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ORIGINAL ARTICLE

Saliva of patients with Type I diabetes: effect of smoking on activity of lysosomal exoglycosidases

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OBJECTIVE: The aim of our study was to evaluate the influence of smoking on the activity of *N*-acetyl-β-hexosaminidase (HEX), its isoenzymes A (HEX-A) and B (HEX-B) and β-galactosidase (GAL), in the saliva of patients with Type I diabetes.

METHODS: In the supernatant HEX and its isoenzymes A and B, and β-galactosidase were determined by the method of Chatteriee *et al* in modification of Zwierz *et al* (mKat kg^{-1} of protein). Protein was determined by the Lowry *et al* method (mg ml^{-1}).

RESULTS: The results presented here suggest that diabetes and smoking modify activity of HEX and its isoenzymes, but only combination of diabetes and smoking give a significant increase in the specific activity of HEX and its isoenzymes.

CONCLUSIONS: Type I diabetes slightly changes the composition of saliva. Smoking cigarettes significantly modifies the composition and properties of saliva in healthy individuals and patients with Type I diabetes.

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Keywords: saliva; Type I diabetes; lysosomal exoglycosidases; smoke

Introduction

Human saliva has antibacterial, antiviral and antifungal activities (Geigy Scientific Tables, 1981; Young and Schneyer, 1981; Mandel, 1989; Edgar, 1992). It participates in the digestion of complex carbohydrates and lipids and protects oral cavity against free radicals (Geigy Scientific Tables, 1981; Young and Schneyer, 1981; Nakamura and Slots, 1983; Zambon *et al*, 1985; Mandel, 1989; Edgar, 1992). It was reported that diabetes as well as smoking can influence the composition of saliva and that smoking aggravates complications of diabetes in the oral cavity and salivary glands (Garrett, 1987; Maciejewski *et al*, 1999).

The enzymes in saliva are derived from salivary glands (Sobotta and Hammersen, 1998), polymorphonuclear leucocytes, epithelial cells, plasma and dietary constituents (Nakamura and Slots, 1983). *N*-acetyl-β-hexosaminidase and β-galactosidase are lysosomal exoglycosidases that degrade oligosaccharide chains of glycoconjugates, such as glycoproteins and glycolipids that constitute cell membranes, and glycosaminoglycan chains of proteoglycans that constitute extracellular matrix. *N*-acetyl-β-hexosaminidase releases *N*-acetylglucosamine and *N*-acetylgalactosamine, and β-galactosidase releases galactose from non-reducing end of oligosaccharide chains of glycoconjugates (Zwierz *et al*, 1989, 1999). Changes in the levels of these enzymes could be associated with breakdown of the periodontal ligament or the oral mucosa.

There are several reports that salivary enzymes are altered in the pathological states of the oral cavity. There are many associations of salivary enzyme levels and periodontal disease levels. Previously (Chauncey, 1961; Cimasoni *et al*, 1977) unspecified proteolytic enzyme activity of the gingival crevicular fluid was found to correlate positively with the degree of periodontal destruction. Later it was reported (Nakamura, 1979) that activity of acid phosphatase (ACP) and elastase-like protease as well as collagenase, dipeptidyl peptidase IV and cathepsin D (Ando, 1980) increased with increasing severity of periodontal disease. Nakamura and Slots (1983) reported that increase in activities of lysosomal: α- and β-galactosidase, α- and β-glucuronidase, α- and β-glucosidase, α- and β-mannosidase and HEX positively correlated with the severity of periodontal disease. Zambon *et al* (1985) reported that periodontal treatment caused significant reductions in activities of salivary caprylate esterase-lipase, leucine, valine and cystine

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aminopeptidases, trypsin, β-galactosidase, β -glucuronidase and α-glucosidase.

Other associations between salivary enzyme levels and oral health have been reported. Quinn *et al* (1994) reported that saliva of 34 critically ill patients had significantly increased levels of activity of α-mannosidase, α-fucosidase, β-hexosaminidase and α-sialidase in comparison to saliva from 23 healthy persons. Increase in sialidase activity correlated with increased adherence of several gram-negative bacteria to epithelial cell monolayers and pure glycoproteins. Quinn concluded that exoglycosidase activity in saliva increases during critical illness. By altering normal cell surface carbohydrates, exoglycosidases may facilitate bacterial adherence and respiratory tract colonization.

