Original Research
Community Dental Health

Analysis of salivary parameters of mucopolysaccharidosis individuals

Patrícia Luciana Serra NUNES[a]
Filipe Atahide FONSECA[b]
Luiz Renato PARANHOS[c]
Cauane BLUMENBERG[d]
Valentim Adelino Ricardo BARÃO[e]
Elizabeth Soares FERNANDES[f]
Rebeca Garcia FERREIRA[g]
Walter Luiz SIQUEIRA[h]
Michelle Foigel SIQUEIRA[h]
Eduardo Buozi MOFFA[b]

[a]Universidade Federal do Maranhão - UFMA, Hospital Universitário, São Luis, MA, Brazil.

[b]Centro Universitário das Faculdades Associadas – UNIFAE, Department of Dentistry, São João da Boa Vista, SP, Brazil.

[c]Universidade Federal de Uberlândia – UFU, Department of Community and Preventive Dentistry, Uberlândia, MG, Brazil.

[d]Universidade Federal de Pelotas – UFPel, Social Medicine Department, Post-graduate Program in Epidemiology, Pelotas, RS, Brazil.

[e]Universidade Estadual de Campinas – Unicamp, Piracicaba Dental School, Department of Prosthodontics and Periodontology, Piracicaba, SP, Brazil.

[f]Centro Universitário das Faculdades Associadas – UNIFAE, Department of Physiotherapy, São João da Boa Vista, SP, Brazil.

[g]University of Saskatchewan, College of Dentistry, Saskatoon, SK, Canada.

[h]Faculdades Pequeno Príncipe, Programa de Pós-graduação em Biotecnologia Aplicada à Saúde da Criança e do Adolescente, Curitiba, PR, Brazil.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Eduardo Buozi Moffa
E-mail: Eduardo.moffa@prof.fae.br

https://doi.org/10.1590/1807-3107bor-2022.vol36.0011

Submitted: May 13, 2021
Accepted for publication: August 31, 2021
Last revision: September 16, 2021

Abstract: Mucopolysaccharidosis (MPS) is a heterogeneous group of rare, chronic, progressive and systemic inherited disorders resulting from deficiency or lack of lysosomal enzymes responsible for the degradation of glycosaminoglycans. Products of nitrosative stress have been previously detected in blood and urine samples of patients with MPS. However, it is unclear whether they are present in the saliva of MPS patients and also if they correlate with salivary parameters such as flow and pH. This study compared the salivary levels of NO_X (NO_2^- + NO_3^-), nitrite (NO_2^-), protein (albumin), erythrocyte and leukocyte numbers, as well as the salivary flow rate and pH values of samples obtained from 10 MPS patients and 10 healthy subjects. MPS patients exhibited higher salivary levels of NO_X and NO_2^- when compared to healthy subjects ($p < 0.05$). Albumin was only detected in six saliva samples of MPS patients and, erythrocytes and leukocytes were detected in 60% and 40% of the MPS patients, respectively. In addition, salivary flow rate and pH averages were statistically lower in this group when compared to healthy samples ($p < 0.05$). Overall, the data indicates that the salivary levels of NO products can be used in combination with other heath indicators to monitor MPS disorders.

Keywords: Mucopolysaccharidoses; Saliva; Oxidative Stress.

Introduction

Mucopolysaccharidosis (MPS) is a heterogeneous group of rare, chronic, progressive and systemic inherited disorders resulting from deficiency or lack of lysosomal enzymes responsible for the degradation of glycosaminoglycans (GAGs). In MPS, GAG oligosaccharide accumulation occurs within the lysosomes of cells, inducing cell and tissue damage and dysfunction.[1] MPS is classified into seven groups (I, II, III, IV, VI, VII and IX) according with the type of enzyme deficiency involved in the disorder. With the exception of type II MPS, which is associated with the X chromosome, all other MPS consist in autosomal recessive disorders.[2]

The clinical characteristics of MPS vary with its type and, the heterogeneity of these signs is considerable.[2] Despite the correlation between some genotypes and phenotypes, individuals with identical enzyme deficiency may have distinct clinical magnitude (from mild to severe).[3] The most commonly observed signs of MPS include umbilical and inguinal hernia, macrocephaly, short stature, marked skeletal

alterations, corneal opacity, claw hands, facial dimorphism, macroglossia, included teeth, gingival hyperplasia and dental enamel defects.[4] Diagnosis is based on traditional clinical and laboratorial criteria based on quantitative/qualitative urinary GAG analyses (*e.g.* dermatan sulphate; heparan sulphate; keratan sulphate and creatine) and enzyme activity assays (*e.g.* α-L-iduronidase, iduronate sulphatase and β-galactosidase). In addition, when available, molecular studies are important tools to identify MPS type.[5]

MPS types that prevalence at birth can vary among developed, underdeveloped and emerging countries.[2] In Norway, more than 60% of the MPS cases fall into the type I group.[6] In contrast, data from Taiwan indicate that only 6% of the Taiwanese cases of MPS are classified as type I, and more than 50% as type II.[7] In Brazil, it is estimated that 37% of the MPS individuals are type II. Conversely, in the United States, 31.7% of the MPS cases correspond to type IV. Thus, there is great inconsistency in the presentation of the disorder throughout the world.[2]

