UNIVERZITA KOMENSKÉHO V BRATISLAVE
PRÍRODOVEDECKÁ FAKULTA

ŠTUDENTSKÁ VEDECKÁ KONFERENCIA PriF UK 2021

ZBORNÍK RECENZOVANÝCH PRÍSPEVKOV

eŠVK
PRIF UK
2021

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Zborník recenzovaných príspevkov

21. Apríl 2021
Bratislava, Slovenská republika
Univerzita Komenského v Bratislave
ISBN 978-80-223-5132-4

Non-invasive diagnostics of MPS IIIA by MALDI-TOF

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Abstract

Mucopolysaccharidosis type IIIA (MPS IIIA), also known as Sanfilippo syndrome A, is severe, progressive disorder caused by mutations in the *SGSH* gene and is inherited in an autosomal recessive manner. Incidence of this disease differs from 1.71 to 3.53 per 100 000 what classifies this syndrome among rare disorders. This disease affects growth, functions of central nervous system, lungs, liver and/or spleen. Usual diagnostic approaches towards MPS IIIA are biochemical genetic test (or enzymatic assay) and selective screening. In presented work, MALDI-TOF analysis of permethylated urine samples was used for diagnostics of MPS IIIA.

Keywords: *Mucopolysaccharidosis; Sanfilippo syndrome A; MALDI-TOF; diagnostics*

Introduction and objectives

Mucopolysaccharidoses (MPS) are a group of rare, inherited lysosomal storage disorders (LSDs), that are clinically characterised by abnormalities in multiple organ systems and reduced life expectancy. Each MPS disorder is caused by a deficiency in the activity of a single, specific lysosomal enzyme required for glycosaminoglycan (GAG) degradation and is characterized by an accumulation of partially degraded GAG fragments in urine or blood [1]. One of known MPS types is MPS IIIA (or Sanfilippo syndrome A), which is caused by deficiency in one of four enzymes involved in degradation of heparan sulfate (HS). MPS IIIA usually affects central nervous system, what is characterized by behavioral problems, gradual loss of speech or decline of cognition. Other symptoms of MPS IIIA are coarse facial features, thick and coarse eyebrow and hypertrichosis. Unfortunately, in case of MPS IIIA, enzyme replacement therapy is not efficient. Thus, MPS IIIA is treated by substrate reduction therapy, where genistein is orally administered [2]. Usually, MPS diseases are diagnosed by genetic analysis or enzymatic assay, which is considered as the most reliable diagnostic method. Due to limited infrastructure, this method is often replaced with other diagnostic methods, such as cetylpyridinium chloride (CPC), thin-layer chromatography (TLC) or enzyme-linked

immunosorbent assay (ELISA). To assay and quantify total urinary GAGs dye-spectrometric methods including alcian blue (AB) and dimethylmethylene blue (DMMB) are used. AB and DMMB methods work on principle of dye interactions with high specificity to sulphated GAGs by ionic interactions and are used as feasible, reproducible and cheap tool for the MPS screening [3]. Mucopolysaccharidoses and other LSDs are currently diagnosed also by spectral methods, especially mass spectrometry (MS). Advances of this analytical method (low sample concentration required, non-invasive approach of diagnostic method – urine samples) lead to acquiring of new biomarkers of LSDs and therefore to more frequent use of MS in diagnostics [4]. Based on our knowledge, there are no studies of MPS IIIA diagnostics by MALDI-TOF published up to this date. In this work, MALDI-TOF analysis of permethylated urine samples was used to obtain the group of possibly new biomarkers for diagnostics of MPS IIIA. Because this disease is rare, only samples obtained from two patients were analysed. Samples were internally marked as Sample 1 (female, 21 years) and Sample 2 (male, 6 years). Diagnosis for both patients was confirmed both genetically and enzymatically. As a negative control, urine from healthy individual was processed under the same conditions.

Materials and methods

Samples were prepared as follows: 50 μL of each urine sample was dissolved in water (LC-MS quality), mixed on vortex and then frozen at – 80 °C. After freezing, the samples were lyophilized overnight and then permethylated. First step of permethylation was addition of DMSO into the NaOH and mixing into slurry. Approximately 150 μL of this slurry was added into each sample and then equivalent volume of iodomethane was added into the mixture and mixed vigorously on vortex. Mixed samples were incubated on shaker (50 min, 25 °C, 2000 rpm). After mixing, samples were extracted with chloroform, washed with ice-cold water until neutral pH value and dried at laboratory temperature. Dried, permethylated samples were then dissolved in 10 μL of methanol/water solution (50/50).

