin-dependent diabetes mellitus (Kawamura et al. 1992).

Non-enzymatic glycation of reactive amino groups in model proteins increased the rate of free radical production at physiologic pH by nearly fifty-fold over non-glycated protein (Mullarkey et al. 1990). Kakkar et al. (1995) suggest that oxidative stress occurs in diabetic state and that oxidative damage to tissues may be a contributory factor in complications associated with diabetes.

The main reason for the decline of salivary SOD activity may be increased glycation of the enzyme or deleterious effect of increased free radicals by glycated

proteins on SOD activity in diabetes mellitus. Inactivation of SOD demonstrated by both in vitro and in vivo studies may be important for the development of diabetic complications because the enzyme has a crucial role in protecting the body against the damaging effects of the superoxide radicals. We conclude that the decline both in sialic acid and SOD in saliva may be a possible factor leading to oral complications of diabetes mellitus.

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