Saliva is a hypotonic fluid composed of elements produced by the salivary glands and constituents from blood plasma, such as hormones and cytokines, among others.[8] Although saliva is not an filtrate of the blood plasma, changes in the systemic levels of substances such as cortisol, can be detected in it, indicating that this fluid could be potentially used as a source of disease markers s for diagnostic purposes.[9,10] The use of saliva to monitor health and disease parameters is attractive as its collection is a non-invasive, easy and painless method that does not require specially trained individuals to collect samples,[11] opening new perspectives for diagnosis, early detection and monitoring of oral and systemic diseases.[12]

Changes in the redox status may occur in lysosomal storage diseases (LSD) such as MPS. Although the pathogenic mechanisms of LSD have been widely studied, they are not yet fully understood. In this context, reactive oxygen species (ROS) and nitrogen reactive intermediates (NRIs) may lead to increased lipid peroxidation of the lysosomal membrane and extravasation of its enzymes into the cytoplasm,[13] therefore contributing to MPS. Inflammatory cell activation culminates in the up-regulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an important enzymatic protein complex that reduces O_2 into reactive metabolites such as superoxide ($O_2^{\bullet-}$).[14] $O_2^{\bullet-}$ is an important substrate for the formation of hydrogen peroxide (H_2O_2) and peroxynitrite, the latter resulting from the reaction of $O_2^{\bullet-}$ with nitric oxide (NO). Of note, peroxynitrite, can structurally alter cellular proteins, leading to cell death.[15] Interestingly, blood and urine samples of type IV MPS individuals presented with an increased activity of superoxide dismutase, an enzyme responsible for the conversion of $O_2^{\bullet-}$ into H_2O_2.[15] Similarly, the RNIs have also been identified in blood and urine samples of MPS individuals.[13,14] However, their salivary levels in MPS patients are yet to be established, as well as their correlation with other health salivary parameters. This study investigates the amounts of (NO_2^- + NO_3^-), nitrite (NO_2^-), protein (albumin), erythrocytes, leukocytes, pH and flow rate in saliva samples from MPS patients and healthy subjects. Possible correlations between these salivary parameters and the clinical signs of MPS such asvisible plaque, gingival bleeding on probing , caries and enamel defects were also determined.

Methodology

Ethical criteria and protocol

A clinical study with twenty individuals aged 5–22 years old was conducted after approval by the Ethics Committee of the Universidade CEUMA (UNICEUMA; Protocol Number: 1.756.478). The Informed Consent and Assent forms were signed by the guardians and/or participants. The protocol of this study followed the recommendations of the 2010 CONSORT statement (http://www.consort-statement.org).

Study design and participants

This cross-sectional study divided individuals into two groups (control and MPS). The control group consisted of 10 systemically healthy individuals with good oral hygiene who attended the dental clinic of UNICEUMA, in São Luís,

Maranhão, Brazil. The MPS group was composed of 10 individuals with biochemical or molecular diagnosis of mucopolysaccharidosis regularly treated at the Hospital Materno Infantil, (São Luís, Maranhão, Brazil).

Individuals who made use of antibiotics or anti-inflammatory drugs in the month prior to the saliva collection were excluded from the study. For the control group, individuals with active carious lesions or periodontal disease were also excluded. A pilot study was carried out to evaluate the methodology and to calibrate examiners/evaluators. Individuals who participated in the pilot study were not included in the main study

Procedures

A questionnaire was applied to all participants for collection of demographic, socioeconomic and general health information. A detailed clinical examination was performed to assess the presence of dental caries,[16] periodontal disease[17] and enamel defects.[16] The participants were asked not to eat two hours prior to the saliva collection. Saliva samples were acquired following dental prophylaxis with additive-free prophylactic paste,[18] and were collected between 9–10 AM in order to minimize possible effects of the circadian rhythm.

Stimulated saliva samples were collected following chewing of plastic film (Parafilm®, 6.25 cm^2, Neenah, United States). The saliva collected within the first minute was discarded. Samples were then, deposited in sterile tubes kept on ice at every minute for 10 min. At the end of the last collection, the participants were instructed to expel the samples with the plastic film. Salivary flow was calculated as total saliva volume normalized by the collection time.[18]

All samples were centrifuged at 4°C, at 4000 rpm for 20 minutes, to remove debris and microorganisms present in the saliva. After centrifugation, the supernatant was collected and stored at -80°C, for further analysis.

NO_2^-/NO_3^- content was measured by the Griess colorimetric reaction, an indicator NO production. For this, NO_3^- was reduced to NO_2^- by incubating 80 µL of sample with 20 µL of 1U / ml nitrate reductase and 10 µL of 1 mM NADPH for 30 min, at 37°C, in a 96- well plate. Then, 100 µl Griess reagent, a 1: 1 mixture of 1% sulfanylamide and 0.1% of 5% naphthyl-enediamine-bi-chlorohydrate in orthophosphoric acid (H_3PO_4) were added per well and incubated for 15 min, at 37°C. The absorbance of each sample was determined at 550 nm in a spectrophotometer (MB-580/Heales, Shenzhen, China) and compared to a standard nitrite curve. Results were expressed as µM.