Health of the surfaces of the oral cavity depends on salivary mucins and epithelial cell components (Kleinberg and Westbay, 1992; Yaegaki and Sanada, 1992), both of which contain numerous glycoproteins (Levine *et al*, 1987). Since the proteolysis of glycoproteins depends on initial removal of the carbohydrate side-chains (Gottschalk and Fazekas De St Groth, 1960), we have considered the possibility that deglycosylation is an initial step in the destruction of the mucosal surfaces of oral cavity. β-galactosidase is one of the important enzymes responsible for the removal of both O- and N-linked carbohydrate side-chains (De Jong and Van Der Hoeven, 1987; Van Der Hoeven and Camp, 1991; Homer *et al*, 1994).

Data on the composition of salivary components of diabetic patients are scarce and inconsistent. The aim of our study was to evaluate the influence of smoking on the activity of *N*-acetyl-β-hexosaminidase (HEX), its isoenzymes A (HEX-A) and B (HEX-B) and β-galactosidase (GAL), in the saliva of patients with diabetes Type 1.

Patients and methods

Each study group consisted of 20 adults (10 women and 10 men) of age 20–35 years, peridontally healthy; gingival index (GI) 0 or 1; index: decayed-missing-filled (DMF) < 3, pocket depths were less than 3 mm (mean 2.73 mm), without dental calculus and with good oral hygiene. Persons under pharmacologic treatment, women with hormonal substitutive therapy, hormonal contraception and pregnancy were excluded from the study. There were four groups:

1. Control group not smoking (CNS): mean age 30 ± 5 years, healthy, non-smokers, mean pH 7.52, mean buffer capacity of saliva 10.23, mean number of missing teeth 0.8.
2. Control group smoking (CS): mean age 29 ± 6 years, healthy, smokers, mean pH 7.35, mean buffer capacity of saliva 11.48, mean number of missing teeth 0.9.
3. Diabetic Type 1 patients, non-smoking (DNS): mean age 28 ± 5 years, patients with diabetes Type 1, mean period of diabetes duration 11 ± 4 years, mean pH 7.31, mean buffer capacity of saliva 12.11, mean number of missing teeth 3.05, mean body mass index (BMI) = 22.65 kg m^{-2}, glycemia in fasting state 7.30 mmol l^{-1}, glycemia after meal 7.46 mmol l^{-1}, glycemia during collection of saliva 8.22 mmol l^{-1}, $HbA_{1c} = 7.47\%$.
4. Diabetic Type 1 patients smoking (DS): mean age 29 ± 6 years, with diabetes Type 1, mean duration of diabetes 10 ± 6 years, mean pH 7.26, mean buffer capacity of saliva 14.27, mean number of missing teeth 3.5, mean body mass index (BMI) = 23.60 kg m^{-2}, glycemia in fasting state 12.45 mmol l^{-1}, glycemia after meal 11.55 mmol l^{-1}, glycemia during collection of saliva 11.52 mmol l^{-1}, $HbA_{1c} = 8.27\%$.

Collection of saliva. One to 3 h after meal 3–5 ml of unstimulated saliva was collected into polyethylene tubes and kept on ice. Smokers' saliva was collected at least 1 h after last smoking and 1–3 h after the last meal. Collected saliva was centrifuged 20 min at 12 000 *g* at a temperature of 4°C and supernatant was stored at −70°C until analysis. In the supernatant, HEX and its isoenzymes A and B and β-galactosidase were determined by the method of Chatteriee *et al* (1975) in modification of Zwierz *et al* (1989) (mKat kg^{-1} of protein). Protein was determined by the Lowry *et al* (1951) method (mg ml^{-1}). The results were analyzed by a Statistica 6.0 StatSoft (StatSoft Polska, Krakov, Poland) according to ANOVA and *post hoc* test (test NIR). Statistical significance of differences was regarded at $P < 0.05$.

This study was performed with the consent of The Bioethical Commission of the Medical University of Bialystok.