1 μL of sample was added to MALDI plate. To each sample, 1 μL of Na-DHB (2,5-dihydroxybenzoic acid dissolved in TA30 with 1 mM NaOH) was added as matrix and dried at laboratory temperature. MALDI TOF and MALDI-TOF/TOF analyses were performed on MALDI spectrometer *UltrafleXtreme II (Bruker Daltonics, Germany)*. MALDI-TOF analysis was performed in reflectron positive ionisation mode. The results were then evaluated with software programs *flexAnalysis 3.4 (Bruker Daltonics, Germany)* and *GlycoWorkbench (www.eurocarbdb.org)*.

Results and discussion

Our overall aim was to obtain possible biomarkers for MPS IIIA in urine samples using MALDI-TOF/TOF mass spectrometry. According to literature, many signals of biomarkers are common for different LSDs. Performing MALDI-TOF analysis, spectra shown on Fig. 1 were obtained. For example, signal with *m/z* value 1240.6 Da, which is published as biomarker for mucolipidosis II (ML II) or Gaucher syndrome [5]. For MALDI-TOF/TOF analysis, signals with highest intensity in MALDI-TOF spectra were chosen. Signals that are often considered as biomarkers for other lysosomal storage disorders such as Pompe disease, galactosialidosis, mucolipidosis II, Gaucher syndrome or MPSs were confirmed.

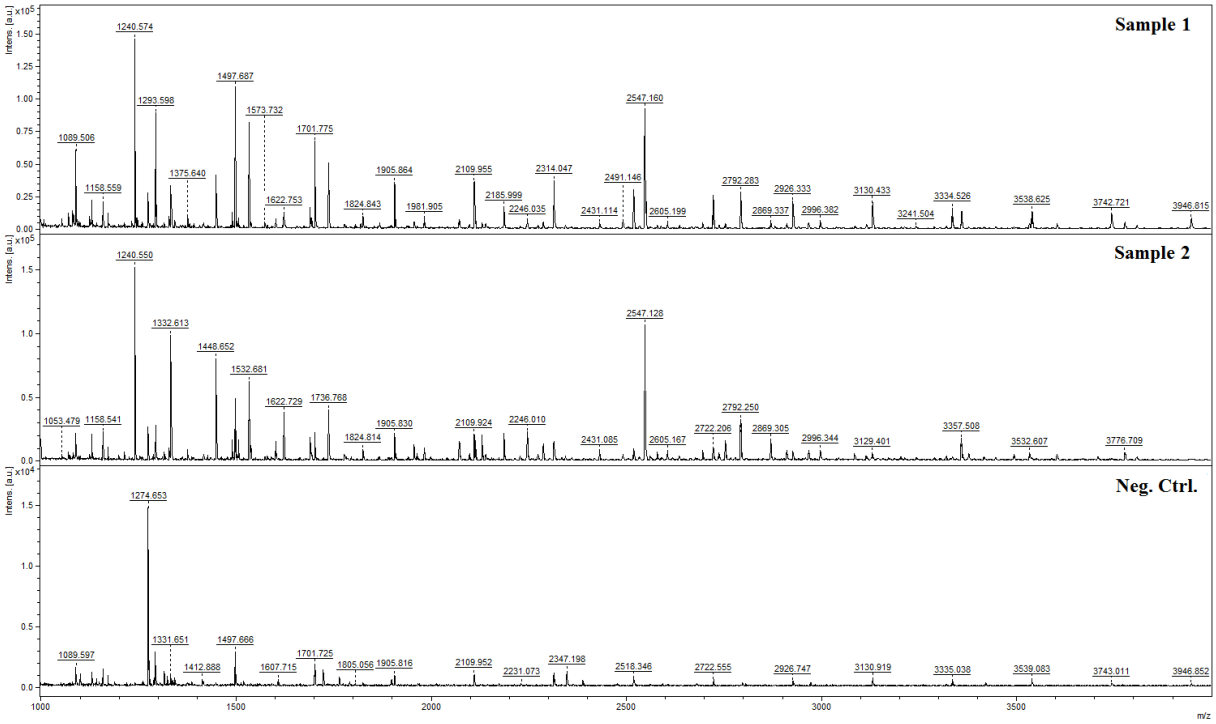

Fig. 1. MALDI-TOF spectra of permethylated urine samples of MPS IIIA patients (Sample 1, Sample 2) and negative control urine sample (Neg. Ctrl.)

