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Salivary exoglycosidases in gestational diabetes

Egzoglikozydazy ślinowe w cukrzycy ciążowej

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: As exoglycosidases have been described as potential markers of salivary gland pathology, we decided to check the possibility of the use of these enzymes in the detection of salivary gland involvement in gestational diabetes.

Materials and methods: For this purpose diabetic pregnant women were compared to pregnant and non-pregnant healthy women. The activities of total HEX as well as GLU in the saliva were determined in duplicate according to Marciniak et al. The activities of GAL, FUC, and MAN in the saliva were determined in duplicate according to Zwierz et al.

Results: It was found that the specific activities of exoglycosidases in the saliva of diabetic pregnant women significantly increased in comparison to healthy pregnant and non-pregnant women.

Conclusion: Increased specific activity of exoglycosidases suggests that gestational diabetes provokes structural/functional alterations in salivary glands and changes in the salivary glycoconjugates metabolism.

Keywords: **gestational diabetes • saliva • lysosomal exoglycosidases**

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Introduction

Saliva is an aqueous fluid found in the oral cavity. It is composed of a complex mixture of secretory organic and inorganic products from the salivary glands, and other substances from gingival sulcus fluid, food deposits and blood-derived compounds. Saliva plays an important role in esophageal physiology, the digestive process and gastric cell protection. It also takes part in mastication, speech, deglutition, gustatory sensitivity, tissue lubrication, mucosal protection against antibacterial, antifungal and antiviral activity, ionic balance regulation and enamel remineralization. These functions are essential for the maintenance of oral and general health and a good quality of life. There are several etiologies of salivary gland hypofunction, including medication and systemic disease [6,7,20,28].

Diabetes mellitus is the most frequent metabolic disease with salivary implications. It is well documented that diabetes type 1 and 2 influences morphology and function of the salivary gland as well as saliva composition [14]. Diabetes-induced sialosis is a disorder of the salivary gland parenchyma and it is accompanied by painless bilateral swelling of the major salivary glands, especially the parotid gland [2]. In terms of salivary gland morphology the following changes are observed: reduction in acinar volume, growth retardation, weight reduction, and decline in the number of salivary ducts and in the density of secretory granules [21]. Diabetic sialosis, like other degenerative diseases, is a multifactor biochemical process that is due in part to the action of several enzymes [5].

Salivary exoglycosidases (N- acetyl-β-D- hexosaminidase (HEX), β- galactosidase (GAL), α-mannosidase (MAN), α-fucosidase (FUC), β-glucuronidase (GLU)) are the group of enzymes that are responsible for the degradation of oligosaccharide chains of glycoconjugates during organogenesis, growth and normal tissue turnover. It has been found that the specific activity of salivary exoglycosidases increases during salivary gland dysfunction. Bierć et al. [3] noted a significant increase in activities of β-glucuronidase, β-galactosidase, and HEX with its isoenzymes in salivary gland tumors. The authors hypothesize that salivary exoglycosidases may take part in tumor development and metastasis. Knaś et al. [7] reported that a combination of diabetes and smoking causes a significant increase in the specific activity of salivary HEX and its isoenzymes. Waszkiewicz et al. [25] suggested that a significant increase in the salivary HEX and its isoenzymes activity after a large single dose of ethanol can be a marker of a harmful level of drinking, which is damaging the salivary glands and/or oral mucosa.

In spite of the large amount of accumulated evidence that the salivary glands and oral health are profoundly affected in type 1 and 2 diabetes, the relationship between salivary gland function and gestational diabetes mellitus has not been explored. As exoglycosidases were described as a potential marker of salivary gland pathology, we decided to assess the possibility of the use of these enzymes in the detection of salivary gland impairment in gestational diabetes. For this purpose diabetic pregnant women were compared to pregnant and non-pregnant healthy women.