Additionally, the presence of NO_2^-, albumin, erythrocytes, leukocytes and, pH values in saliva samples were measured by using reagent strips (Biocon 10, VerconDiagnostic, Belo Horizonte, Brazil). For this, each reagent spot of the strips was covered with 20 µl of saliva and incubated for 120 or 60 seconds, for leukocyte and other markers, respectively. After this period, a semi-quantitative reading was performed by visually comparing the color chart and the color intensities reflected by each area. For evaluation of NO_2^- levels, a score (ranging from 1 to 3) was assigned, in which: 1=absence of NO_2^-, 2 = small/moderate amount of NO_2^- and 3 = large amount of NO_2^-. Protein (albumin) quantification results were also presented in scores (ranging from 1 to 3), where: 1=presence of albumin, 2 = moderate amounts of albumin and 3 = large amounts of albumin. For the evaluation of erythrocytes and leukocytes, the score ranged from 1 to 4 where: 1= absence, 2=traces, 3=moderate and 4= large amounts of white or red cells. The pH evaluation (5 to 9) was assessed by visually performing a comparison between the color chart and the color intensities of the strips after the addition of saliva.

Data analysis

Data are represented as mean ± SD or median and interquartile range (25–75th; IQR), depending on their distribution. Accordingly, we used parametric (unpaired t-test) or non-parametric (Mann-Whitney) tests to determine the significance of differences between groups in the NO_x values and NO_2, albumin, pH, erythrocytes and leucocytes scores. Correlations between the different parameters were determined using Spearman's rho. Socio-demographic and oral health data on the number of decayed and permanent teeth were compared between groups by Fisher's exact test. The medians of continuous variables such as visible plaque index, gingival bleeding on probing

index and salivary flow were compared by Wilcoxon ranking tests. Correlations between the evaluated parameters were measured by Spearman's correlation test. Significance level of 5% was considered for all analyses. Analyses were performed using Stata 16.0 software (StataCorp LLC, CollegeStation, USA).

Results

According to Table 1, MPS and control groups were composed of 60% of individuals aged 5 to 12 years old and 40% of individuals aged 13 to 22 years old. Both groups showed similar gender distribution, and most MPS individuals identified themselves as African Americans (70%). When inquired about their oral health, 70% of the individuals on the MPS group and 100% the individuals on the control group rated themselves as excellent and 30% of the patients from the MPS had a poor oral health. MPS patients and control groups were comparable in regards to numbers of decayed permanent and deciduous teeth (Table 1), the data shows that 100% of the patients of the control group did not have any type of decayed teeth, however for the MPS group 33% of patients presented at least one or more permanent/deciduous decayed teeth. Median and IIQR values observed for visible plaque index was higher in the MPS group compared with the control group (52.7 (17.5–97.9) versus 0; p< 0.001). The median of the gingival bleeding index was also

Table 1. Patients characteristics.

Characteristics	Healthy subjects	MPS	p-value*
	n (%)	n (%)	
Age[a] (years)			1.000
5–12	6 (60%)	6 (60%)	
13–22	4 (40%)	4 (40%)	
Gender[a]			1.000
Male	5 (50%)	5 (50%)	
Female	5 (50%)	5 (50%)	
Skin color[a]			0.350
White	5 (50%)	2 (20%)	
Brown	5 (50%)	7 (70%)	
Black	0 (0%)	1 (10%)	
Oral health[a]			0.211
Good	10 (100%)	7 (70%)	
Poor	0 (0%)	3 (30%)	
Decayed permanent teeth[a]			0.087
0	10 (100%)	6 (67%)	
1 +	0 (0%)	3 (33%)	
Decayed deciduous teeth[a]			0.417
0	3 (100%)	4 (67%)	
1 +	0 (0%)	2 (33%)	
	Median (IQ)	Median (IQ)	
Visible plaque index (%)[b]	0.0 (0.0–0.0)	52.7 (17.5–97.9)	< 0.001
Gingival bleeding index (%)[b]	0.0 (0.0– 0.0)	16.2 (0.0–45.5)	0.022
Salivary flow rate (mL/min)[b]	0.8 (0.6–1.5)	0.3 (0.2–0.3)	0.007

IQ: Interquartile Range; [a]Fisher's exact test; [b]Wilcoxon rank test.

higher in the MPS group in comparison with the control group (16.2 (0.0–45.5); versus 0; p = 0.022). Salivary flow rate was significantly higher (p = 0.007) in healthy subjects when compared to MPS, 0.8 and 0.3 mL/min, respectively (Table 1). Median and IQR values observed for albumin present in saliva are as follows: healthy subjects 1.0 (1.0–1.0) and MPS 2.0 (1.0–2.0) with a statistical difference between groups, p = 0.0087 (Figure A). In addition, the pH median and IQR values for healthy subjects and MPS where 7.0 (7.0–7.5) and 7.0 (6.0–7.0) being statistically different, p = 0.0074 (Figure B).