Using MALDI-TOF/TOF analysis of signal with *m/z* value 1240.6 Da, structure corresponding with Neu5Ac2Hex1HexNAc1 was assigned. MALDI-TOF/TOF spectrum of this signal is shown on Fig. 2. As was mentioned before, this signal is biomarker for some other LSDs as well, such as MPS I, MPS II or ML II. Second signal analysed by MALDI-TOF/TOF analysis was signal with *m/z* value of 1497.7 Da (Fig. 3). This signal represents Hex7 oligosaccharide and as a biomarker is characteristic for Pompe disease and could also originate from saccharide-rich food intake. Third signal chosen to MALDI-TOF/TOF analysis was 2547.1 Da (Fig. 4). According to literature, this signal is biomarker for ML II or

galactosialidosis and corresponds with biantennary N-glycan structure Neu5Ac2Hex5HexNAc3 [5].

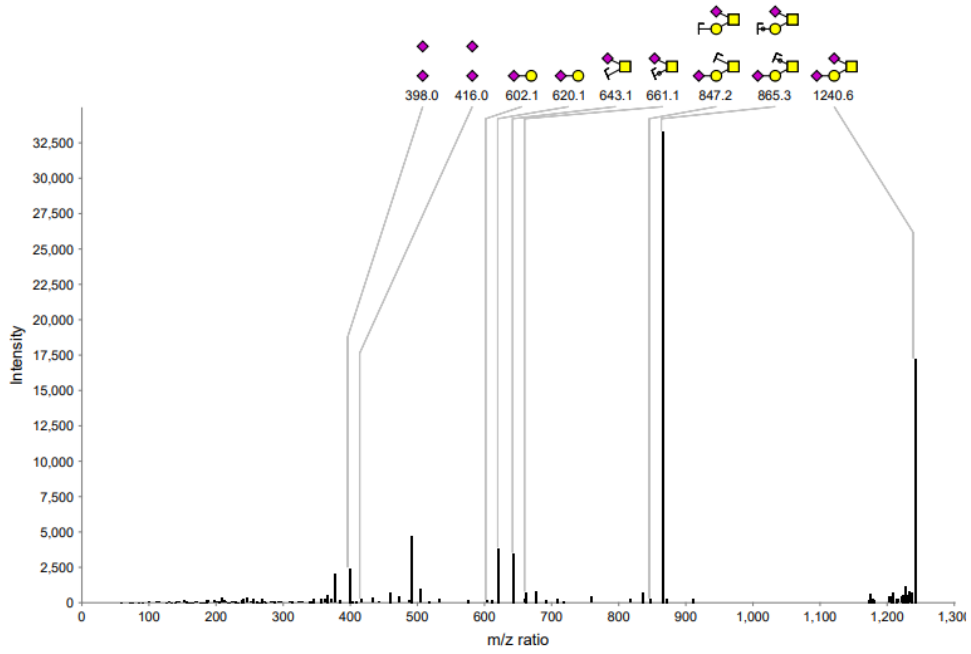

Fig. 2. MALDI-TOF/TOF spectrum of signal 1240.6 Da

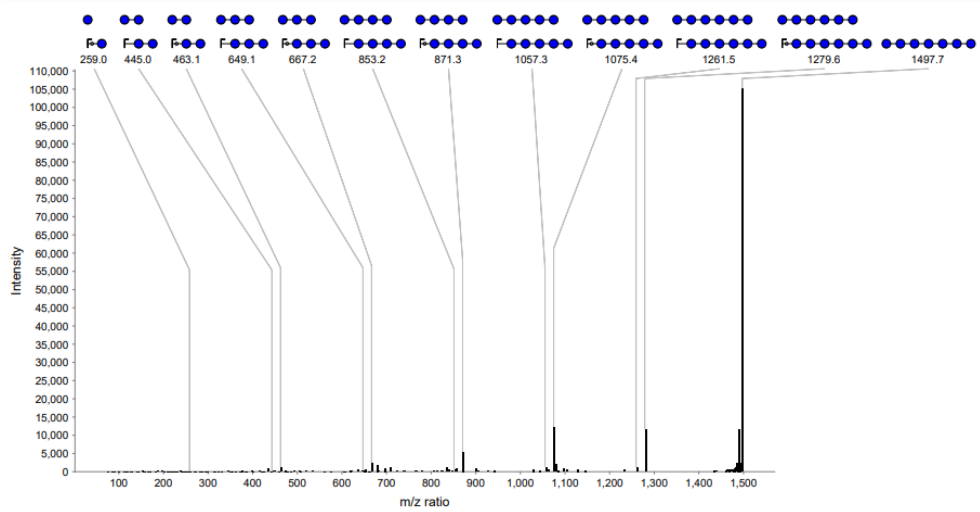

Fig. 3. MALDI-TOF/TOF spectrum of signal 1497.7 Da

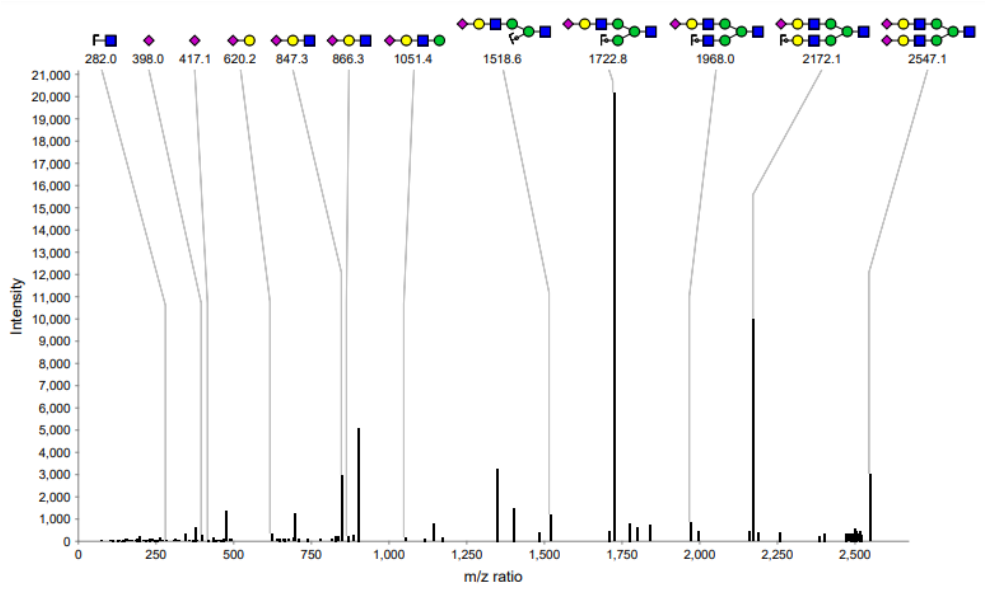

Fig. 4. MALDI-TOF/TOF spectrum of signal 2547.1 Da

Using MALDI-TOF/TOF analysis of both urine samples, other structures were identified and could be considered as biomarkers for MPS IIIA (Tab. 1).

Tab. 1. Table of signals analysed with MALDI-TOF/TOF analysis and their corresponding structures

Signal – *m/z* value (Da)	Structure
1130.5	Hex4HexNAc1
1171.5	Hex3HexNAc2
1736.7	Neu5Ac1Hex4HexNAc2
1824.8	Hex5HexNAc3
1905.8	Hex3HexNAc5
2185.9	Neu5Ac1Hex5HexNAc3

Signals analysed by MALDI-TOF/TOF analysis were identified and assigned to their structures and these signals could be considered as biomarkers for MPS IIIA even despite the fact, that some of these signals are biomarkers for other LSDs. None of these signals were present in negative control urine sample. Results from this