ORIGINAL ARTICLE

Salivary Metabolites in Patients with Mucopolysaccharidosis

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ABSTRACT

Objective: To identify the salivary metabolites profile of Mucopolysaccharidosis (MPS) types I, II, IV, and VI patients. **Material and Methods:** The participants were asked to refrain from eating and drinking for one hour before sampling, performed between 7:30 and 9:00 a.m. Samples were centrifuged at $10.000 \times g$ for 60 min at 4ºC, and the supernatants (500µl) were stored at -80ºC until NMR analysis. The salivary proton nuclear magnetic resonance (^{1}H-NMR) spectra were acquired in a 500 MHz spectrometer, and TOCSY experiments were used to confirm and assign metabolites. Data were analyzed descriptively. **Results:** Differences in salivary metabolites were found among MPS types and the control, such as lactate, propionate, alanine, and N-acetyl sugar. Understanding these metabolite changes may contribute to precision medicine and early detection of mucopolysaccharidosis and its monitoring. **Conclusion:** The composition of low molecular weight salivary metabolites of mucopolysaccharidosis subjects may present specific features compared to healthy controls.

Keywords: Mucopolysaccharidoses; Saliva; Magnetic Resonance Spectroscopy; Metabolomics.

Introduction

Mucopolysaccharidosis (MPS) is a rare group of inherited lysosomal storage disorders caused by genetic defects that result in the absence or severe deficiency of lysosomal hydrolases, responsible for the degradation of glycosaminoglycans (GAGs) [1]. There are seven different MPS disorders (I, II, III, IV, VI, VII, and IX) with an estimated birth prevalence of 1.04 to 4.8/100,000 [2]. The MPS is an autosomal recessive condition, except for MPS-II, which is X-linked recessive [3].

The abnormal accumulation of GAGs leads to dysfunction in multiple organs, affecting the cardiovascular, respiratory, gastrointestinal, skeletal, and central nervous systems. Children with MPS often experience respiratory infections, otitis media with hearing loss, hepatosplenomegaly, joint stiffness, limited function/contracture, and cognitive/behavioral problems [4]. Oral cavity complications include macroglossia, malocclusion (anterior open bite, anterior/posterior crossbite), limited mouth opening, impacted teeth, and delayed dental eruption [4-6]. In addition, supernumerary teeth, conoid teeth, taurodontism, partial and complete bony teeth, and root dilaceration are more common in MPS individuals [7]. Given the importance of early diagnosis in managing patients with MPS, it is crucial to understand the metabolite changes that contribute to this condition's development. By analyzing the salivary low molecular weight metabolites composition of MPS patients, we can identify critical markers for early detection and monitoring of this disorder.

The non-invasive nature of saliva sampling makes it a valuable tool for medical research, providing crucial metabolic information about both systemic and oral conditions—additionally, the simplicity and obtaining biological samples, especially in pediatrics, where invasive procedures can be challenging. Salivary research is a growing field due to the high metabolic information of systemic and oral conditions. Alterations in salivary metabolites can be fundamental for the early detection and monitoring of systemic disorders [8-12]. Although some studies have described the oral characteristics of MPS patients [5-7,13,14], the salivary low molecular metabolic composition remains unknown.

This study could bring new insights into the molecular mechanisms underlying MPS and provide potential biomarkers for early detection and monitoring of the condition. This information would be valuable not only for precision medicine but also for developing new treatments that target the underlying metabolic defects in MPS. The analysis of salivary low molecular weight metabolites in MPS patients holds great potential for improving our understanding of this rare and debilitating condition. It could lead to better management and care for patients affected by MPS. This study aims to identify the salivary low molecular weight metabolites composition of patients with mucopolysaccharidosis.

Material and Methods

Study Design and Ethical Clearance

This is an observational, cross-sectional study of a group of patients with MPS described as a case series, which was submitted and approved by the local Ethics Committee (56788816.1.0000.5257) and was carried out following The Code of Ethics of the World Association (Declaration of Helsinki).

Participants

From a group of MPS patients, four being representatives of different types of MPS (I, II, IV, VI), attending two referral centers for MPS in Rio de Janeiro, Brazil (*Instituto de Puericultura e Pediatria Martagão Gesteira* - IPPMG and *Instituto Fernandes Figueira* - IFF), and healthy patient (Department of Pediatric Dentistry and Orthodontics – UFRJ) were included. Informed consent was obtained from all participants or the children's

legal guardians before study initiation. We had participants without systemic disease as a control group. We included patients with MPS after undergoing genetic testing. The inclusion criteria were subjects diagnosed.