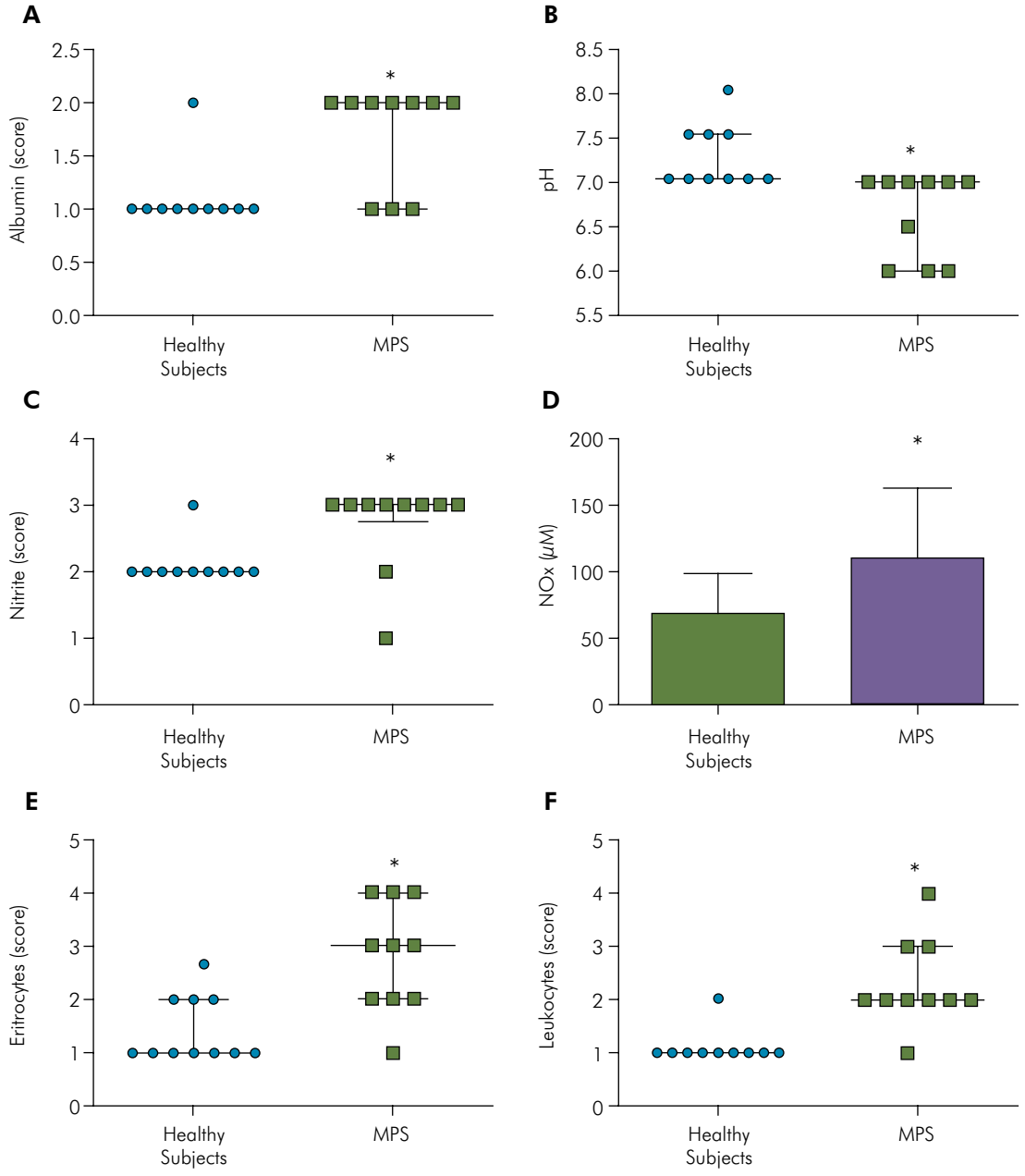

Figure.. Median and IQR values observed for albumin present in saliva (Figure A). Salivary pH median and IQR values for healthy subjects and MPS (Figure B). Amounts of nitrite observed in healthy and MPS patients (Figure C). Salivary NOx values (Figure D). Erythrocytes (Figure E) and Leukocytes (Figure F) scores in the saliva.

Table 2. Spearman's correlations coefficient between the Clinical/Biochemical parameters and NO^x levels in saliva (n = 20).

Variables	Healthy subjects			MPS		
	NO^x	pH	Protein	NO^x	pH	Protein
Visible plaque index	-	-	-	0.109	-0.233	0.898**
Gingival bleeding index	0.174	-0.266	-	-0.025	-0.007	0.909**
Salivary flow rate	-0.337	0.385	-	-0.559	0.219	-0.436
Number of decayed teeth	-	-	-	0.082	-0.125	0.877**
Number of teeth with defects	-	-	-	-0.225	-0.187	0.800*

*p < 0.05; **p < 0.001

Higher amounts of nitrite were observed in MPS patients, 3.0 (2.75–3.0), when compared to the control group, 2.0 (2.0–2.0), p = 0.0105 (Figure C). Figure D, presents the mean and standard deviations of NO^x in saliva, showing that patients with MPS had a higher amount (p=0.0386) of nitrite and nitrate (111.6 ± 51.8) in their saliva when compared to the healthy subjects (69.8 ± 28.5).

