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Activity of N-acetyl-β-D-hexosaminidase in the saliva of children with type 1 diabetes

Aktywność N-acetylo-β-D-heksozoaminidazy w ślinie dzieci z cukrzycą typu 1

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

Summary

Background/Aim: Type 1 diabetes is one of the most common chronic diseases in children. The aim of the study was to evaluate the catabolism of glycoconjugates in saliva of children with type 1 diabetes, by measurement of the activity of N-acetyl-β-D-hexosaminidase (HEX) in their saliva.

Material/Methods: The study was performed in 65 children with type 1 diabetes and 39 healthy children. Salivary HEX activity was determined spectrophotometrically by the method of Zwierz et al. in the modification of Marciniak et al. Protein was determined by the bicinchoninic acid method (BCATM Assay Protein Kit). Concentration of the HEX activity was expressed in pKat/mL and HEX specific activity in pKat/μg of protein.

Results: A significant increase in the concentration and the specific activity of HEX in the saliva of children with type 1 diabetes, compared to healthy children, was found.

Conclusions: Type 1 diabetes increases salivary catabolism of glycoconjugates reflected by the significant increase in the activity of HEX in the saliva of children with type 1 diabetes compared to healthy children. The salivary HEX activity may be used in the diagnosis of children with type 1 diabetes after confirmation of our results on a larger cohort of children with type 1 diabetes.

Key words: **N-acetyl-β-D-hexosaminidase (HEX) • type 1 diabetes • children • saliva**

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Introduction

In Poland and worldwide, a steady increase in the incidence of type 1 diabetes in children has been reported [2,5]. The growing number of cases of type 1 diabetes in children is influenced by adverse genetic and environmental factors, especially dietary habits, and drugs used to treat other diseases [4]. Type 1 diabetes is a metabolic disorder of diverse etiology, characterized by chronic hyperglycemia with disturbances in the metabolism of carbohydrates, fats and proteins, as a result of failure of secretion and/or insulin action [17]. Glycation of proteins has a huge influence on the development of complications in type 1 diabetes. Glycation changes enzyme activity, accumulation of proteins in basement membrane of the blood vessels, causes vascular wall damage and endothelial dysfunction, reduces the affinity of hemoglobin for oxygen, and increases free radical formation and prothrombotic activity [18]. Type 1 diabetes is frequently accompanied by macro-and microvascular complications proceeding with damage of tissue [3]. Enzymes degrading tissue constituents, among them glycoconjugates (glycoproteins, proteoglycans, and glycolipids), take part in removal of the damaged tissue constituents [9,13,19,23,24].

N-acetyl-β-D-hexosaminidase (HEX) (EC 3.2.1.52) is the most active of the lysosomal exoglycosidases. HEX participates in lysosomal degradation (cleavage of single amino sugar residues from the non-reducing end of oligosaccharide chains of glycoconjugates [23,25].

The aim of the study was to evaluate the catabolism of salivary glycoconjugates of children with type 1 diabetes, by measuring the activity of N-acetyl-β-D-hexosaminidase in their saliva.

Material and methods

The saliva was collected from 65 children with type 1 diabetes (25 girls and 40 boys) aged 7-17 years (mean age 12.98± 3.07) treated in the Second Department of Pediatrics, Medical University of Bialystok. Glycosylated hemoglobin concentration ranged from 5.8 to 11.9 (mean 8.31± 1.59). Duration of type 1 diabetes ranged from 3 months to 16 years (mean 5.2 years). Investigated children had a healthy oral cavity (without clinical evidence of inflammation in the oral cavity) according to the clinical investigation performed by one qualified dentist at the Department of Pedodontics, Medical University of Bialystok, and correct values of serum acute phase protein (CRP). Children suffering from diseases with a documented increase in HEX activity were excluded from the study. The control saliva was derived from 39 healthy children (18 girls and 21 boys) aged from 6 to 17 years (mean age 11.41± 2.89 years).

Unstimulated saliva (4 mL) was collected by the spitting method under standardized conditions, directly into plastic tubes immersed in ice [1,8], at 9-10 AM, at least two hours after the last meal to minimize the influence of circadian rhythms. Each collected saliva sample was centrifuged at 14,000 x g, for 20 min at 4°C to remove cells and debris, and supernatant was frozen at -80°C. The supernatant was thawed just before determination of the activity of HEX and protein concentration. The determinations were performed in duplicate.

HEX activity was determined by the method of Zwierz et al. [24] as modified by Marciniak et al. [6] as follows: to 10 µl of suitably diluted salivary supernatant were added 40 µl of 0.1 M phosphate – citrate buffer, pH 4.7 and 30 µl of 20 mM p-nitrophenyl-N-acetyl-β-D-glucopyranoside (Sigma, St. Louis, MO, USA) in 0.1 M phosphate-citrate buffer, pH 4.7. The mixture was incubated for 60 min at 37°C. The reaction was stopped by adding 200 µl of 0.2 M borate buffer at pH 9.8. HEX activity, corresponding to the quantity of released p-nitrophenol from p-nitrophenyl-N-acetyl-β-glucopyranoside, was measured at λ = 405 nm with a microplate reader ELX 800 and KC junior computer program (Bio-Tek Instruments, Winooski, VT, USA). Protein concentration was determined by the bicinchoninic acid (BCA) method (PIERCE BCA Protein Assay Kit).

The results were statistically analyzed using the statistical package SPSS ® 7.1 for Windows PL. The level of statistical significance of differences was $p < 0.05$.

The study was approved by the Ethics Committee at the Medical University of Bialystok (No. RI-002/53/2008).

