

Research Article



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EVALUATION OF DENTAL CARIES, SALIVARY pH, AND THE ALTERATION OF TOOTH MICROBIALS IN CHILDREN WITH MUCOPOLYSACCHARIDOSIS

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ABSTRACT

Background: A class of lysosomal storage diseases known as mucopolysaccharidosis (MPS) is brought on by the accumulation of polysaccharides within cells. This results in systemic and oral symptoms that are visible on radiographs and in clinical settings.

Aim: The purpose of this study was to evaluate dental caries, salivary pH, and the alteration of tooth microbiota in children with mucopolysaccharidosis.

Methods: In this study, 100 children with mucopolysaccharidosis were compared to 100 healthy controls who were between the ages of 3 and 15. Saliva pH and an index of decaying, missed, and filled teeth as well as decayed filled and removed teeth were measured and recorded for each patient.

In terms of microbiological analysis, saliva was inoculated into blood agar, Candida CHROMagar, MacConkey agar, and Mitis salivarius agar. The CFUs (colony-forming units) were then measured and counted based on colony features and gram staining.

The research's findings indicated that the study groups had substantially greater levels of streptococcus viridans species ($p=0.00001$), Candida species ($p=0.003$), and overall microbial load ($p=0.00006$). Salivary pH was more acidic in participants with mucopolysaccharidosis ($p=0.00001$), and the study groups had higher rates of caries for both permanent ($p=0.02$) and primary dentition ($p=0.007$).

Conclusion: in comparison to children in normal health, those with mucopolysaccharidosis had a larger microbial load, more acidic saliva, and a higher prevalence of dental cavities. As a result, their healthcare regimen has to include routine dental examinations, prevention, and treatment.

Keywords: pH, mucopolysaccharidosis, decaying, missed, decayed removed or filled teeth, dental caries

INTRODUCTION

A collection of illnesses known as mucopolysaccharidosis are brought on by a lack of certain lysosomal enzymes, which disrupts the breakdown of glycosaminoglycans (GAGs). The cause of the rise in GAG storage in the lysosomes of different body tissues is that it causes malfunctions in the affected body's organs and organ systems. 1, 2

In individuals with mucopolysaccharidosis, oral manifestations such as gingival hyperplasia, an increased risk of malocclusion, hypoplastic enamel, sharp, thin cusps, microdontia, high arched palate, spaced dentition, delayed tooth eruption, anterior open bite, and/or macroglossia were evaluated both radiographically and clinically.^{3,4}

However, information about dental caries and changes in oral microbiota in patients with mucopolysaccharidosis is limited and contentious in the literature. 5. Therefore, the current study sought to evaluate dental caries, salivary pH, and the alteration of tooth microbiota in children with mucopolysaccharidosis.

MATERIALS AND METHODS

Assessing the impact of salivary pH, dental caries, and changes in tooth microbiota in children with mucopolysaccharidosis were the goals of the current clinical evaluation research. The research participants came from the Institute's Department of Pedodontics and Preventive Dentistry. Prior to their involvement in the study, all individuals gave their written and verbal informed consent.

200 participants, ages 3 to 15, were evaluated for the research; 100 of them had a diagnosis of mucopolysaccharidosis, while the remaining 100 were healthy controls. Following their inclusion, these participants were examined orally using mouth mirrors and sterile explorers. The decayed, extracted, and filled (DEF) or decayed, missing, and filled (DMFT) indices were used in accordance with WHO (World Health Organization) guidelines and standards. Consequently, the study's validity and trustworthiness were guaranteed by the accuracy and dependability of all the data collected.

The individuals' mouths were sampled for unstimulated saliva in the morning, at least two hours after brushing, breakfast, or drinking anything. Throughout the whole investigation, each subject's saliva sample was collected at the same time. However, prior to research enrollment, brushing and oral hygiene could not be standardized. Additionally, because each sample had a variable level of mental development and accompanying obstacles, the period of each sample collection varied.

Participants were instructed to expectorate into a calibrated broadmouth container, and each kid subject had about 2 milliliters of unstimulated saliva collected. A pH-evaluating strip was placed into the saliva sample for one minute after the sample was moved to the sterile test tube. The manufacturer's instructions were followed, and the color shift was compared to them. For the microbiological test, these saliva samples were then promptly taken to an icebox that was kept between 4 and 8 degrees Celsius. Following that, each sample was put onto four distinct culture media: blood agar, MacConkey agar, MS (mitis salivarius) agar, and Candida CHROM agar.

An inoculating loop with a diameter of 4 mm was used to streak the obtained samples under aseptic circumstances after each culture plate was cut into four equal sections. After that, culture plates were sealed and kept at 37 °C in an incubator.