Erythrocytes were detected in 30% of the individuals of the control group in contrast with 90% of MPS patients (Figure E). I In both groups, three individuals presented traces of hemoglobin in their saliva, with values ranging from 0.1-10 RBC/µL of sample, in addition, six patients from the MPS presented a moderate or large amount of hemoglobin in their saliva with a variation of 50 to 250 RBC/µL.

Leukocytes were detected in the saliva of 10% of the healthy individuals and in 90% of the MPS patients. Among the MPS patients, six presented traces, two moderate amounts and one, a large amount of leukocytes in their saliva. In addition, the three MPS individuals with the highest amounts of leucocytes also presented the highest levels of salivary NO_x (Figure F).

Gingival bleeding on probing and visible plaque indexes were positively correlated with protein in the MPS group (r = 0.909 and r = 0.898, respectively). In addition, positive correlations were observed between the number of decayed teeth and protein (r = 0.877), and also, between the number of teeth with enamel defects (r = 0.800) and protein levels in MPS individuals (Table 2).

Discussion

Saliva has been used as a tool for the diagnosis, early detection, progression and monitoring of various systemic diseases.[8,9] Although the pathogenic mechanisms of MPS have been widely studied, they are not fully understood. Thus, the evaluation of nitrosative stress parameters and other biomarkers in saliva of MPS individuals may help understanding the underlying causes of the disease. According to our results, the salivary parameters evaluated in this clinical study can be considered potential biomarkers for disease monitoring.

The evaluation of NO_x levels is an indirect way to measure nitrite and nitrate levels in saliva.[19] To date, this is the first study to quantify salivary nitric oxide and nitrite levels in MPS patients. Based our data, it was found that the MPS group presented higher NO^x production and nitrite in saliva samples when compared to the control group (p = 0.00386). Blood and urine samples analysis from patients with type II MPS undergoing long-term Enzyme Replacement Therapy (ERT), showed a positive correlation between GAG levels and urinary nitrate and nitrite concentrations, as well as between IL-1β and NO^x plasma concentrations, presenting increased plasma and urine ROS/NRIs. These findings reinforce that, even under ERT, a NO-dependent inflammation is present in this serious disease.[13]

Interestingly, individuals with type II MPS under ERT exhibited an increase in NO and also in IL-1β.[13] Individuals with type II MPS under ERT showed increased NO, $NO_2^{\bullet}$ and $NO_3^- + NO_2^-$, but not superoxide dismutase activity or ONOO- levels [13]. NLRC4 inflammassome has been associated with pathogenic microorganisms, while NLRP3 has been associated with sterile inflammation; however, recent studies[20,21] have shown that NLRC4 can be activated in the absence of microorganisms by molecular patterns associated with damage.

Studies have indicated that GAG accumulation would be responsible for triggering inflammation and that even in individuals under ERT, there is an increase in proinflammatory mediators.[13,22,23,24] Thus, we believe in the potential involvement of NO in the induction and maintenance of inflammatory states in MPS individuals.

NO is considered one of the salivary components with promising potential for prediction of inflammatory events and its role in the pathogenesis of oral diseases has been investigated [2,25]. A recent study associated total salivary nitrate and nitrite levels with periodontal health in 74 individuals.[25] The authors observed that the increase in salivary nitrate and nitrate concentrations was related to periodontal changes and that periodontal treatment led to a decrease in the levels of these markers. The same study concluded that salivary nitrate and nitrite levels are associated with defense mechanisms and suggested that salivary glands may respond to infectious oral diseases, increasing the release of NO.[25]

In the present study, positive correlations were observed between protein and gingival bleeding on probing, visible plaque indexes, number of decayed teeth and number of teeth with defects in MPS patients, with a significant statistical difference vs control. When we correlated the same parameters with only the dosed nitrite in reagent strips, a weak correlation was obtained. It is known that there is an increase in the production of oral nitric oxide during the beginning of dental plaque deposition.[26] Similar studies suggest that this increase results from overexpression of the gingival cell-induced nitric oxide synthase (iNOS) enzyme. Such excess is not only derived from bacterial proliferation, but also from cytokine production stimulated by the presence of bacterial plaque.[27,28] Overall, the protein increase is mostly involved in tissue protection and inflammatory control, suggesting the activation of protective pathways, including antibacterial activity, regulation of the inflammatory process, antioxidants, and protease inhibitors characterizing the host responses. The negative moderate correlation between salivary flow rate and NO_x in the MPS patients indicates that oxidative stress causes changes in the structure of the salivary gland and is involved in hyposalivation. The accumulation of GAGs in the cells causes an inflammatory response and it is mediated by neutrophils migrating from the blood stream to the gingival tissues and into the oral cavity through the GCF or saliva. We speculate that the increase in oral erythrocytes, leucocytes and NO_x in MPS patients is related to cell disruption caused by the GAG accumulation.