Materials and methods

Each study group consisted of 25 women of age 23-30 years, DMFT index (decay, missing, filled teeth) 8-15, gingival index (GI) 0 or 1, with good oral hygiene (Oral Hygiene Index-Simplified OHI-S) 0.35-0.7. There were three groups:

Pregnant women with gestational diabetes (GD) (the third trimester of the first pregnancy) treated in the Department of Endocrinology, Diabetology and Internal Disease, Medical University of Bialystok. Screening for GD was carried out routinely in all pregnancies, between 22 and 30 weeks of gestation, by a 2-hour oral glucose tolerance test (OGTT) after a 75-g oral glucose load according to WHO criteria. GD was defined as a glucose level ≥140 mg/dL after 2-h 75-g OGTT. Women known to have diabetes mellitus before the test or other pregnancy complications (gestational hypertension, gestosis) were excluded from the study. Women with GD were treated with a diabetic diet (White class G_1);

Generally healthy pregnant women (P) (the third trimester of the first pregnancy) who attended the Maternity and Gynecological Private Hospital in Bialystok;

Non-pregnant women (NP) recruited from the Private Institution of Health Care "Stomatology Dr Knas and Company".

The non-diabetic control women were generally healthy, non-smoking and did not take any medication (with the exception of vitamins).

Clinical dental (DMFT index) and gingival status (GI index), and oral hygiene (OHI-S index) examinations were performed by one dentist (G.G.) under standardized conditions in the Private Institution of Health Care "Stomatology Dr Knas and Company", Bialystok, Poland, in a dental chair using portable equipment with fibreoptic light, suction device and compressed air (Table I). All examinations were done by using diagnostic dental tools (dental mirror, probe and periodontal probe).

In 30 patients the inter-rater agreement between the examiner (G.G.) and another dentist (A.Z.) was assessed. The reliability for GI was r=0.94, for DMFT was r= 0.97, and for OHI-S was r= 0.98.

Written informed consent was obtained from each participant after the aims and methodology of the study were explained. The study was approved by the Ethics Committee of the Medical University of Bialystok (permission number R-I-003/226/2006).

Unstimulated whole saliva collection

Women were instructed to refrain from food and beverages, except water, for one hour before saliva collection. All salivary samples were collected between 9 a.m. and 11 a.m. During saliva collection the patient was seated on a chair and protected from any stimulation. The whole saliva was collected in a plastic tube placed on ice by the spitting method. The secretion rate of unstimulated whole saliva was measured during the time required to obtain 5 mL of saliva. Immediately after collection, the salivary samples were centrifuged (3,000xg, 20 min, 4°C). The resulting supernatants were divided into 50 µL portions and frozen (-80°C).

Analytical methods

As a substrate for determination of HEX activity, 4-nitrophenyl-N-acetyl-β-glucosaminide (Sigma, St. Louis, MO, USA) was used, as well as p-nitrophenyl-β-D-glucuronide (Fluka) for β-GLU and p-nitrophenyl-β-D-galactopyranoside (Sigma) for β-GAL, p-nitrophenyl-α-D-fucopyranoside (Sigma) for α-FUC and p-nitrophenyl-α-D-mannopyranoside for α-MAN (Sigma). Activities of total HEX as well as GLU in saliva were determined in duplicate by the Marciniak et al. method [13]. Activities of GAL, FUC, and MAN in saliva were determined in duplicate according to Zwierz et al. [30].

Protein content in saliva was determined in duplicate by Lowry's method [10].

Spectrophotometric measurements were carried out at 405 nm using microplate reader Elx800™.

Statistics

Statistical analysis was done using Statistica 10.0 (StatSoft Cracow Poland) according to ANOVA and post hoc test. Pearson's correlation coefficient was used to determine the association between two variables. Results are expressed as means± SD. The statistical significance was defined as $p< 0.05$.

Results

The specific activity of all salivary exoglycosidases examined was significantly higher in the whole saliva of women with gestational diabetes (GD) in comparison to both other groups (Figure 1). The specific activity of GAL, FUC, and GLU in the whole saliva of pregnant healthy women was significantly lower in comparison to healthy non-pregnant women; however, specific activity of HEX and MAN did not differ between these two groups (Figure 1).

The salivary flow in diabetic pregnant women was significantly lower in comparison to the salivary secretion rate in the healthy pregnant and non-pregnant control groups ($p=0.00$ and $p=0.03$, respectively). The salivary flow in the pregnant healthy group was similar to that of the healthy non-pregnant group, $p= 0.98$ (Figure 2).

There were no significant differences regarding OHI-S, GI, DMFT, PD indexes between examined groups.

There were no correlations between HbA_{1C}, pre-prandial glycemia, OHI-S, GI, DMFT, PD indexes and the specific activity of salivary exoglycosidases.

Discussion

The present study demonstrates that the salivary gland function and enzymatic properties of saliva of pregnant women with gestational diabetes and their pregnant and non-pregnant controls exhibit significant differences.

