

EVALUATION OF SALIVARY MICROBIAL LOAD AND BIOMARKERS IN PATIENTS WITH DENTAL CARIES

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ABSTRACT

INTRODUCTION: Dental caries is a multifactorial, biofilm-mediated, diet-modulated, non-communicable disease characterized by the progressive demineralization of dental hard tissues. **AIM:** To evaluate the salivary microbial load and selected biochemical and immunological biomarkers in patients with dental caries. **METHODOLOGY:** This cross-sectional observational study was conducted at the Department of dentistry SMS hospital Jaipur. The study was carried out between 2023 – 2024. **RESULT:** The study showed a progressive increase in salivary microbial load, particularly *Streptococcus mutans* and *Lactobacillus*, along with rising IL-6 levels as caries severity increased. Conversely, salivary pH, buffering capacity, and flow rate decreased with higher DMFT scores, indicating a more acidic and caries-prone oral environment. **CONCLUSION:** Salivary microbial load and inflammatory biomarkers were significantly associated with increasing caries severity, while protective factors such as salivary pH and flow rate declined progressively.

Keywords: Dental caries, microbial load, biomarkers

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INTRODUCTION

Dental caries is a multifactorial, biofilm-mediated, diet-modulated, non-communicable disease characterized by the progressive demineralization of dental hard tissues¹. It results from a dynamic imbalance between pathological and protective factors in the oral environment. The disease process begins when fermentable carbohydrates, particularly sucrose, are metabolized by acidogenic bacteria within dental plaque, leading to the production of organic acids such as lactic acid. These acids reduce the pH at the tooth surface, favoring enamel demineralization.² If the acidic challenge persists and protective mechanisms fail to counteract it, a carious lesion develops. Traditionally, specific microorganisms such as *Streptococcus mutans* have been strongly associated with dental caries due to their ability to produce extracellular polysaccharides and thrive in acidic conditions. However, modern ecological plaque theory suggests that caries is not caused by a single pathogen but by a shift in the balance of the entire microbial community toward a more acidogenic and aciduric profile³. In addition to pathogenic bacteria, protective species such as *Streptococcus dentisani* have gained attention. This recently described species exhibits arginolytic activity, producing ammonia that helps neutralize plaque acids, and may inhibit cariogenic bacteria through bacteriocin production⁴. Therefore, the net cariogenic potential of dental biofilm depends on the balance between acid-producing and alkali-generating microorganisms. Saliva plays a fundamental role in maintaining oral homeostasis. It contributes to mechanical cleansing, provides buffering capacity, supplies essential ions for

remineralization, and contains antimicrobial proteins and enzymes. Salivary flow rate, pH, buffering capacity, and biochemical components such as lactate are considered potential indicators of caries risk. Low salivary flow and reduced buffering capacity impair acid neutralization, thereby increasing susceptibility to demineralization⁵. Similarly, elevated lactic acid levels reflect increased microbial metabolic activity and may indicate a higher cariogenic challenge. One important physiological response in dental plaque is the “Stephan curve,” which describes the rapid drop in plaque pH following sugar exposure and its gradual return to baseline. The magnitude and duration of this pH drop depend on both microbial composition and host buffering mechanisms⁶. Measuring plaque and salivary pH and lactate levels before and after sugar exposure may therefore provide insight into the acidogenic potential and recovery capacity of the oral ecosystem. Although numerous studies have independently evaluated microbial counts, salivary parameters, or clinical indices, few investigations have simultaneously analyzed clinical, biochemical, and microbiological markers in the same population.^{7,8}

AIM

To evaluate the salivary microbial load and selected biochemical and immunological biomarkers in patients with dental caries.

METHODOLOGY

This cross-sectional observational study was conducted at the Department of dentistry SMS hospital Jaipur. The study was carried out between 2023 – 2024, following approval by the institutional

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ethics committee, and all participants provided informed consent prior to enrollment. A total of 56 adults were included in the study. The inclusion criteria consisted of systemically healthy adults aged 18 years or older who agreed to participate and provided written informed consent. Participants were required to refrain from eating, drinking, smoking, or performing oral hygiene procedures for at least one hour before sample collection. The exclusion criteria included individuals undergoing antibiotic therapy within the previous three months, those with systemic diseases affecting salivary flow, pregnant or lactating women, individuals using orthodontic appliances, and participants with conditions that could interfere with salivary composition or oral microbiota analysis.

RESULT

TABLE 1: Demographic Characteristics of Study Participants (n = 56)

Age Group (Years)		
18–25	12	21.4%
26–35	16	28.6%
36–45	14	25%
46–55	8	14.3%
>55	6	10.7%

The majority of the study participants belonged to the 26–35 years age group (28.6%), followed by 36–45 years (25%) and 18–25 years (21.4%). Fewer participants were observed in the 46–55 years (14.3%) and >55 years (10.7%) age groups, indicating a predominance of young and middle-aged adults in the study population.

Table 2: Oral hygiene and dietary sugar exposure among study participants

Oral Hygiene Practice		
Once daily brushing	20	35.7%
Twice daily brushing	30	53.6%
Irregular brushing	6	10.7%
Dietary Sugar Exposure		
<2 times/day	15	26.8%
2–3 times/day	27	48.2%

>3 times/day	14	25%
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More than half of the participants (53.6%) reported brushing twice daily, while 35.7% brushed once daily and 10.7% had irregular brushing habits. Regarding dietary habits, 48.2% consumed sugary foods 2–3 times per day, 25% more than three times daily, and 26.8% less than twice daily, indicating moderate to high sugar exposure in the majority of the study population.