Results

The concentration of protein in saliva was investigated in all groups and the concentration of protein found in our experiment is lower than that reported by other authors. The lowest concentration of protein was noted in smoking healthy individuals (16.4 mg ml^{-1}). Diabetes caused decreased protein concentration (21.56 mg ml^{-1}) in comparison to healthy control groups (23.38 mg ml^{-1}). A distinct increase in the concentration of protein in saliva was noted in the saliva of smoking persons (26.42 mg ml^{-1}) in comparison to the remaining groups. Therefore, activity is expressed as kg $protein^{-1}$.

Figure 1 shows significantly higher specific HEX activity in the group of smoking diabetic patients in comparison to control groups and non-smoking diabetic patients. It was observed that there was a tendency for the specific HEX activity in the group of non-smoking diabetic patients to increase in comparison to non-smoking healthy individuals, but an insignificant increase in HEX activity in the group of smoking healthy persons in comparison to non-smoking healthy persons was observed. However, smoking alone did not increase the levels of the isozyme, but tended to decrease it.

Figure 2 demonstrates a significant increase in activity of isoenzyme A of HEX in the group of smoking diabetic patients in comparison to the other three

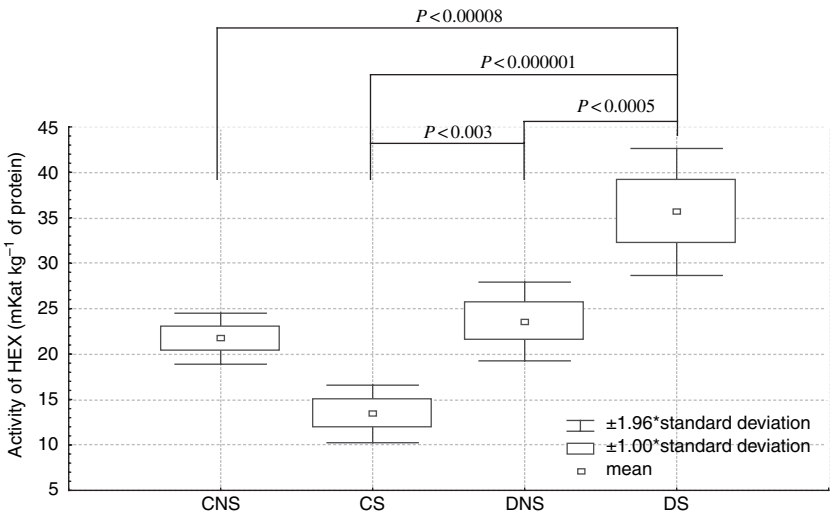

Figure 1 Activity of *N*-acetyl-*β*-hexosaminidase (HEX) in saliva. CNS, control non-smoking group; CS, control smoking group; DNS, diabetic patients not smoking; DS, diabetic patients smoking. Activity was determined by the method of Chatteriee *et al* (1975) in modification of Zwierz *et al* (1989)

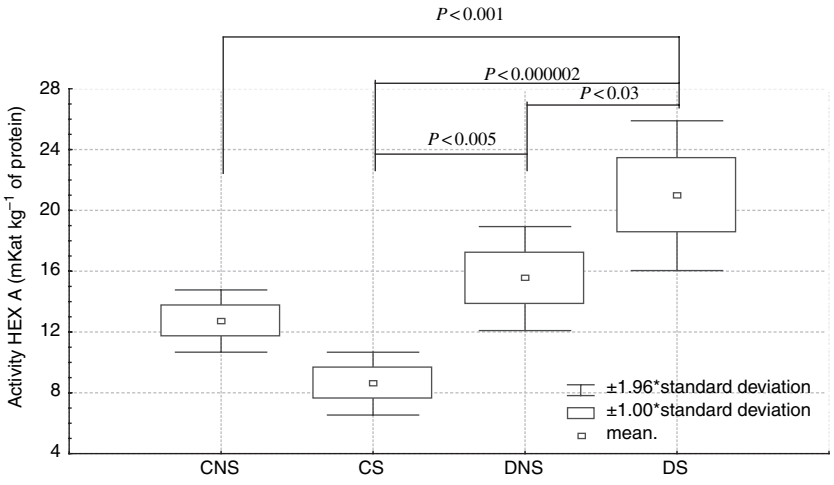

Figure 2 Activity of isoenzyme A of *N*-acetyl-*β*-hexosaminidase (HEX A) in saliva. CNS, control non-smoking group; CS, control smoking group; DNS, diabetic patients not smoking; DS, diabetic patients smoking. Activity was determined by the method of Chatteriee *et al* (1975) in modification of Zwierz *et al* (1989)

groups. There was a tendency of increased isoenzyme A of HEX activity in the group of diabetic non-smoking patients when compared with healthy non-smoking persons and their levels were significantly higher than healthy smokers. However, smoking alone did not increase the levels of the isozyme, but tended to decrease it.