MPS and gender/age-matched children with good general health. Exclusion criteria included inability or unwillingness to sign the informed consent form diagnosis of a medical condition that required medication and a history of uncharacterized systemic disease, immunocompromised individuals, bleeding oral lesions, and antibiotic intake within three months prior to saliva collection. We already have knowledge of the salivary profile of healthy patients in this age range due to our previously published data [8,9].

Saliva Sample Collection

Unstimulated whole saliva was collected from one representative patient of each MPS type I, II, IV, and VI before the oral examination and from one systemic and oral healthy patient. For saliva sample collection, participants were required to expectorate saliva into a plastic universal tube with sodium azide (6 µl) immersed in ice. They refrained from eating and drinking for one hour before sampling, performed between 7:30 and 9:00 a.m. The samples were centrifuged at $10.000 \times g$ for 60 min at 4ºC, and the supernatants (500µl) were stored at -80ºC until NMR analysis.

Sample Preparation and Nuclear Magnetic Resonance (NMR) Measurements

^{1}H-NMR (proton nuclear magnetic resonance) spectra were acquired using a 500 MHz Avance III spectrometer (Bruker Biospin, Rheinstetten, Germany) at 25°C. Saliva samples were prepared by mixing 500 µL of saliva supernatant, 100 µL of sodium phosphate buffer (pH 7.0) (containing 60 µL of deuterated water (D_2O; Cambridge Isotope Laboratories Inc., USA), and 10 µL of 20 mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS; Sigma-Aldrich, Milwaukee, USA)). The CPMG (Carr-Purcell-Meiboom-Grill) pulse sequence was used for saliva samples at 298 K with 1024 scans. After spectra acquisition, edge effects were evaluated by overlaying all spectra using Topspin (Bruker Biospin, Rheinstetten, Germany). The assignment strategy included using the Human Metabolome database (http://www.hmdb.ca/) [15] and previous studies [8,9].

Data Analysis

Data were analyzed descriptively. After spectrum acquisition on a 500 MHz Advance III spectrometer, edge effects were evaluated by overlaying all spectra using Topspin (Bruker Biospin). Spectra and spectral regions that could not be corrected for phase and baseline were excluded from the analysis. Each NMR spectrum was analyzed from 0-8 ppm, excluding the water region (4.5–5.5 ppm). Our assignment strategy was implemented. TOCSY experiments confirmed and assigned metabolites [8-12]. The metabolomics workflow is reported in Figure 1.
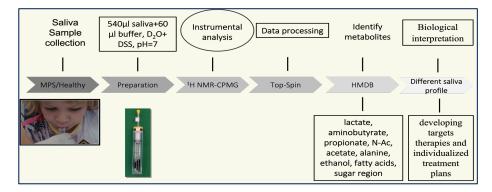

Figure 1. MPS and healthy saliva metabolomics workflow diagram.

Results

The sample consisted of 13 subjects (3 MPS-I, 5 MPS-II, 1 MPS-IV, 4MPS-VI), nine males and five females, with ages ranging from 2-38 years. The subjects were gender and age-matched and were subjects with good general health. Saliva from a 7-year-old female healthy patient was chosen as an example of healthy control. The spectra from healthy subjects as well as MPS patients demonstrate the assignment of metabolites in the 0.0-4.5 ppm region, such as fatty acid (0.86 ppm), propionate (1.04/2.7 ppm), ethanol (1.16, 3.65 ppm), lactate (1.30-1.38 ppm/ 4.10-4.18 ppm), alanine (1.48 ppm), acetate (1.92 ppm), N-AC (2.02-2.06), aminobutyrate (1.88, 2.29, 3.02 ppm), and the sugar region (4.10- 3.86 ppm). Figure 2 shows the representative NMR spectra of salivary samples from patients (0- 4.5 ppm): (a) healthy, (b) MPS type I, (c) MPS type II, (d) MPS type IV and (e) MPS type VI. The evaluation of the NMR spectra suggests lower lactate, propionate, alanine, and N-AC levels in healthy subjects compared to MPS patients.
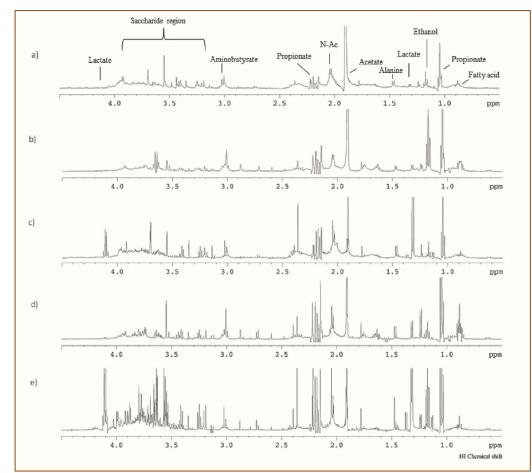