The significant reduction in salivary gland function measured by the unstimulated saliva flow rate in women with gestational diabetes identifies the salivary glands as one of the major target organs of gestational diabetes. Similar findings have been reported in the case of type 1 and 2 diabetes [14]. It can be assumed that the reduction in saliva secretion in gestational diabetes mellitus, as in the case of type 1 and 2 diabetes, is associated with diabetes-induced neuropathic changes in the salivary gland parenchyma and presence of autoimmune lymphocytic salivary gland infiltrate [4, 15]. Reductions in salivary flow have been reported to be more frequent in diabetic pregnant women with a high level of glycated hemoglobin, suggesting that the function of salivary glands might deteriorate as a result of poorly controlled glycemia [22]. In our study, all pregnant diabetic women were under constant care of the Department of Endocrinology, Diabetology and Internal Disease, Medical University of Bialystok; therefore impaired salivary gland function is not exclusive for poorly controlled disease but also is present in well-controlled women.

The specific activity of all exoglycosidases was examined in the whole saliva of gestational diabetic women and an increased level of activity was recorded. These results were compared with both healthy pregnant and non-pregnant women. The mechanism responsible for increasing lysosomal exoglycosidases specific activity outside the cell in saliva has not been explained.

The observed changes in the specific activity of these enzymes do not appear to be conditional on the pregnancy. In healthy pregnant women, a significant decrease was observed for the specific activity of GAL, GLU and FUC, with unchanged specific activity of HEX and MAN.

Nakamura and Slots [16] reported increased activities of salivary β- and α- galactosidase, β- and α-glucuronidase, β- and α-glucosidase, β- and α-mannosidases as well as hexosaminidase in parallel with an increase in the inflammatory process in the periodontium. We did not find

