Salivary hexosaminidase B in children with type 1 diabetes

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ABSTRACT

Introduction: Children type 1 diabetes is accompanied by inflammation and microangiopathy pro-ceeded with increased degradation of tissues. Nacetyl-β-hexosaminidase (HEX) is the most active of exoglycosidases degrading oligo-saccharide chains of glycoconjugates (glycoproteins, glycolipids and proteoglycans).
Purpose: To evaluate the hexosaminidase B (HEX B) activity in the saliva of children with type 1 diabetes.
Materials and methods: The study was performed in 35 children with type 1 diabetes and 20 healthy children. Salivary HEX B activity was determined by the colorimetric, and protein by bicinchoninic acid methods. The HEX B activity concentration was expressed in pKat/mL and specific activity in pKat/µg of protein.
Results: A significant increase in the concentration and the specific activity of HEX B in the saliva of children with type 1 diabetes, as compared to healthy children, was found.
Conclusion: Children suffering from type 1 diabetes have increased catabolism of salivary glycoconjugates by HEX B, which potentially may be useful in the diagnosis of type 1 diabetes in children.
Key words: hexosaminidase isoenzyme B (HEX B), type 1 diabetes, children, saliva.

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INTRODUCTION

Type 1 diabetes in children, caused mostly by the immunological destruction of pancreatic β cells, which create insulin deficiency, proceeds with hyperglycaemia [1]. Hyperglycaemia is connected with damage and disturbance of the function of many organs [2]. Autoimmunological and degradative processes, accompanying type 1 diabetes, increase the catabolism of glyco-conjugates (glycoproteins, glycolipids and proteo-glycans) which may be reflected by the increase in the activity of lysosomal hydrolases and among them - exoglycosidases [3]. One of the most active exoglycosidases is N-acetyl-β-hexosaminidase (HEX) [4]. Complications of type 1 diabetes include microangiopathy, neuropathy and predispositions to the specific atheromatosis [5]. Type 1 diabetes in children requires early diagnosis and entire life therapy with insulin to avoid complications [6]. In early diagnosis of type 1 diabetes in children (especially young children) one should take into consideration non invasive diagnostic methods. One of the body fluids obtained by a non invasive method is saliva. Saliva is a body fluid containing small concentrations of enzymatic proteins [7]. Therefore, we chose the activity of isoenzyme B of N-acetyl-β-hexo-saminidase (HEX B), the most active of exogly-cosidases, as an indicator of the increased catabolism of salivary glycoconjugates in type 1 diabetes in children, and type 1 diabetes itself.

MATERIALS AND METHODS

The study was approved by the Ethics Committee at the Medical University of Białystok (No. RI-002/53/2008). Written informed consent was obtained from each participant parents following the explanation of the nature, purpose, and potential risks of the study.

Participants and procedure

The saliva was collected from 35 children with type 1 diabetes aged 7 - 17 years (mean age 12.46 ± 2.98) treated in the Second Department of Pediatrics, Medical University of Bialystok. Investigated children had healthy oral cavity, (without clinical evidence of inflammation in the oral cavity) according to clinical investigation performed by one qualified dentist at Department of Pedodontics, Medical University in Bialystok, and correct values of serum acute phase protein (CRP). Children who suffered from diseases with documented increase in the HEX B activity were excluded from the study. The control saliva derived from 20 healthy children aged from 6 to 15 years (mean age 11.20 ± 2.65 years).

An unstimulated saliva (4 mL) was collected by spitting method under standardized conditions, directly into plastic tubes immersed on ice [8,9], at 9-10 AM, at least two hours after last meal to minimize the influence of circadian rhythms. Each collected saliva sample was centrifuged at 14,000 x g, for 20 min at 4^0C to remove cells and debris, and supernatant was frozen at -80^0C. The determinations were performed in duplicates.

Isoenzyme B N-acetyl-β-D-hexosaminidase assay

HEX B activity was determined by the method of Zwierz et al. [10] as modified by Marciniak et al [11] as follows: to 10 µl of suitably diluted salivary supernatant was added 40 µl of 0.1 M phosphate – citrate buffer, pH 4.7, mixed and mixture was preincubated 180 minutes at 50^0C to deactivate thermolabile isoenzyme A of N-acetyl-β-D-hexosaminidase. Then, 30 µl of 20 mM substrate (p-nitrophenyl-N-acetyl-β-D-glucosaminide-Sigma, St. Louis, MO, USA) in 0.1 M phosphate-citrate buffer, pH 4.7 was added. The mixture was incubated for 60 min at 37^0C. The reaction was stopped by adding 200 µl of 0.2 M borate buffer at pH 9.8. HEX B activity, corresponding to the quantity of released p-nitrophenol from p-nitrophenyl-N-acetyl-β-glucosaminide, was measured at λ = 405 nm with a microplate reader ELX 800 and KC junior computer program (Bio-Tek Instruments, Winooski, VT, USA).

Protein assay

A protein concentration was determined by bicinchoninic acid BCA method (PIERCE BCA Protein Assay Kit).

