

Hyposalivation, acidic saliva, decayed teeth and oral yeast prevalence in children with mucopolysaccharidosis

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OBJECTIVE: Patients with mucopolysaccharidosis present several alterations of the stomatognathic complex, however, no data is available on saliva biochemistry and yeast colonization. The aim of the study was to evaluate caries experience as well as saliva biochemistry and microbiology parameters in patients with mucopolysaccharidosis.

METHOD: The sample consisted of twelve participants with mucopolysaccharidosis followed in the Metabolic Disease Unit of the Centro Hospitalar de S. João and twelve healthy participants followed at the Faculty of Dental Medicine, University of Porto. To all participants, Decayed, Missing, Filled Teeth (DMFT) index was evaluated. In addition, saliva was collected to evaluate biochemical parameters (flow rate, pH, sodium, potassium, chloride, calcium, phosphate, α -amylase and IgA) and the microbiological profile (total microorganisms, mutans streptococci and yeasts) of all participants.

RESULTS: In comparison to controls, the mucopolysaccharidosis patients presented a higher prevalence of decayed teeth, lower salivary flow and pH values. They also presented also lower calcium and higher phosphate ions in saliva. No differences were found between groups regarding oral microbial load for total microorganisms, mutans streptococci and yeasts as well as oral prevalence of mutans streptococci. However, MPS patients presented higher prevalence of oral *Candida* in comparison to controls.

CONCLUSION: The higher prevalence of decayed teeth and higher oral yeast colonization in MPS patients may be related to the lower saliva calcium concentration, pH and flow.

KEYWORDS: mucopolysaccharidosis; oral health; pediatrics; candida, salivary pH.

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INTRODUCTION

Mucopolysaccharidosis (MPS) are a heterogeneous group of genetic disorders caused by deficiency of the enzymes responsible for the degradation of glycosaminoglycans, causing the accumulation of these macromolecules in lysosome present in various tissues and organs. The intracellular and extracellular accumulation of these non-metabolized substances causes dysfunction in multiple organs and systems.¹⁻³

MPS are classified into seven major groups (I, II, III, IV, VI, VII, IX) according to the deficient enzyme and the accumulated substrate.^{4,5} Type II is linked to chromosome

X, whereas all the others are autosomal recessive disorders.^{4,6,7} It is estimated that the overall incidence of MPS is around 4:100,000 live births (0.04%).^{8,9} In Portugal, the prevalence of birth is estimated to be of 4.8:100,000.¹⁰

Each type of MPS is associated with a wide range of clinical heterogeneity.^{2,4} Classically, MPS presents a particular phenotype with occurrence of dwarfism, macrocephaly, coarse facial changes, umbilical and inguinal hernias, delayed motor skills, bone dysplasias, dysostoses, limited joint mobility, hearing loss, ophthalmological involvement (corneal clouding, glaucoma, optic atrophy) progressive neurodegenerative disease, heart disease (valvular disease, cardiomyopathy, arrhythmias), respiratory distress (obstructive/restrictive disease) and

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hepatosplenomegaly,^{1-3,5,7,11} requiring always the support of a multidisciplinary team.⁶

MPS patients also present oral manifestations that are observed either clinically or radiographically. Macroglossia, anterior open bite, gingival hyperplasia, spaced dentition high palate, condylar defects and thick lips are common oral features described in association with MPS.^{5-7,11-17} In this population, teeth have been described as microdontics, peg-shaped and hypoplastic. Delayed eruption of teeth may also be present and associated to the presence of cystic lesions or thickness of the dental follicle.^{12,18,19} However, conflicting results regarding caries occurrence have been reported.^{6,7,12,15,20} So, the aim of this study was to assess caries, as well as biochemical and microbiological salivary parameters in patients with MPS and compare this information with that of a healthy population.

■ MATERIAL AND METHODS

The sample consisted of all 12 patients with MPS followed in Metabolic Diseases Unit of Centro Hospitalar de S. João, EPE/Faculty of Medicine, University of Porto (Feb 2013 - Feb 2014) with a positive diagnosis resulting from enzyme assay;⁴ the control group consisted of twelve healthy participants followed at Faculty of Dental Medicine, University of Porto; patients and controls were between 5 and 28 years old.

The research project was approved by the ethics committees of the Faculty of Dental Medicine and of the Centro Hospitalar de S. João. An informed, free and clear consent was provided for and signed by all participants or by their parents/legal guardians. Storage and processing of data guaranteed confidentiality of all information, thereby respecting the rules of conduct expressed in the Declaration of Helsinki.