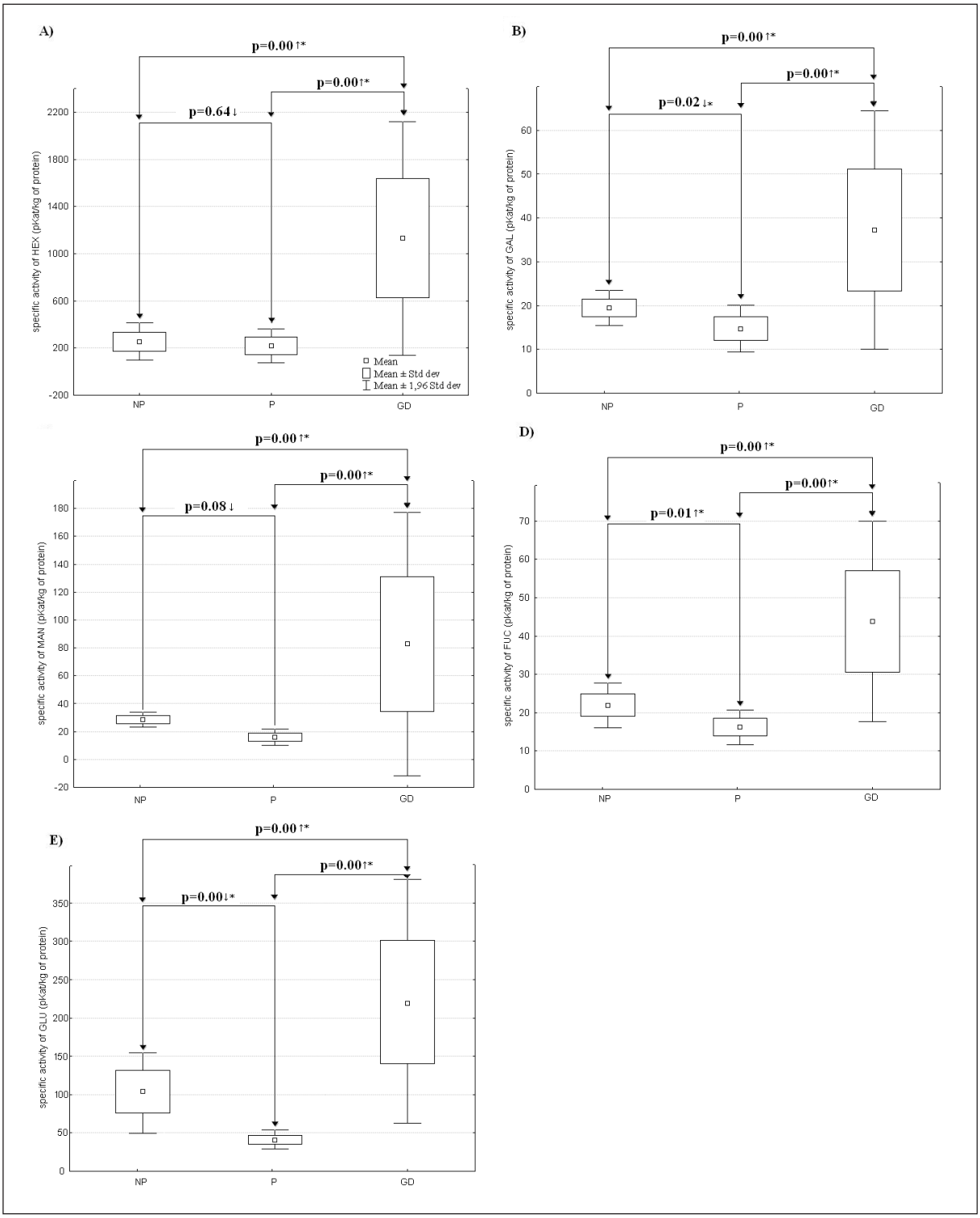

Fig. 1. The specific activity of lysosomal exoglycosidases (pKat/kg of protein) in mixed saliva of non-pregnant (NP), pregnant (P) and gestational diabetes (GD) women. Abbreviations: HEX- N-acetyl-β-D-hexosaminidase, GAL-β-galactosidase, MAN-β-mannosidase, FUC-β-fucosidase, GLU-β-glucuronidase

any significant differences regarding GI, OHI-S and DMFT between examined groups; nor did we observe a local inflammatory state which might cause a significant increase in the specific activities of salivary exoglycosidases in the saliva of GD women. The lack of correlation between activity of salivary lysosomal exoglycosidases and parameters of the gingival, hygiene and dental status suggests that the increase in lysosomal exoglycosidase activities may be due to damage to the tissue of salivary glands caused by diabetes.