Statistical analysis

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

RESULTS

HEX B activity concentration in unstimulated saliva of children with type 1 diabetes ranged from 74.33 to 406.52 pKat/mL (mean 259.68 ± 127.74 pKat/mL), and in the saliva of healthy children from 30.04 to 111.54 pKat/mL (mean 59.34 ± 27.32 pKat/mL) (Figure 1). Data collected on Figure 1 shows that HEX B activity concentration in the saliva of children with type 1 diabetes was significantly higher as compared to the concentration of HEX B activity in the saliva of healthy children ($p<0.0001$).

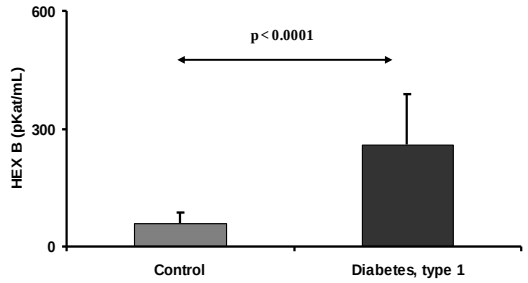

Figure 1. The activity concentrations of HEX B (pKat/mL) in unstimulated saliva of children with type 1 diabetes

The specific activity of HEX B in unstimulated saliva of children with type 1 diabetes ranged from 0.0560 to 1.0817 pKat/µg protein (mean of 0.3569 ±0.2113 pKat/µg protein), and in the saliva of healthy children from 0.0272 to 0.2159 pKat/µg protein (mean 0.0825 ±0.0485 pKat/µg protein) (Figure 2). Figure 2 shows that the specific activity of HEX B in the saliva of children with type 1 diabetes was significantly higher as compared to the specific activity of HEX B in saliva of healthy children ($p<0.0001$).

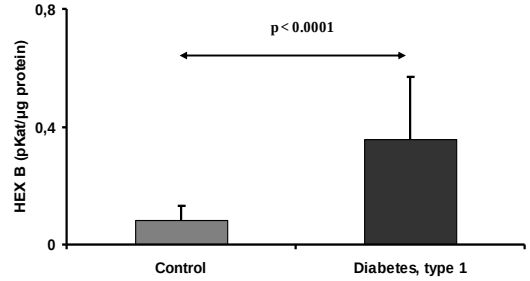

Figure 2. The specific activity of HEX (pKat/µg protein) in unstimulated saliva of children with type 1 diabetes

DISCUSSION

Hyperglycaemia accompanying type 1 diabetes disturbs micro- and macrocirculation by damaging capillary and precapillary blood vessels, causing difficulty in nutrients supply and elimination of useless products of metabolism [12]. Therefore, extremely important is avoiding hyperglycaemic complications in children resulted from increased degradation of cells and extracellular matrix, by early diagnosis and treatment of type 1 diabetes. One of possibilities avoiding complications of the type 1 diabetes is an early diagnosis by monitoring the catabolism of glycoconjugates. Glycoconjugates, important constituents of cell membranes (glycolipids and glycolproteins) as well as extracellular matrix (proteoglycans and glycoproteins) are degraded by the lysosomal exoglycosidases [3]. To the lysosomal exoglycosidases belong: N-acetyl-β-hexosaminidase (HEX) releasing N-acetyl-hexosamines from non reducing ends of the acid (HEX A) and neutral oligosaccharides (HEX B) [4], β-galactosidase (GAL) releasing galactose, α- and β-mannosidases releasing mannose, α-fucosidase releasing fucose and β-glucuronidase hydrolysing glucuronides [3]. It was reported that HEX B may be a marker of damage to the cell [13].

We observed a significant increase in the concentration (Figure 1) and specific activity (Figure 2) of HEX B in the saliva of type 1 diabetes children, in comparison to the healthy children. Our results give evidence for the significant increase in the catabolism of glycoconjugates containing neutral oligosaccharides in balanced type 1 diabetes. Our results agree with report of Severini et al. [14] who observed a significant increase in the serum and urinary HEX activity in diabetic patients. Severini et al. [14] reported that in serum HEX B to HEX A ratio significantly decreases, and in urine significantly increases in diabetic group, in comparison to the control group. We confirmed results of Kamada et al. [15] who reported a significant increase in the specific HEX activity in submandibular glands of streptozocin-induced diabetes in rats. Płocica et al. [16] reported significant increase in the activity of HEX and GAL in the saliva of adult persons with periodontitis, suggesting that increase in the activity of salivary exoglycosidases may reflect immunological processes and accompany the degradation of tissues. As type 1 diabetes has an immunological background, the significant increase observed by us in the concentration and specific activity of the salivary HEX B, may be related to the autoimmunological destruction of the salivary glands.

CONCLUSIONS

1. Type 1 diabetes in children increases the catabolism of salivary glycoconjugates, which is reflected by the increase in the salivary activity of HEX B.
2. The salivary HEX B activity may be potentially used in the diagnosis of children type 1 diabetes, after confirmation of our results on a larger cohort of children with type 1 diabetes.

Conflicts of interest

We declare that we have no conflicts of interest.

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