Figure 3 shows that the specific activity of isoenzyme B significantly was increased in the group of diabetic smoking patients in comparison to remaining groups and in the group of non-smoking diabetic patients in comparison to persons in the smoking control group. The specific activity of isoenzyme B in control smoking healthy persons significantly decreased in comparison to both non-smoking healthy group and the diabetic group.

The specific activity of *β*-galactosidase (Figure 4) was significantly decreased in the diabetic non-smoking group in comparison to control healthy non-smoking and control smoking group. In the diabetic patient smoking group, specific activity of *β*-galactosidase was significantly decreased in comparison to control non-smoking healthy persons and smoking healthy persons. The specific activity of *β*-galactosidase had a tendency to decrease in healthy smoking persons in comparison to healthy non-smoking persons and in diabetic smoking patients, in comparison to non-smoking diabetic patients.

Discussion

Our results show that increase in activity of HEX and its isoenzymes A and B as well as *β*-galactosidese may be caused by diabetes and smoking. In the saliva of non-smoking diabetic patients, we found a tendency of total HEX inactivity to increase in comparison to control non-smoking healthy persons. In healthy smoking persons the specific activity of HEX showed a tendency to decrease in comparison to healthy non-smoking persons. But only combination of smoking and diabetes caused significant increase in the specific activity of total HEX in comparison to the remaining

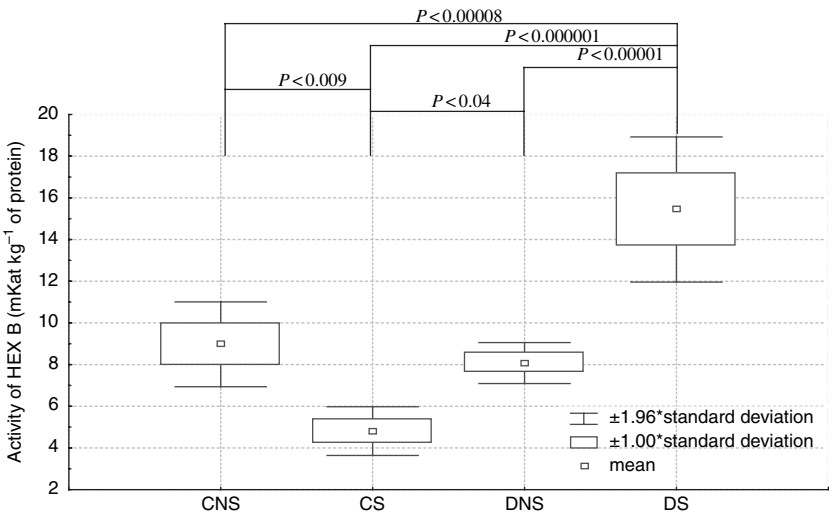

Figure 3 Activity of isoenzyme B of *N*-acetyl-*β*-glucosaminidase (HEX B). CNS, control non-smoking group; CS, control smoking group; DNS, diabetic patients not smoking; DS, diabetic patients smoking. Activity was determined by the method of Chatteriee *et al* (1975) in modification of Zwierz *et al* (1989)