The total number of decayed, missing and filled teeth (DMFT) was recorded for each patient and control in order to characterize the epidemiological history of caries in both groups, using a mirror and explorer in accordance with World Health Organization criteria and methods.²¹ The examiner was an experienced dentist, with high intra-examiner reliability (Kappa = 0.89). Saliva was collected to evaluate the biochemical and microbiological profile. To collect saliva, the participants were asked to chew paraffin pellets (Ivoclar Vivadent, NY, USA) over a 5 minutes period, for stimulated salivary secretion. The salivary pH was measured immediately after saliva collection using pH indicator paper (5.0-8.0, Duotest, Germany). Saliva for biochemistry analysis was frozen directly at -80 °C, whereas the saliva collected for microbiological analysis was mixed 1:1 with Brain Heart Infusion broth (Cultimed, Barcelona, Spain) with 15% glycerol and then frozen at -80 °C until assayed. The saliva volume was registered with

graduated pipettes for stimulated salivary flow rate (mL/min) calculation.^{22,23}

The biochemical evaluation of collected saliva was performed using the automated analyser Pentra C200 (Horiba ABX Diagnostics, Switzerland). In brief, salivary IgA was determined by immunoturbidimetry; potassium and chlorine were evaluated by potentiometry using ion selective electrodes; phosphate was detected by UV phosphomolybdate; and α -amylase was detected by an enzymatic photometric assay using 4,6-ethylidene (G7)-p-nitrophenyl (G1)- α -D maltoheptaoside (EPS-G7) as the substrate.^{22,23}

For microbiological analysis, the saliva samples were rapidly defrosted in a 37 °C water bath, thoroughly homogenized and serially diluted up to 10⁻⁴ in 0.9% sterile NaCl solution. The samples were immediately plated in triplicate (i) on Brain Heart Infusion agar culture media (Cultimed, Barcelona, Spain) to assess the number of total microorganisms from oral cavity; (ii) on Mitis Salivarius agar (BD Difco, Barcelona, Spain) containing 0.2 units of bacitracin/ml plus 20% sucrose to detect *Streptococcus mutans*; (iii) on Sabouraud agar (Cultimed, Barcelona, Spain) supplemented with chloramphenicol to evaluate the presence of fungi. The Brain Heart Infusion agar was incubated aerobically at 37 °C for four days; Mitis Salivarius with bacitracin was incubated anaerobically for five to seven days at 37 °C; Sabouraud agar was incubated aerobically for 48 h at 37 °C. Colonies were counted, and the results were expressed in Log₁₀ of colony forming units per mL of saliva (CFU/mL).^{22,23}

Statistical analysis

The analyses were performed using the statistical analysis software Statistical Package for Social Sciences (SPSS) 21.0 for MAC OS. The categorical variables were summarized as relative and absolute frequencies (%) and the continuous variables were described using the mean \pm standard deviation. When appropriate, the chi-square independence test was used to analyze hypotheses regarding the categorical variables; the Mann-Whitney U Test was used for the continuous variables. A level of 0.05 was considered significant.

■ RESULTS

The mean age of both MPS and control groups did not differ significantly (16.5 \pm 7.5 vs. 14.4 \pm 6.3, p = 0.506). Gender distribution between groups did not differ significantly (p = 0.637): the MPS group included six males, whereas the control group included eight males. The MPS group included one patient with MPS I, one patient with MPS II, one with MPS IIIC, one with MPS IV and eight patients with MPS VI.

Although no differences were observed in the Decay-missing-filled-teeth (DMFT) index (7.7 \pm 1.4 vs. 4.5 \pm 1.2, p = 0.098), MPS patients presented significantly

greater prevalence of decayed teeth (5.7 ± 1.0 vs. 1.5 ± 0.5 , $p = 0.001$) and lower prevalence of filled teeth than the control group (0.7 ± 0.4 vs. 2.7 ± 0.8 , $p = 0.037$), as shown in Figure 1.

Salivary flow, and chemical analysis of saliva for are shown in Table 1. MPS patients presented reduced salivary flow rates and a lower pH in comparison to controls. They also presented a reduction of 35% in calcium ions and an increase of 44% in phosphate ions. Other analyzed ions (chlorine, potassium, and sodium) presented no statistically significant differences between the two groups. Values for α -amylase and IgA did not differ between the two groups.

Figure 2 exhibits microbial prevalence and oral loads. No differences between groups regarding prevalence or microbial load (7.58 ± 0.11 vs. 7.43 ± 0.13 CFU/ml, $p = 0.725$). The oral Mutans streptococci group also exhibited similar prevalence and microbial load (4.35 ± 0.37 vs. 4.59 ± 0.16 CFU/ml, $p = 0.880$), between MPS patients and controls. We found a higher prevalence ($p < 0.05$) of yeast in the MPS vs. the control group as shown in Figure 2; however, the yeast load did not differ between groups (2.95 ± 0.23 vs. 2.57 ± 0.16 , $p = 0.392$).