While MS agar and CHROMagar plates were incubated aerobically for 48 hours at 37°C, MacConkey agar and blood agar plates were incubated for 24 hours. An identification chart based on traits, color, and morphology was used to evaluate MS agar colonies. Gram-staining and colony morphology were used to evaluate microbial growth on MacConkey agar and blood agar. On every medium plate, the colony-forming units, or CFUs, were counted and recorded using the unaided eye. To distinguish between gram-positive and gram-negative microorganisms, Gram-staining was used. The collected data was statistically evaluated using the Student t-test, ANOVA (analysis of variance), Chi-square test, and SPSS (Statistical Package for the Social Sciences) software version 24.0 (IBM Corp., Armonk, NY, USA) for evaluating descriptive measures. The findings were presented as frequency, percentages, mean, and standard deviation. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

Assessing the impact of salivary pH, dental caries, and changes in tooth microbiota in children with mucopolysaccharidosis were the goals of the current clinical evaluation research. 200 participants, ages 3 to 15, were evaluated for the research; 100 of them had a diagnosis of mucopolysaccharidosis, while the remaining 100 were healthy controls.

Following their inclusion, these participants were examined orally using mouth mirrors and sterile explorers. The decayed, extracted, and filled (DEF) or decayed, missing, and filled (DMFT) indices were used in accordance with WHO (World Health Organization) guidelines and standards. There were 41860 ± 23715 gram-positive CFUs in the control group and 63288 ± 28543 CFUs in the study group, which was substantially higher in the study group with $p=0.00006$, according to the Mann-Whitney test results in the study participants. However, study groups had 406 ± 356.764 gram-negative bacterial CFUs, which was non-significantly greater than the control group's 280 ± 256.103 CFUs ($p=0.1094$).

The study group's mean CFUs ($63,696 \pm 28,769$) were substantially greater than the control group's ($42,142 \pm 23,856$), with a p-value of 0.00006 in the overall microbial load (Table 1).

The mean CFUs for streptococcus mutans were considerably greater in the study groups (24420 ± 14027.311) than in the control group (15626 ± 13602) with $p=0.0001$, according to the distribution of the mean count of viridans in study individuals. In terms of streptococcus mitis, the study groups' mean CFUs ($22216 \pm 11043 \pm 169$) were considerably larger than those of the control group (12480 ± 7011.475), with a p-value of 0.0002. The research groups' mean CFUs for Streptococcus salivarius were 314 ± 270.568 , which was considerably greater than the control group's 202 ± 193.342 ($p=0.02$).

The study groups' mean CFUs for streptococcus viridans total counts were $46,972 \pm 23139.725$ compared to the control group's 28310 ± 18259 mean CFUs ($p=0.00001$) (Table 2). Based on the distribution of the mean candidal counts in the research participants, the study participants had 178 ± 203.142 CFUs, which was considerably higher than the controls' 86.00 ± 137.972 CFUs ($p=0.003$) (Table 3). The research group's CFUs were 3 and 6.98, whereas the control group's were 4 and 7.23, based on the mean pH distribution of the study participants. With $p=0.00001$, the research group's mean CFU for PH was considerably lower than that of the controls (Table 4).

Regarding the distribution of mean DMFT among research participants, the study group's mean DEF was 4.32 ± 5.123 , which was considerably higher than the control group's mean of 170 ± 1883 ($p=0.02$). In contrast to the research group, which had a mean DMFT of 1.18 ± 1.967 with $p=0.007$, the control group's mean DMFT was 0.12 ± 0.493 (Table 5).

DISCUSSION

200 participants, ages 3 to 15, were evaluated in this study; 100 of them had a diagnosis of mucopolysaccharidosis, while the remaining 100 were healthy controls. Following their inclusion, these participants were examined orally using mouth mirrors and sterile explorers. The decayed, extracted, and filled (DEF) or decayed, missing, and filled (DMFT) indices were used in accordance with WHO (World Health Organization) guidelines and standards.

There were 41860 ± 23715 gram-positive CFUs in the control group and 63288 ± 28543 CFUs in the study group, which was substantially higher in the study group with $p=0.00006$, according to the Mann-Whitney test results in the study participants. However, study groups had 406 ± 356.764 gram-negative bacterial CFUs, which was non-significantly greater than the control group's 280 ± 256.103 CFUs ($p=0.1094$). With a mean CFU of $63,696 \pm 28,769$ in the study group and $42,142 \pm 23,856$ in the control group, the study group's overall microbial burden was considerably greater ($p=0.00006$). These findings were consistent with those of Zhou J et al. (2020) and Ponciano S et al. (2017), who also found comparable demographics and distributions of gram-positive, gram-negative, and total microbial load in their respective investigations.

According to the research's findings, the mean CFUs for streptococcus mutans were considerably greater in the study groups (24420 ± 14027.311) than in the control group (15626 ± 13602) with a p-value of 0.0001. In terms of streptococcus mitis, the study groups' mean CFUs ($22216 \pm 11043 \pm 169$) were considerably larger than those of the control group (12480 ± 7011.475), with a p-value of 0.0002.

The research groups' mean CFUs for Streptococcus salivarius were 314 ± 270.568 , which was considerably greater than the control group's 202 ± 193.342 ($p=0.02$). The research groups' mean CFUs for streptococcus viridans total counts were $46,972 \pm 23139.725$ compared to the control group's 28310 ± 18259 mean CFUs ($p=0.00001$). These results aligned with the findings of Antunes LA et al. (2013) and Çelik B et al. (2021), where the authors' stated distribution of the mean count of viridans in study participants was similar to the current research's findings.