work might be useful for the future diagnostic approach towards MPS IIIA diagnostics. However, small number of samples and therefore no statistical confirmation of these results is a major limitation of this study. Although single observed signals are not specific biomarkers for MPS IIIA, their overall combination, consisted of particular m/z and intensity values, represent unique and specific fingerprint that could be considered as a new set of biomarkers for MPS IIIA.

Conclusion

In this work were analysed permethylated urine samples of two different patients – one male (6 years) and one female (21 years). The main goal of this work was to obtain new signals as biomarkers for diagnostics of MPS IIIA disease using MALDI-TOF analysis. Using this method were identified some signals and they were assigned to their structures as well. Many of these signals are considered as biomarkers for other LSDs, such as ML II, Gaucher syndrome or Pompe disease. These signals might be also considered as biomarkers for MPS IIIA. However, because of small number of samples, more analyses are needed for statistical confirmation of identified signals as biomarkers of MPS IIIA.

Acknowledgement

This work was supported by grants from Ministry of Health of the Slovak Republic under the project registration number 2019/7-CHÚSAV-4 - VEGA 2/0060/21 and from the Slovak Research and Development Agency under the contract no. APPV-18-0336. This publication was created with the support of the Operational Program Integrated Infrastructure for the project Study of structural changes of complex glycoconjugates in the process of inherited metabolic and civilization diseases, ITMS: 313021Y920, co-financed by the European Regional Development Fund.

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