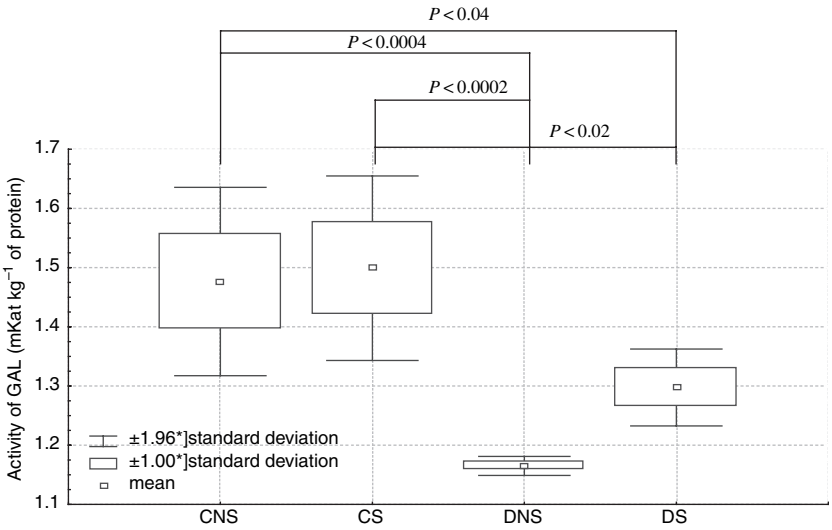

Figure 4 Activity of *β*-galactosidase (GAL). CNS, control non-smoking group; CS, control smoking group; DNS, diabetic patients not smoking; DS, diabetic patients smoking. Activity was determined by the method of Chatteriee *et al* (1975) in modification of Zwierz *et al* (1989)

groups. The isoenzymes of HEX behaved similarly, with the exception of tendency of specific isoenzyme B to show decreased activity in non-smoking healthy diabetic patients. The results presented here suggest that diabetes and smoking modify the activity of HEX and its isoenzymes, but only combination of diabetes and smoking give a significant increase in the specific activity of HEX and its isoenzymes.

Our results (significant decrease in HEX-B, no significant decrease in HEX and HEX-A) are consistent with those of Nagler *et al* (2000, 2001) who found significant reduction (by 83%, 57% and 77%), respectively, in activities of amylase, lactate dehydrogenase (LDH) and ACP after exposure to only one cigarette smoke (CS). CS also reduced salivary peroxidase activity by more than 80%, which may be of great importance to the clinical set up, as peroxidase is considered a pivotal enzyme of the salivary antioxidant system. Similar results were reported in Zappacosta *et al* (2002) who demonstrated an *in vitro* significant decrease of some enzymatic activities (LDH, aspartate aminotransferase and amylase), both in plasma and saliva, following exposure to CS. All enzymatic activities showed a significant decrease following the smoking of a single cigarette, probably due to the interaction between smoke aldehydes and -SH groups of the enzyme molecules. It is concluded that the loss of salivary enzyme activities may be due to various agents in the CS (mostly smoke aldehydes). After 30 min, the level of activity returned to 90–100% of the presmoking level, presumably due to the secretion of new saliva into the oral cavity (Reznick *et al*, 2003). There is lack of information on long-term smoking on exoglycosidases in saliva. Our research was instituted only for the long-term effect of smoking (stimulation) on the activity of exoglycosidases in the saliva of patients with diabetes Type 1.

In the case of *β*-galactosidase we observed decrease in specific activity in both groups of diabetic patients. While smoking tended to elevate the enzyme in the diabetics, suggesting that smoking can cause increases in the levels of this enzyme, diabetes decreased the specific

activity of β-galactosidase. We were not able to find in the literature any data on the activity of lysosomal exoglycosidases in the saliva of diabetic Type 1 patients, so it is impossible to compare our results with the results of other authors.

We believe that increase in the activity of lysosomal hexosaminidase and its isoenzymes may mask visible periodontical changes and determination of those three enzymes in saliva may be used as a marker of periodontal disease. Diabetes Type 1 is the systemic disease that changes the activity of many organs including salivary glands and oral cavity tissues. Diabetes Type 1 modifies basic biochemical processes and changes slightly the composition of the saliva (Lamey and Lewis, 1991; Williams *et al*, 1992; Soames and Southam, 1993; Müller, 2001). Smoking significantly changes the composition of the saliva simultaneously to strengthen complications of the diabetes within the oral cavity. Our results show that changes caused by the diabetes and smoking differ. Three of the enzymes were most increased in diabetics that smoked, which may explain the higher levels of periodontal attachment loss in diabetics who smoke. As our study analyzed saliva of patients with healthy oral cavity, we can advance the argument that diabetes Type 1 and cigarette smoking initiate biochemical reactions that could affect periodontal and oral health.

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