Figure 2. Representative ¹H NMR saliva spectra differences among groups. a) saliva sample from a healthy patient, b) patient with MPS type I, c) patient with MPS type II, d) patient with MPS type IV, and e) patient with MPS type VI. Resonance assignments of specific metabolites.

Discussion

Mucopolysaccharidoses are a rare group of genetic disorders that result from defects in the degradation of glycosaminoglycans (GAGs) due to a lack of functional lysosomal hydrolases [1]. There are seven different MPS disorders (I, II, III, IV, VI, VII, and IX), and a study in Brazil found a total of 600 cases diagnosed between 1994 and 2012, with the most common being MPS I, II, IV, and VI [16]. This study analyzed the salivary

metabolites of patients with MPS Types I, II, IV, and VI using 1H-NMR metabolomics. We found differences in the salivary profiles between MPS patients and healthy subjects.

To our knowledge, this is the first study of metabolomics analyses in salivary samples of patients with different types of mucopolysaccharidosis. Previous metabolomics analyses have recently shed light on the biological profile of inherited rare diseases such as mucopolysaccharidosis type III [17] and type VI [18], using urine samples of MPS patients through tandem mass liquid spectrometry combined with ultra-high-resolution liquid chromatography. Changes in amino acid profiles and biochemical patterns were found in MPS patients compared with the healthy controls.

^{1}H-NMR metabolomic demonstrated different metabolite profiles, as already been published [8,9]. This concept was successfully used to identify metabolic changes in saliva samples of children who have type I diabetes [8], patients with renal diseases [9,10], lupus erythematosus [11], and Covid 19 [12]. We found differences in the salivary biochemical patterns between the MPS patients and the healthy subjects, including changes in the lactate, aminobutyrate, propionate, N-Ac, acetate, alanine, ethanol, fatty acids, and saccharides. The results showed differences in the NMR spectrum among the MPS types compared to the healthy subject salivary profiles. Further studies should be performed to analyze differences in the biochemical patterns between MPS types and healthy ones. Differences in the salivary low molecular weight analysis by NMR between healthy subjects and patients may contribute to the knowledge of the metabolic pathways in mucopolysaccharidosis.

The results of this study suggest that saliva may be a suitable source for studying metabolite changes in individuals with MPS and that the "omic" approach with saliva in MPS is a promising mechanism for evaluating the health status or condition. It is a limitation of the study that only a restricted number of subjects were investigated. Still, this study shows the potential of using saliva profiles for early diagnosis of MPS, which can induce the search for early treatment and help alleviate the clinical oral manifestations. In addition to the benefits of early diagnosis, our findings open the door for further studies in the field of MPS. Using saliva as a source of information could benefit children and infants, who often have difficulties providing blood or urine samples.

Moreover, the non-invasive nature of saliva collection makes it easier for patients to comply with follow-up evaluations. Additionally, the results of this study can be used as a foundation for future studies on personalized medicine for patients with MPS. The identification of specific metabolic changes in saliva can provide a basis for developing targeted therapies and individualized treatment plans.

Conclusion

Using saliva as a source for metabolomics analysis holds great potential for diagnosing and managing MPS. The results of our study provide evidence that NMR spectroscopy can be used to identify differences in the salivary metabolite profiles between healthy subjects and individuals with MPS. Further studies are necessary to confirm these results and to understand the mechanisms underlying the metabolic changes observed in MPS patients. In light of these findings, it is important to continue exploring the potential of saliva analysis to diagnose and manage MPS and other metabolic diseases.

Authors' Contributions

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APCV	https://orcid.org/0000-0001-7219-1123	Conceptualization, Methodology, Software, Formal Analysis, Data Curation, Supervision and Project Administration.

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| IPRS | https://orcid.org/0000-0003-0449-6624 | Conceptualization, Data Curation, Writing - Review and Editing, Supervision, Project Administration and Funding Acquisition. |

All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

Financial Support

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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