During the inflammatory response, NO production in the oral cavity related to periodontal disease occurs via iNOS. Fibroblasts, inflammatory infiltrate cells, and other periodontal cells begin to produce NO via iNOS. Despite being a defense mechanism, excessive amounts of this free radical may contribute to tissue destruction in pathogenic periodontitis.[23,26] Considering that MPS individuals in this study presented plaque and gingival bleeding on probing indexes and high NO_x levels, added to plaque retentive factors, such as enamel defects, it is suggested that these individuals are more likely of developing periodontal diseases.

Some plasma-derived diagnostic markers, such as albumin, can be found in saliva. Albumin is the major plasma protein and its concentration in saliva depends on periodontal health, presence (or absence) of teeth and oral mucosa integrity.[29] Helmerhorst et al.[29] demonstrated that plasma-derived albumin concentration increased after oral hygiene ceased and returned to baseline levels after oral hygiene resumption in individuals with gingivitis. The present study evaluated the albumin levels in saliva from individuals in both groups. When the control group was compared to the MPS, it was possible to observe that MPS presented higher levels of albumin, where 70% of the patients presented albumin in a range of 30 to 100 mg/dL, according to Carda et al.[30] the usual amount of albumin in saliva should not be higher than 30 mg/dL. There was strong correlation between this plasma protein and gingival bleeding on probing index ($r = 0.909$, $p < 0.001$) and visible plaque index ($r = 0.898$, $p < 0.05$), confirming that of periodontal health influences the albumin concentration in saliva.

When the presence of erythrocytes in saliva is analyzed by the reagent strips, tree samples

from the control group had red blood cells, even though clinically bleeding on probing was not detected, this could possibly be explained by trauma occurring during dental prophylaxis or even by chewing the plastic film. Interestingly, 90% of the patients of MPS group presented higher amounts of RBC in their saliva. In addition, individuals with type II MPS have elevated saliva leukocyte levels. This could be explained by the continued use of Risperidone, since this drug consists of an anti-psychotic that may present leukocytosis as adverse effect. Moreover, we discovered that the same individual was making use of unsuitable enzyme doses. Thus, it is possible that this may have decreased the expected protective effect of ERT and may have contributed to both the leukocytes increase and the higher NO^x levels. In contrast, this individual did not have a higher level of salivary albumin, despite the presence of leukocytosis, which makes it difficult to attribute high saliva leukocyte levels to local inflammatory factors such as periodontal disease. As this is an unusual finding, it is likely that such an occurrence is not of local inflammatory nature, such as gingivitis, but of a systemic nature, since local inflammatory response would cause the presence of albumin in saliva.

The average plaque index found in our results was 54.08%, and half of individuals had indexes greater than 50% and three of these indexes greater than 95%. These results support the hypothesis that MPS individuals are more likely of having caries and periodontal disease. Only three individuals had no gingival bleeding on probing Orofacial alterations observed in these individuals, such as malocclusion, mouth opening limitations, macroglossia and enamel defects,[31,32] in addition to motor and intellectual limitations, may have contributed to the oral condition observed in this study.

A strong correlation between the number of teeth with enamel defects and the number of decayed teeth was also observed. The enamel of MPS individuals is fragile and more porous,[33,34,35] especially those with type IV MPS. These defects topographically modify the dental enamel, facilitating bacterial adhesion.[36] Some dental structural alterations have been described in type I PMS, such as narrow dentinal tubule, dentin deposition in irregular wave pattern, small cracks in the enamel-dentinal junction, altered arrangement of enamel prisms.[35] Deficiency of the N-acetyl galactosamine-6-sulfatase enzyme in IVA type MPS results in the pathological accumulation of GAG keratan sulfate and chondroitin 6-sulfate in the secretory phase ameloblast lysosomes, contributing to the formation of defective enamel.[37]

Stimulated salivary flow < 0.8ml/min is considered a pathological condition and has been considered a strong indicator of increased caries risk.[37,38,39] In the present study, salivary flow rate and pH of stimulated saliva were reduced in MPS individuals when compared to controls, corroborating previous studies.[38] Eight subjects had salivary flow rate less than or equal to 0.3 ml / min. We believe that the causes of hyposalivation in this group of individuals are related to the orofacial characteristics that influence chewing performance[40] and the possible GAG accumulation in the salivary glands, since there is evidence of GAG accumulation in organs such as liver, kidneys and spleen in IIIC type MPS models.[40]

In addition, it was possible to observe that MPS individuals presented lower salivary pH values when compared to the heathy group, which does not corroborate literature findings about the influence of salivary pH reduction on the dental caries process.[39] However, some studies have shown correlation between these two findings.[39] A positive correlation between salivary flow and pH was observed in our results, corroborating a study[38] in which MPS individuals presented lower salivary flow and pH averages compared to healthy controls.[38] Furthermore, the correlation between visible dental plaque index with salivary flow and pH was investigated, resulting in negative correlation among these parameters. These data reinforce the increased risk of caries in these individuals.

In conclusion, our data indicate that the salivary parameters evaluated in this study could help understanding the pathophysiology of mucopolysaccharidosis. Thus, we suggest the quantification of these salivary parameters to comprehensively monitor the disease stage and development.