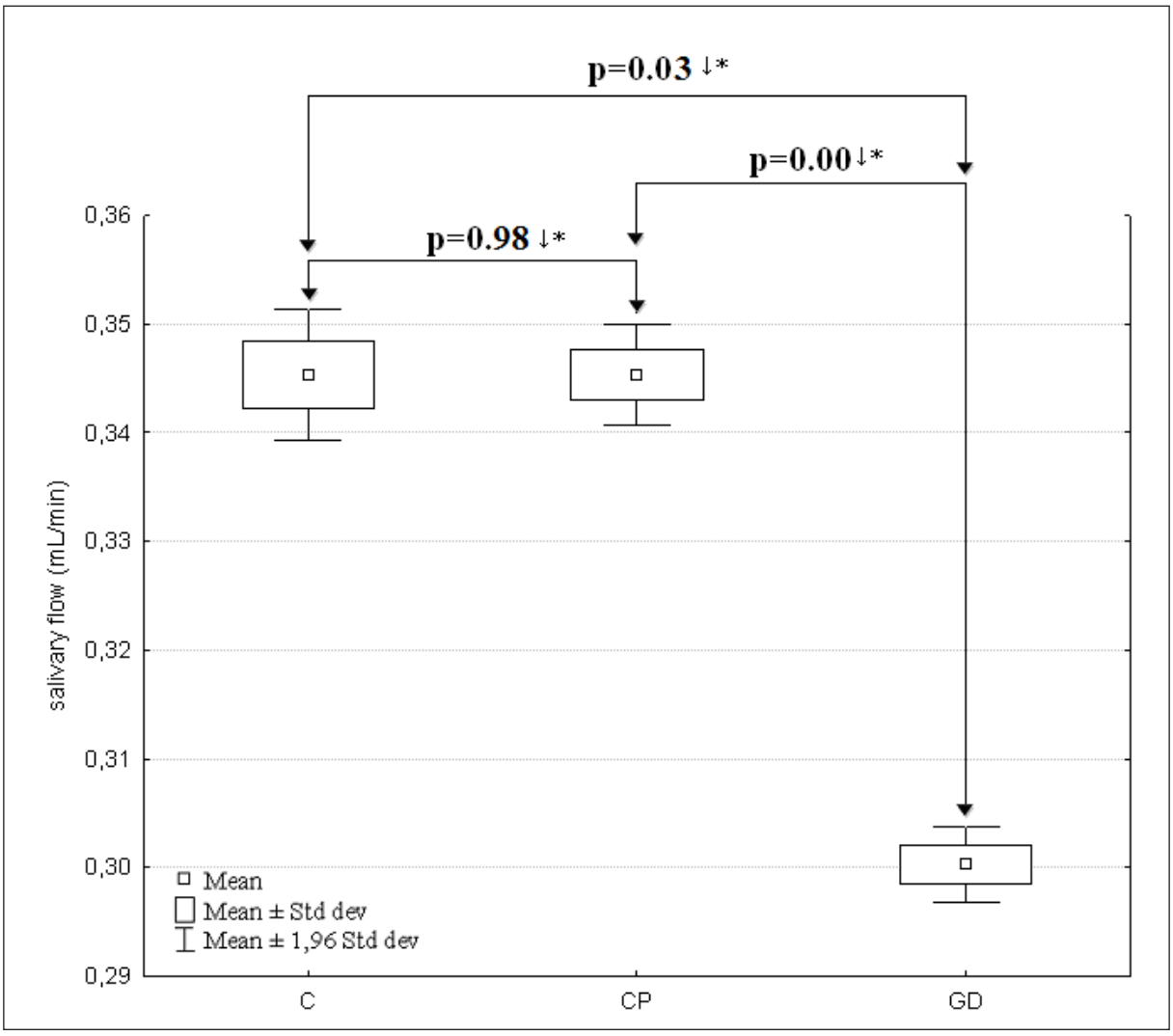

Fig. 2. Salivary flow in non-pregnant (NP), pregnant (P) and gestational diabetes (GD) women (mL/min)

The pathogenesis and nature of changes in the salivary glands are not well understood. Perhaps these changes, as with insulin-dependent and insulin-independent diabetes mellitus, involve degenerative changes and the appearance of lymphocyte infiltration of B and T cells as well as reduced acinar structure, and adipose tissue hypertrophy, which leads to degeneration of the organ [4,15]. The development of diabetic complications is influenced by the glycation of structural and functional proteins, which causes a change of enzyme activity, accumulation of basement membrane proteins in the vascular wall and endothelial dysfunction, decreased affinity of hemoglobin for oxygen and enhanced formation of free radicals (reactive oxygen species – ROS) [19]. The possible role of oxidative stress in the pathogenesis of diabetes mellitus has been suggested previously [19,22]. ROS are known to modify proteins and lipids in the lysosomal and cellular membranes, increasing cell and lysosomal membrane fragility and thus releasing exoglycosidases and other hydrolases via cytosol into the saliva [26]. Literature data [17, 18] reported a significant increase in the activity of lysosomal exoglycosidases in the inflammatory state. The observed increase in specific activity of exoglycosidases, particularly GLU (which is treated as a marker of neutrophil infiltration and release of primary granules by neutrophils) [8, 9], is evidence of the inflammatory component of salivary gland damage in the course of gestational diabetes.

Whatever the reason for the increase in activity of salivary exoglycosidases, they may be responsible for accelerated degradation of salivary glycoconjugates comprising the oral mucosa, as well as the glycoproteins of the acquired pellicle. It has been proven that oral cavity health depends on soluble salivary glycoproteins [29] as well as epithelial cell components [29] containing numerous cell membrane glycoproteins. Glycoproteins present in saliva are an important part of oral defense and maintenance of ecological balance (they take part in bacterial clearance and regulate bacterial colonization of oral tissue [11, 12]); also they are part of innate and adaptive immune systems [23,24], provide fluid layers with highly effective lubricating properties [1] and maintain pH of saliva [27]. Accelerated catabolism of

oligosaccharide chains of salivary glycoproteins with significantly reduced secretion of salivain women with gestational diabetes may be partially responsible for significantly more frequent oral infections such as caries and gingivitis or periodontitis, as reported by a different study [22].

Conclusion: Gestational diabetes increased the activity of salivary exoglycosidases, which may be followed by subsequent degradation of salivary glycoproteins and deterioration of oral health. The elevation in the specific activities of salivary exoglycosidases proves loss of balance between degradation of older and synthesis of new elements of the salivary gland, and may help to prove that salivary glands are damaged by gestational diabetes.

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The authors have no potential conflicts of interest to declare.