The distribution of the mean candidal counts in the research participants showed that the study participants had 178 ± 203.142 CFUs, which was substantially greater than the control group's 86.00 ± 137.972 CFUs ($p=0.003$). The research group's CFUs were 3 and 6.98, whereas the control group's were 4 and 7.23, based on the mean pH distribution of the study participants. The mean CFU for PH in the research group was significantly lower than that of the controls ($p=0.00001$).

These findings were in line with those of Coppa GV et al. (2011) and James A. et al. (2012), who likewise documented the distribution of mean pH and candidal counts in study individuals similar to the current study in their separate investigations.

When analyzing the distribution of mean DMFT across research participants, the control group's mean DEF was 4.32 ± 5.123 , which was substantially greater than the study group's mean of 170 ± 1883 ($p=0.02$). In comparison to the research group, which had a mean DMFT of 1.18 ± 1.967 with $p=0.007$, the control group's DMFT was substantially lower at 0.12 ± 0.493 .

These findings were in line with those of Poswar FO et al. (2012) and Braunlin EA et al. (2011), who found that the mean DMFT distribution in study participants was similar to what the authors reported in their investigations.

CONCLUSIONS

Considering its limitations, the present study concludes that subjects diagnosed with mucopolysaccharidosis have a higher microbial load, more acidic saliva, and further, a higher incidence of caries compared to normal healthy child subjects. Hence, a regular dental assessment, prevention, and treatment must be included in their healthcare regime. In the future, further studies from a multi-institutional setup might be needed for further exploration of the issue.

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| | Minimum (CFU/ml) | Maximum (CFU/ml) | Mean ± S. D | p-value |
|-------------------------------|-------------------------|-------------------------|--------------------|----------------|
| Gram-positive bacteria | | | | |
| Control group | 13,000 | 115,600 | 41860±23715 | 0.00006 |
| Study group | 15,500 | 125,000 | 63288±28543 | |
| Gram-negative bacteria | | | | |
| Control group | 0 | 1000 | 280±256.103 | 0.1094 |
| Study group | 0 | 1200 | 406±356.764 | |
| Total microbial load | | | | |
| Control group | 13,000 | 116.600 | 42,142±23,856 | 0.00006 |
| Study group | 15,500 | 126,000 | 63,696±28,769 | |

Table 1: Distribution of total microbial load with Mann-Whitney test in study subjects

| | Minimum (CFU/ml) | Maximum (CFU/ml) | Mean ± S. D | p-value |
|-----------------------------|-------------------------|-------------------------|--------------------|----------------|
| Streptococcus mutans | | | | |
| Control group | 2200 | 60,000 | 15626±13602 | 0.0001 |

| | | | | |
|--|--------|---------|------------------|----------------|
| Study group | 2500 | 55,500 | 24420±14027.311 | |
| Streptococcus mitis | | | | |
| Control group | 1300 | 25500 | 12480±7011.475 | 0.0002 |
| Study group | 8200 | 53000 | 22216±11043±169 | |
| Streptococcus salivarius | | | | |
| Control group | 0 | 1000 | 202±193.342 | 0.02 |
| Study group | 0 | 1300 | 314±270.568 | |
| Total streptococcus viridans count. | | | | |
| Control group | 7000 | 87,900 | 28310±18259 | 0.00001 |
| Study group | 13,000 | 103,100 | 46,972±23139.725 | |

Table 2: Distribution of the mean count of viridans in study subjects

| Mean candidal counts | Minimum (CFU/ml) | Maximum (CFU/ml) | Mean ± S. D | p-value |
|----------------------|------------------|------------------|---------------|--------------|
| Control group | 0 | 200 | 86.00±137.972 | 0.003 |
| Study group | 0 | 500 | 178±203.142 | |

Table 3: Distribution of the mean candidal counts in study subjects

| S. No | Mean pH | Minimum (CFU/ml) | Maximum (CFU/ml) | Mean ± S. D | p-value |
|-----------|----------------------|------------------|------------------|--------------|----------------|
| 1. | Control group | 4 | 7.23 | 67000±0.3336 | 0.00001 |
| 2. | Study group | 3 | 6.98 | 61000±0.4603 | |

Table 4: Distribution of the mean pH in study subjects

| S. No | | Minimum (CFU/ml) | Maximum (CFU/ml) | Mean ± S. D | p-value |
|-----------|---------------|------------------|------------------|-------------|--------------|
| 1. | Deft | | | | |
| a) | Control group | 0 | 3 | 1.70±1883 | 0.02 |
| b) | Study group | 0 | 16 | 4.32±5.123 | |
| 2. | DMFT | | | | |
| a) | Control group | 0 | 1 | 0.12±0.493 | 0.007 |
| b) | Study group | 0 | 6 | 1.18±1.967 | |

Table 5: Distribution of mean DMFT in study subjects