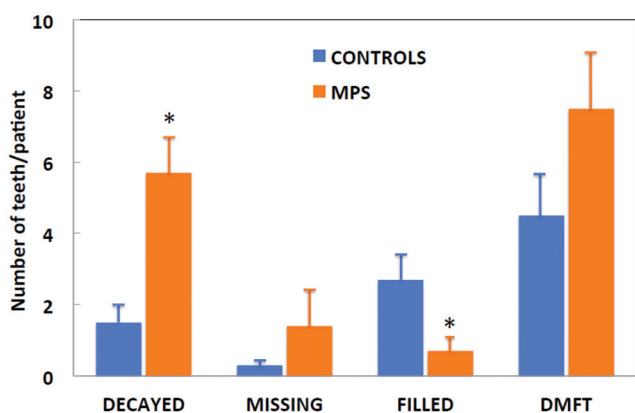


Figure 1 - Number of decayed, missing, or filled teeth/patient and respective Decayed-Missing-Filled teeth index (DMFT) in normal (controls) children and in children with mucopolysaccharidosis (MPS). * $p < 0.05$.

DISCUSSION

Mucopolysaccharidosis patients observed in this study presented a high prevalence of caries activity compared with control participants, a result which conflicts with some of the existing reports on the subject.^{5-7,12,15,20,24-27} We suggest that the increase of caries in this MPS population may be associated to the presence of reduced levels of salivary flow, at an acidic pH in comparison to controls. Also, the higher prevalence of oral *Candida* may be due to the acidic oral environment, and may actually contribute to it.

Dental Caries is a multifactorial disease that affects people of all ages. It is determined by the coexistence of three main factors, namely host factors, frequency of ingestion of carbohydrates and the presence of acidogenic and acidophilic microorganisms in the oral biofilm.²⁸ Social and behavioral determinants may also play an important role in its etiology.^{28,29} Thus, dental caries result from the dynamic balance between aggressive factors that lead to demineralization vs. protective factors that lead to remineralization.²⁹

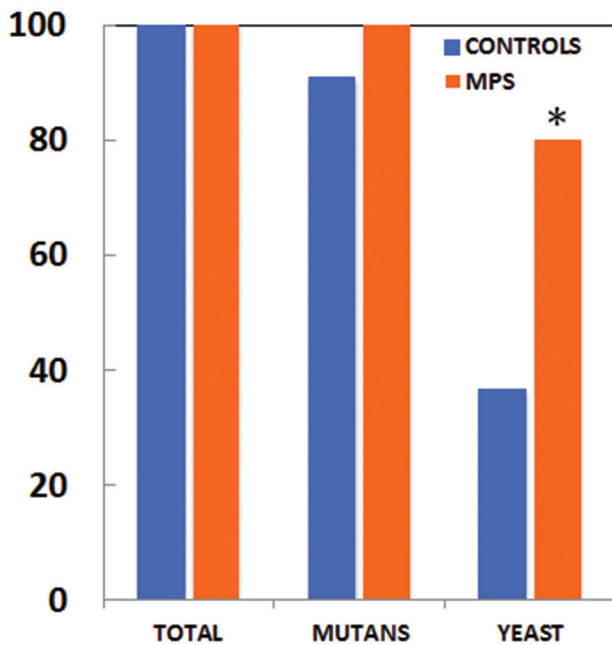
We found low levels of calcium in patients with mucopolysaccharidosis and this can be associated to medication such as the calcium channel blocker nifedipine. This concept is supported by previous findings indicating that calcium channel blockers lead to salivary secretion with reduced calcium concentrations.³⁰⁻³² The presence of vitamin D deficiency, quite common in the MPS population, may also negatively impact bone mineralization, and alter calcium concentrations in the saliva.^{33,34} We should note that the patients included in this study were on Vitamin D supplementation. Three different reports suggest that Vitamin D induces higher calcium intake, probably by enhancement of calcium absorption.³⁵⁻³⁷ Interestingly, patients diagnosed with vitamin D deficiency and low calcium values have more dental enamel defects and caries when compared with healthy children.^{34,38,39} The relation between a low calcium content in saliva and caries has also been reported.^{36,37} It is suggested that lower values

