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Selected antibacterial factors in the saliva of diabetic patients

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ABSTRACT

Objective: Diabetes mellitus leads to many systemic complications, including changes in the morphology, function of the salivary glands, and the composition of saliva.
Design: The study comprised a randomly selected 156 adults, of both genders, aged from 21 to 79, out of which patients with diabetes type 1 and 2, and healthy subjects forming two control age- and gender matched to the ill subjects. In unstimulated mixed saliva, total protein, peroxidase, myeloperoxidase and immunoglobulin A were measured as well as salivary flow rate. The periodontal condition was assessed with the use of GI, mSBI and PSR index. The obtained data were analysed with the use of *U* Mann–Whitney's test, Spearman's rang correlation and Chi-square test at a significant level of $p < 0.05$ with use of Statistica 9.0 software.
Results: Type 1 diabetics in comparison to healthy age and gender matched control group had a lower salivary flow rate ($p < 0.01$), a higher content of total protein ($p < 0.01$), myeloperoxidase ($p < 0.001$) and immunoglobulin A ($p < 0.001$). Similarly, type 2 diabetics in comparison to control subjects had a higher level of total protein concentration ($p < 0.01$), myeloperoxidase ($p < 0.05$) and immunoglobulin A ($p < 0.001$). We also found worse periodontal condition.
Conclusion: Within the limitation of the study it may be stated that diabetes type 1 and 2 can cause abnormalities in salivary glands function resulting in the diminishing of salivary flow rate and the increase in total protein content. Higher levels of myeloperoxidase and IgA in the saliva can be linked to worse periodontal condition in the diabetic patients.

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1. Introduction

Diabetes mellitus is a metabolic disorder of multifactorial etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defective secretion/activity of insulin. Four main types of the disease have been defined,[1] but regardless of the type the main cause of the complications is insufficient control of glucose levels in the blood, which leads to the formation of advanced glycation end-products (AGEs) in the plasma and tissues. One of the first oral symptoms of diabetes reported by patients is xerostomia and later, a greater susceptibility of oral tissues to trauma, opportunistic

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infections, greater accumulation of plaque, susceptibility to periodontal diseases, greater risk of caries, delayed wound healing, oral paraesthesia (including burning mouth or tongue) and altered taste sensation.[2–6] Furthermore, diabetic patients revealed changes in the morphology, function of the salivary glands, and the composition of their secretions, including antibacterial agents in the saliva, which may have a negative impact on the condition of the oral cavity. Antibacterial agents in the saliva are usually divided into two main categories: non-immunoglobulin (innate) and immunoglobulin (acquired) factors. The major components of the non-immunoglobulin group are peroxidase and myeloperoxidase systems, lysozyme, lactoferrin, agglutinins, histidine-rich protein, anionic proteins and phagocytic cells.[7,8] Peroxidase activity in the saliva is derived from salivary peroxidase (SPO) and myeloperoxidase (MPO). The enzymes catalyze the oxidation of the thiocyanate ion (SCN^-) by hydrogen peroxide (H_2O_2) to generate hypothiocyanite anion ($OSCN^-$) which is a strong oxidizing agent that plays an important role in the control of bacteria and prevention of the oral accumulation of potential cytotoxic levels of the hydrogen peroxide. Peroxidase is secreted by the acinar cells of salivary glands. Myeloperoxidase is produced by polymorphonuclear leukocytes and monocytes as well. Peroxidase and myeloperoxidase differ in their substrate specificity so that MPO can oxidize chloride to OCl^-, whereas SPO cannot. Immunoglobulin A (IgA) is the main salivary immunoglobulin being the first line of defence against pathogens which invade the mucosal surface.[7–9]

The aim of this study was to estimate and compare the levels of peroxidase, myeloperoxidase and immunoglobulin A in the saliva, and salivary flow rate as well as periodontal condition in type 1 and 2 diabetic patients with healthy control subjects.

2. Subjects and methods

The study consisted of a randomly selected sample of 156 adults, of both gender, aged from 21 to 79, out of which 34 with diabetes type 1 (group D1) and 59 with diabetes type 2 (group D2). There were also 63 healthy subjects forming two control groups age- and gender matched to the ill subjects (groups C1, $n = 30$ and C2, $n = 33$) (Table 1). The inclusion criteria included well-established diabetics type 1 and 2 for at least 1 year, recruited from the ill of the outpatient diabetic clinic. The exclusion criteria concerned the subjects with any other systemic illnesses or medications other than those for diabetes. The control groups comprised healthy non-diabetic subjects without any systemic diseases or medications.

The subjects with both types of diabetes were divided into subgroups in relation to the level of glycemic control of the disease, i.e. HbA1C in the blood 8.5% or less (well controlled, subgroups A1 and A2, respectively) and HbA1C higher than 8.5% (poorly controlled, subgroups B1 and B2, respectively) and according to the time of diagnosis, i.e. less than 10 years (subgroups E1 and E2, respectively) or 10 years and over (subgroups F1 and F2, respectively).

Samples of unstimulated mixed saliva were collected from all subjects during the morning hours at least one hour after eating or drinking. The subjects were sitting with their head bent down and mouth open, and the collected saliva from the mouth floor was taken with a plastic pipette into a graded test tube which was placed on ice. The time needed for the collection of the saliva was noted in minutes with a stopwatch. Basing on the time needed for the salivary sample collection, and the measurement of its volume, salivary flow rate was calculated as ml/min (V). The salivary samples were centrifuged (for 13,000 rpm, 15 min) before biochemical assays. In salivary supernatants total protein—P by Lowry et al. method,[10] peroxidase—SPO (by Nbs-SCN method,[11] myeloperoxidase—MPO by Nbs-Cl method[12] and immunoglobulin A—IgA by immunoturbidimetric method (Turbiquant, Dade Behring) were measured. The enzymes activity was expressed in mlU (mlU/ml) and specific activity mlU per 1 mg salivary protein (mlU/mgP).

The periodontal condition was assessed with the use of Gingival Index—GI (according to Löe and Silness), modified Sulcus Bleeding Index—mSBI (according to Muhlemann and Sohn) and Periodontal Screening and Recording—PSR index (recommended by American Academy of Periodontology). Moreover, in the diabetic subjects, the level of glycosylated haemoglobin (HbA1C) was measured with the DCA 2000 Reagent Kit at the time of salivary samples collection.

The approval from the Bioethics Committee of the Medical University was obtained (781/2004).

The obtained data were analysed with the use of *U* Mann–Whitney's test, Spearman's rang correlation and Chi-square test at a significance level of $p < 0.05$ with the help of Statistica 9.0 software.

Table 1 – Characteristics of studied of the study population.

Groups	Age (years)		Sex	
	mean ± SD	Range (min–max)	Male/female	(%)
Diabetes type 1 (D1, $n = 34$)[a]	37.5 ± 12.47[b]	(21–57)	16/18	47.0/53.0
Control 1 subjects (C1, $n = 30$)	37.0 ± 12.03	(21–57)	14/16	46.7/53.3
Diabetes type 2 (D2, $n = 59$)[a]	65.0 ± 9.04[b]	(45–79)	31/28	52.5/47.5
Control 2 subjects (C2, $n = 33$)	63.7 ± 9.65	(45–79)	17/16	51.5/48.5

Significant differences between a–a and b–b at $p < 0.001$ with the use of *U* Mann–Whitney's test.
D1—patients with diabetes type 1, D2—patients with diabetes type 2, C1—control group to D1, C2—control group to D2, SD—standard deviation.

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