References

1. Kingma SD, Bodamer OA, Wijburg FA. Epidemiology and diagnosis of lysosomal storage disorders; challenges of screening. Best Pract Res Clin Endocrinol Metab. 2015 Mar;29(2):145-57. https://doi.org/10.1016/j.beem.2014.08.004
2. Khan AS, Perecha H, Ballhausen D, Wiesbauer A, Rohrbach M, Gautschi M, et al. Epidemiology of mucopolysaccharidoses. Mol Genet Metabol.. 2017 July;121(3):227-40. https://doi.org/10.1016/j.ymgme.2017.05.016
3. Neufeld E, Muenzer J. The online metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2014.
4. Sarmento DJS, Carvalho SH, Melo SL, Fonseca FR, Diniz DN, Bento PM, et al. Mucopolysaccharidosis: radiographic findings in a series of 16 cases. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015 Dec;120(6):e240-6. https://doi.org/10.1016/j.oooo.2015.08.009
5. Slaughter YA, Malamud D. Oral diagnostics for the geriatric populations: current status and future prospects. Dent Clin North Am. 2005 Apr;49(2):445-61. https://doi.org/10.1016/j.cden.2004.10.007
6. Malm G, Lund AM, Månsson JE, Heiberg A. Mucopolysaccharidoses in the Scandinavian countries: incidence and prevalence. Acta Paediatr. 2008 Nov;97(11):1577-81. https://doi.org/10.1111/j.1651-2227.2008.00965.x
7. Lin HY, Lin SP, Chuang CK, Niu DM, Chen MR, Tsai FJ, et al. Incidence of the mucopolysaccharidoses in Taiwan, 1984-2004. Am J Med Genet A. 2009;149(A):960-4. https://doi.org/10.1002/ajmg.a.32781
8. Li Y, St John MA, Zhou X, Kim Y, Sinha U, Jordan RC, et al. Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res. 2004 Dec;10(24):8442-50. https://doi.org/10.1158/1078-0432.CCR-04-1167
9. Sunitha M, Shanmugam S. Evaluation of salivary nitric oxide levels in oral mucosal diseases: A controlled clinical trial. Indian J Dent Res. 2006 Jul-Sep;17(3):117-20. https://doi.org/10.4103/0970-9290.29878
10. Tikhonova S, Booij L, D'Souza V, Crosara KT, Siqueira WL, Emami E. Investigating the association between stress, saliva and dental caries: a scoping review. BMC Oral Health. 2018 Mar;18(1):41. https://doi.org/10.1186/s12903-018-0500-z
11. Brosky ME. The role of saliva in oral health: strategies for prevention and management of xerostomia. J Support Oncol. 2007 May;5(5):215-25.
12. Spielmann N, Wong DT. Saliva: diagnostics and therapeutic perspectives. Oral Dis. 2011 May;17(4):345-54. https://doi.org/10.1111/j.1601-0825.2010.01773.x
13. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964 Feb;22(1):121-35. https://doi.org/10.3109/00016356408993968
14. Amado FM, Ferreira RP, Vitorino R. One decade of salivary proteomics: current approaches and outstanding challenges. Clin Biochem. 2013 Apr;46(6):506-17. https://doi.org/10.1016/j.clinbiochem.2012.10.024
15. Jacques CE, Donida B, Mescka CP, Rodrigues DG, Marchetti DP, Bitencourt FH, et al. Oxidative and nitrative stress and pro-inflammatory cytokines in Mucopolysaccharidosis type II patients: effect of long-term enzyme replacement therapy and relation with glycosaminoglycan accumulation. Biochim Biophys Acta. 2016 Sep;1862(9):1608-16. https://doi.org/10.1016/j.bbadis.2016.05.021
16. Carter HG, Barnes GP. The gingival bleeding index. J Periodontol. 1974 Nov;45(11):801-5. https://doi.org/10.1902/jop.1974.45.11.801
17. Siqueira Junior WL, Nicolau J. Stimulated whole saliva components in children with Down syndrome. Spec Care Dentist. 2002 Nov-Dec;22(6):226-30. https://doi.org/10.1111/j.1754-4505.2002.tb00276.x
18. Siqueira WL, Salih E, Wan DL, Helmerhorst EJ, Oppenheim FG. Proteome of human minor salivary gland secretion. J Dent Res. 2008 May;87(5):445-50. https://doi.org/10.1177/154405910808700508
19. Meschiari CA, Zuardi LR, Gomes VA, Almeida GRC, Novaes Junior AB, Gerlach RF, et al. Salivary, blood and plasma nitrite concentrations in periodontal patients and healthy individuals before and after periodontal treatment. Clin Chim Acta. 2015 Apr;444(444):293-6. https://doi.org/10.1016/j.cca.2015.02.045
20. Donida B, Marchetti DP, Biancini GB, Deon M, Manini PR, Rosa HT, et al. Oxidative stress and inflammation in mucopolysaccharidosis type IVA patients treated with enzyme replacement therapy. Biochim Biophys Acta. 2015 May;1852(5):1012-9. https://doi.org/10.1016/j.bbadis.2015.02.004
21. Donida B, Marchetti DP, Jacques CE, Ribas G, Deon M, Manini P, et al. Oxidative profile exhibited by Mucopolysaccharidosis type IVA patients at diagnosis: increased keratan urinary levels. Mol Genet Metab Rep. 2017 Apr;11:46-53. https://doi.org/10.1016/j.ymgmr.2017.04.005
22. Organização Mundial da Saúde. Levantamentos básicos em saúde bucal. Geneva: Organização Mundial da Saúde ; 1999. p. 21-52 .
23. Donida B, Jacques CE, Mescka CP, Rodrigues DG, Marchetti DP, Ribas G, et al. Oxidative damage and redox in lysosomal storage disorders: biochemical markers. Clin Chim Acta. 2017 Mar;466:46-53. https://doi.org/10.1016/j.cca.2017.01.007
24. Parwani SR, Chitnis PJ, Parwani RN. Salivary nitric oxide levels in inflammatory periodontal disease: a case-control and interventional study. Int J Dent Hyg. 2012 Feb;10(1):67-73. https://doi.org/10.1111/j.1601-5037.2011.00508.x
25. Sánchez GA, Miozza VA, Delgado A, Busch L. Total salivary nitrates and nitrites in oral health and periodontal disease. Nitric Oxide. 2014 Jan;36:31-5. https://doi.org/10.1016/j.niox.2013.10.012