Written informed consent was obtained from each participant's parents following the explanation of the nature, purpose, and potential risks of the study.

Results

HEX activity concentration in unstimulated saliva of children with type 1 diabetes ranged from 112.19 to 1264.79 pKat/mL (mean 494.33± 237.26 pKat/mL), and in the saliva of healthy children from 126.12 to 398.40 pKat/mL (mean 287.06± 111.34 pKat/mL) (Figure 1). Data collected in Figure 1 show that HEX activity concentration in the saliva of children with type 1 diabetes was significantly higher as compared to the concentration of HEX activity in the saliva of healthy children ($p<0.00001$).

Specific activity of HEX in unstimulated saliva of children with type 1 diabetes ranged from 0.1360 to 1.7526 pKat/μg protein (mean of 0.6266± 0.3561 pKat/μg protein), and in the saliva of healthy children from 0.1113 to 0.9908 pKat/μg protein (mean 0.40340± 0.1978 pKat/μg protein) (Figure 2). Figure 2 shows that the specific activity of HEX in saliva of children with type 1 diabetes was significantly higher as compared to the specific activity of HEX in saliva of healthy children ($p<0.001$).

Discussion

Type 1 diabetes is caused by insulin deficiency resulting from a chronic immune process damaging β cells of pancreatic islets of Langerhans [11]. Poorly controlled diabetes leads to complications in the form of macro-and microvascular damage resulting in failure of the different organs [17]. The glycation of functional and structural proteins that changes enzyme activity, increases accumulation of basement membrane proteins, as well as causing vascular wall stiffening and endothelial dysfunction, has a huge impact on development of complications of type 1 diabetes [18]. Metabolic changes resulting from macro-and microvascular complications caused by type 1 diabetes may lead to increased catabolic processes, in particular of glycoconjugates [3].

Glycoconjugates (glycoproteins, glycolipids, proteoglycans) are macromolecules made up of oligosaccharide chains combined with lipids or proteins. Glycoconjugates are present in the cell membranes, extracellular matrix, serum and secretions. Catabolism of glycoconjugates is intimately linked with the processes of damage and regeneration of cellular elements [12]. Participating in lysosomal degradation of oligosaccharide chains of glycoconjugates are lysosomal exoglycosidases present in various tissues and body fluids, e.g. blood serum, urine, saliva, and synovial fluid [1,7,9,12,13,14,15,16,19,20,21,22,24]. Exoglycosidases release a single monosugar from non-reducing ends of oligosaccharide chains of glycoconjugates. Therefore, we decided to investigate whether and how the activity of the most active of exoglycosidases, HEX, behaves in the saliva of children with type 1 diabetes, compared to healthy children. We found a significant increase in the concentration of HEX activity (pKat/mL) (Figure 1) and specific activity (pKat/μg protein) (Figure 2) in the saliva of children with type 1 diabetes, in comparison to the matched control group of healthy children. Our results are in agreement with the report of Severini et al. [10], who observed an increase in the activity of HEX in serum and urine of adults with diabetes. Kamada and colleagues [3] demonstrated increased specific activity of HEX in the submandibular glands of rats with diabetes.

In children with type 1 diabetes increased catabolism of salivary glycoconjugates is reflected by the increase in the salivary activity of HEX. Salivary HEX activity may be used in the diagnosis of children with type 1 diabetes after confirmation of our results on a larger cohort of children with type 1 diabetes.

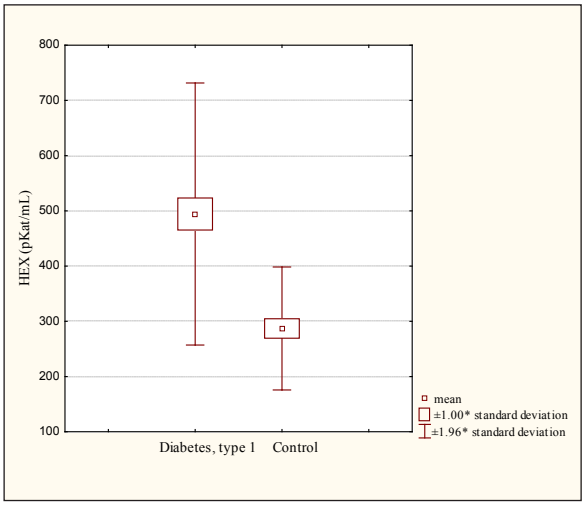

Fig. 1. Activity concentrations of HEX (pKat/mL) in unstimulated saliva of children with type 1 diabetes

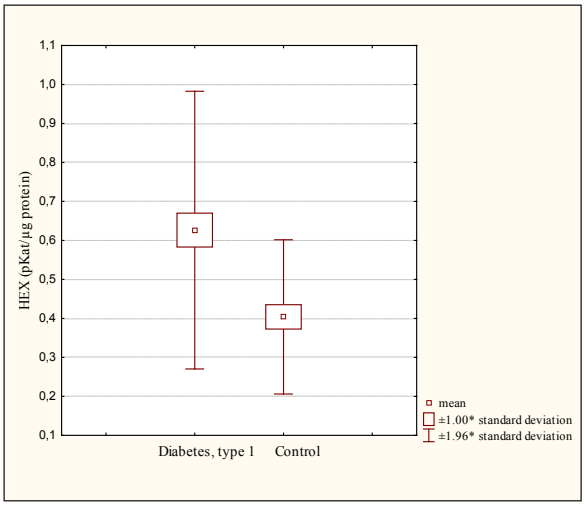

Fig. 2. Specific activity of HEX (pKat/μg protein) in unstimulated saliva of children with type 1 diabetes

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The authors have no potential conflicts of interest to declare.