Table 1- Salivary flow, pH, ions and salivary proteins in control and MPS groups

	MPS	Control	p
Salivary flow rate, ml/min	0.75 ± 0.61	2.46 ± 1.42	0.013
pH	6.66 ± 0.52	7.40 ± 0.47	0.006
Sodium, mmol/L	30.70 ± 18.00	28.96 ± 11.60	> 0.999
Potassium, mmol/L	26.54 ± 10.63	25.06 ± 3.69	0.630
Chlorine, mmol/L	13.26 ± 12.05	7.20 ± 0.60	0.196
Calcium, mg/dL	0.32 ± 0.28	0.49 ± 0.30	0.044
Phosphate, mg/dL	18.76 ± 97.56	13.05 ± 3.47	0.043
α -Amylase, U/L	835.50 ± 618.19	580.41 ± 232.57	0.691
IgA, g/L	11.63 ± 4.90	9.00 ± 5.92	0.146

Values are shown as mean ± SD; p-values were calculated using the Mann-Whitney U T

A. PREVALENCE (PERCENTAGE)



B. LOAD (LOG₁₀ CFU/ML)

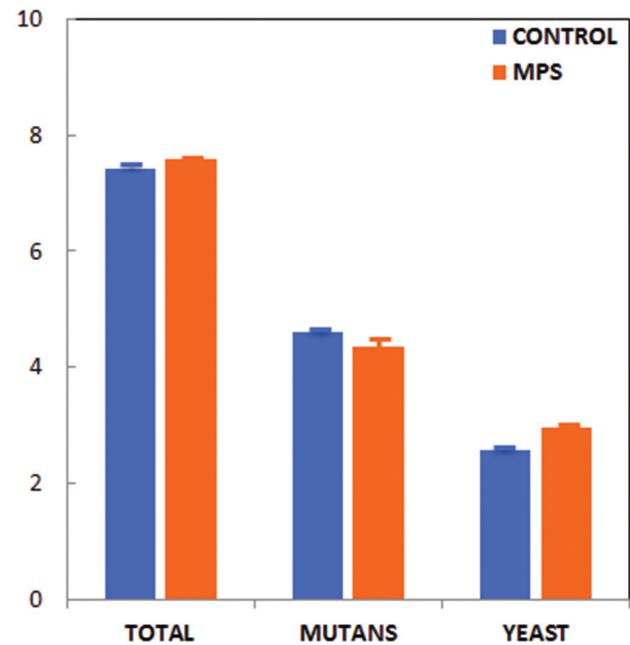


Figure 2 - Prevalence (A) and load of Colony Forming Units - CFU (B) of total bacteria, mutans strptococci and yeast in normal children (controls) and in children with mucopolysaccharidosis (MPS). * p < 0.05.

of calcium enhance enamel demineralization, reduce remineralization and increase alveolar bone loss.^{35,37} In parallel with low calcium levels in saliva, we found high levels of phosphate. Phosphate homeostasis is coordinated by complex cross organ axis.⁴⁰ Parathyroid hormone secreted in response to low serum calcium can increase phosphate efflux from bone, kidney and intestine. Important factors, such as Vitamin D, are involved in maintaining calcium and primarily phosphate balance.⁴⁰⁻⁴² The formation and mineralization of bone and teeth are greatly influenced by calcium and phosphate metabolism since both consist mainly of calcium-phosphate.⁴³ Interestingly, Schroth et al. reported that children with caries were significantly more likely to have low vitamin D, calcium, and elevated PTH levels.⁴⁴

In our study, MPS patients presented reductions of salivary flow and pH. These alterations could be due not only to the genetic changes but also to medication such as nifedipine and furosemide.⁴⁵⁻⁴⁸ Hyposalivation reduces the buffering capacity, the elimination capacity of cariogenic nutrients, and the antimicrobial protection, leading to the appearance of dental caries.^{49,50} The low salivary pH is directly associated with high caries activity not only by promoting teeth demineralization in itself, but also by promoting the growth of acidogenic and aciduric microorganisms, that in turn will further decrease the oral pH.⁴⁹⁻⁵¹ In confirmation of this idea, we found that MPS patients presented increased oral yeast. The most prevalent

oral yeast is *Candida* spp. a well known and significant acidogenic microorganism.⁵² So, the ecological changes in oral environment, such as the reduction in salivary flow and pH, could stimulate the growth of *Candida* spp. and induce a more acidic Stephan pH response.

Interestingly, MPS patients did not present higher loads of mutans streptococci. However, dental caries may occur in their absence, if other microorganisms are present, capable of producing substantial amounts of acid from fermentable carbohydrate; oral *Candida* is a well established candidate for such an action.⁵¹

The higher prevalence of oral *Candida* can also be justified by the immune changes of MPS patients that are still under study.⁵³ Notwithstanding, no significant differences were found between groups regarding oral IgA concentration, the main immunoglobulin present in the oral cavity.