26. Carossa S, Pera P, Doglio P, Lombardo S, Colagrande P, Brussino L, et al. Oral nitric oxide during plaque deposition. Eur J Clin Invest. 2001 Oct;31(10):876-9. https://doi.org/10.1046/j.1365-2362.2001.00902.x
27. Rausch-Fan X, Matejka M. From plaque formation to periodontal disease, is there a role for nitric oxide? Eur J Clin Invest. 2001 Oct;31(10):833-5. https://doi.org/10.1046/j.1365-2362.2001.00903.x
28. Reher VG, Zenóbio EG, Costa FO, Reher P, Soares RV. Nitric oxide levels in saliva increase with severity of chronic periodontitis. J Oral Sci. 2007 Dec;49(4):271-6. https://doi.org/10.2334/josnusd.49.271
29. Helmerhorst E, Dawes C, Oppenheim F. The complexity of oral physiology and its impact on salivary diagnostics. Oral Dis. 2017;18:1-23.
30. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal. 2006 Jul;11(4):E309-14.
31. Fonseca FR, Sarmento DJS, Medeiros PFV, Diniz DN, Santos MT. Patients with mucopolysaccharidosis have tendencies towards vertical facial growth. J Oral Maxillofac Surg. 2014 Dec;72(12):2539-46. https://doi.org/10.1016/j.joms.2014.07.006
32. Carneiro N, Deps TD, França EC, Valadares ER, Pordeus IA, Borfes-Oliveira AC. Oral health of children and adolescents with mucopolysaccharidosis and mother's Sence of Coherence. Spec Care Dentist. 2017 Sep;37(5):223-9. https://doi.org/10.1111/scd.12238
33. Almeida-Barros RQ, Medeiros PF, Azevedo MQA, Ortega AOL, Yamamoto AT, Dornelas SK, et al. Evaluation of oral manifestations of patients with mucopolysaccharidosis IV and VI: clinical and imaging study. Clin Oral Investig. 2018 Jan;22(1):201-8. https://doi.org/10.1007/s00784-017-2100-8
34. Fitzgerald J, Verveniotis SJ. Morquio's syndrome: a case report and review of clinical findings. N Y State Dent J. 1998 Oct;64(8):48-50.
35. Guven G, Cehreli ZC, Altun C, Sençimen M, Ide S, Bayari SH, et al. Mucopolysaccharidosis type I (Hurler syndrome): oral and radiographic findings and ultrastructural/chemical features of enamel and dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 Jan;105(1):72-8. https://doi.org/10.1016/j.tripleo.2007.02.015
36. Vargas-Ferreira F, Zeng J, Thomson WM, Peres MA, Demarco FF. Association between developmental defects of enamel and dental caries in schoolchildren. J Dent. 2014 May;42(5):540-6. https://doi.org/10.1016/j.jdent.2014.02.010
37. Yamakoshi Y, Hu JC, Liu S, Sun X, Zhang C, Oida S, et al. Porcine N-acetylgalactosamine 6-sulfatase (GALNS) cDNA sequence and expression in developing teeth. Connect Tissue Res. 2002;43(2-3):167-75. https://doi.org/10.1080/03008200290001131
38. Leone CW, Oppenheim FG. Physical and chemical aspects of saliva as indicators of risk for dental caries in humans. J Dent Educ. 2001 Oct;65(10):1054-62. https://doi.org/10.1002/j.0022-0337.2001.65.10.tb03449.x
39. Ponciano S, Areias C, Leão-Teles E, Sampaio-Maia B. Hyposalivation, acidic saliva, decayed teeth and oral yeast prevalence in children with mucopolysaccharidosis. MedicalExpress (São Paulo). 2015;2(5):1-7. https://doi.org/10.5935/MedicalExpress.2015.05.02
40. Ericsson Y. Clinical investigation of the salivary buffering action. Acta Odontol Scand. 1959;97(2):131-65. https://doi.org/10.3109/00016355908993928