MPS patients present severe teeth morphological alterations, and in the absence of X-ray imaging, it was not possible to discriminate between primary and permanent dentitions.^{5-7,11-17} So, in the present study, the global DMFT index was calculated although some participants had still primary teeth.

In addition to the higher caries rate, MPS patients presented a lower number of filled teeth, revealing a level of neglected dental care. In other groups of chronic patients this dental carelessness has also been documented.⁵⁴ Additionally, access to dental care may be late and difficult,

delaying the identification of dental problems.⁶ So there is an evident need to educate and motivate not only patients but also their parents or legal guardians to ensure the improvement of oral hygiene routines. Dental treatment must be integrated into a multidisciplinary team working with health professionals from all areas, intervening early in order to improve the quality of life of patients and their families. It is important to encourage regular visits to dentists, in order to avoid complex treatments involving sedation and general anesthesia. Treatment should be based on a preventive protocol promoting the use of fissure sealants, fluoride, dietary control and good oral hygiene habits.

In conclusion, MPS patients present higher prevalence of decayed teeth; therefore preventive measures to avoid caries should be especially promoted in this population. Key factors responsible for the higher prevalence of decayed teeth in MPS patients may include lower salivary calcium concentration, pH and flow. Also, the higher prevalence of oral *Candida* may be due to, but also a contributor to the acidic oral environment.

■ SUMMARY

Patients with mucopolysaccharidosis present higher prevalence of decayed teeth that may be associated to (i) hyposalivation, (ii) lower salivary calcium concentration and pH and/or (iii) higher oral yeast colonization.

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■ CONFLICT OF INTEREST

Sara Ponciano, Cristina Areias, Elisa Leão-Teles and Benedita Sampaio-Maia declare that they have no conflict of interest.

■ AUTHOR CONTRIBUTIONS

All the authors reviewed the literature and conceived the study. Ponciano S and Leão-Teles E were involved in patient selection, monitoring and data collection at Centro Hospitalar de S. João. Ponciano S and Areias C were involved in patient selection, monitoring and data collection at Faculty of Dental Medicine. Sampaio-Maia B and Ponciano S performed the biochemistry and microbiology analysis, the statistical analysis and wrote the manuscript. All authors reviewed the manuscript.

HIPOSALIVAÇÃO, SALIVA ACÍDICA, DENTES CARIADOS E PREVALÊNCIA ORAL DE LEVEDURA EM CRIANÇAS COM MUCOPOLISACARIDOSE

OBJETIVO: Pacientes portadores de mucopolissacaridose apresentam várias alterações do complexo estomatognático; no entanto, não existem dados disponíveis sobre a bioquímica da saliva e ou sobre a colonização por fungos. O objetivo deste estudo foi avaliar a prevalência de cárie dentária bem como parâmetros bioquímicos e microbiológicos em pacientes com mucopolissacaridose.

MÉTODO: A amostra foi constituída por doze participantes com mucopolissacaridose, acompanhados na Unidade de Doenças Metabólicas do Centro Hospitalar de S. João do Porto e por doze participantes saudáveis acompanhados na Faculdade de Medicina Dentária da Universidade do Porto. Para todos os participantes, o índice de dentes cariados, perdidos ou obturados foi avaliado. Além disso, foram recolhidas amostras de saliva de todos os participantes para avaliar os parâmetros bioquímicos (fluxo salivar, pH, sódio, potássio, cloreto, cálcio, fosfato, α -amilase e IgA) e microorganismos tais como *Streptococcus mutans* e leveduras.

RESULTADOS: Em comparação aos controlos, os pacientes com MPS apresentam maior prevalência de dentes cariados assim como fluxo salivar e pH reduzido. Os pacientes com MPS apresentaram também taxas menores de íons de cálcio e maiores de íons de fosfato. Não foram encontradas diferenças entre os grupos quanto à carga microbiana oral por microrganismos totais, *Streptococcus mutans* e leveduras, bem como quanto à prevalência oral de *Streptococcus mutans*. No entanto, os pacientes com MPS apresentaram maior prevalência de candidíase oral em comparação com os controlos.

CONCLUSÃO: A maior prevalência de dentes cariados e a maior colonização oral por leveduras em pacientes com MPS pode estar relacionada com a baixa concentração de cálcio salivar, com o pH ácido e com a hiposalivação.

PALAVRAS-CHAVE: Mucopolissacaridose, saúde oral, pediatria, *Candida*, pH salivar.

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