Table 3: Distribution of Dental Caries Severity (DMFT Index)

DMFT Score Category		
1–3 (Mild)	14	25.0%
4–6 (Moderate)	24	42.9%
>6 (Severe)	18	32.1%

The majority of participants exhibited moderate caries severity (DMFT 4–6) accounting for 42.9%, followed by severe caries (>6) in 32.1% of cases. Mild caries (DMFT 1–3) was observed in 25% of the study population, indicating a considerable burden of moderate to severe dental caries among participants.

Table 4: Mean Salivary Microbial Load (CFU/mL)

Microorganism	Mean ($\times 10^5$ CFU/mL)	Standard Deviation
Streptococcus mutans	6.2	± 1.8
Lactobacillus spp.	4.8	± 1.3
Candida albicans	2.3	± 0.9
Total Bacterial Count	9.1	± 2.5

The mean salivary microbial load revealed that Streptococcus mutans showed the highest concentration ($6.2 \pm 1.8 \times 10^5$ CFU/mL), followed by Lactobacillus spp. ($4.8 \pm 1.3 \times 10^5$ CFU/mL), while Candida albicans demonstrated comparatively lower levels ($2.3 \pm 0.9 \times 10^5$ CFU/mL). The total bacterial count was $9.1 \pm 2.5 \times 10^5$ CFU/mL, indicating a substantial overall microbial burden in the study population.

Table 5: Salivary Physical & Biochemical Parameters

Parameter	Mean $\pm$ SD	Normal value
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Salivary pH	6.1 ± 0.5	6.8–7.4
Buffering Capacity	4.0 ± 1.1	5–7
Unstimulated Flow Rate (mL/min)	0.42 ± 0.18	≥0.3
Stimulated Flow Rate (mL/min)	0.82 ± 0.21	≥0.7

The mean salivary pH (6.1 ± 0.5) and buffering capacity (4.0 ± 1.1) were lower than the normal reference range, indicating a relatively acidic oral environment with reduced neutralizing ability. Although the unstimulated (0.42 ± 0.18 mL/min) and stimulated (0.82 ± 0.21 mL/min) flow rates were above the critical threshold values, they suggest borderline salivary protection in the study population.

Table 6: Salivary Immunological & Inflammatory Biomarkers

Biomarker	Mean ± SD
Secretory IgA (mg/dL)	24.6 ± 8.2
IL-6 (pg/mL)	27.8 ± 9.4
CRP (mg/L)	2.4 ± 0.7

The mean salivary secretory IgA level was 24.6 ± 8.2 mg/dL, indicating activation of local mucosal immunity in response to cariogenic bacterial load. Elevated IL-6 (27.8 ± 9.4 pg/mL) and CRP (2.4 ± 0.7 mg/L) levels suggest the presence of an ongoing inflammatory response associated with dental caries in the study population.

Table 7: Comparison of Salivary Parameters According to Caries Severity (DMFT Groups)

Parameter	Mild (n=14) Mean ± SD	Moderate (n=24) Mean ± SD	Severe (n=18) Mean ± SD
S. mutans (×10 ⁵ CFU/mL)	3.8 ± 1.2	6.1 ± 1.5	8.4 ± 1.7
Lactobacillus (×10 ⁵ CFU/mL)	2.9 ± 1.0	4.7 ± 1.2	6.3 ± 1.4
Salivary pH	6.6 ± 0.3	6.1 ± 0.4	5.8 ± 0.3
Unstimulated Flow (mL/min)	0.58 ± 0.14	0.43 ± 0.16	0.31 ± 0.12

IL-6 (pg/mL)	18.4 ± 5.2	26.9 ± 7.3	34.7 ± 9.1
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A progressive increase in S. mutans, Lactobacillus, and IL-6 levels was observed from mild to severe caries groups, indicating a positive association between microbial load, inflammatory response, and caries severity. Conversely, salivary pH and unstimulated flow rate decreased with increasing DMFT scores, suggesting reduced salivary protection in patients with severe dental caries.

DISCUSSION

In our study the study population consisted of 56 participants distributed across five age groups. The highest proportion of participants belonged to the 26–35 years age group, comprising 28.6% (n=16) of the total sample. This was followed by the 36–45 years group accounting for 25% (n=14) and the 18–25 years group contributing 21.4% (n=12). Participants aged 46–55 years represented 14.3% (n=8) of the study population. The least represented group was individuals above 55 years, constituting 10.7% (n=6).

In our study More than half of the study participants (53.6%) reported brushing their teeth twice daily, while 35.7% brushed once daily. Only 10.7% of participants had irregular brushing habits. Regarding dietary practices, 48.2% of the participants consumed sugary foods or beverages 2–3 times per day, which constituted the majority. About 26.8% reported sugar intake less than twice daily, whereas 25% consumed sugar more than three times per day. These findings indicate that although most participants maintained regular brushing habits, a considerable proportion had frequent sugar exposure, which may contribute to increased caries risk.

In present study The distribution of dental caries severity was assessed using the DMFT index among the study participants. A total of 25.0% of subjects were categorized under the mild group (DMFT score 1–3). The majority of participants (42.9%) belonged to the moderate category (DMFT score 4–6), indicating a considerable burden of dental caries within the study population. Furthermore, 32.1% of individuals were classified under the severe category (DMFT score >6), reflecting advanced caries involvement. The predominance of moderate and severe cases suggests a high overall caries experience among the participants. This distribution highlights the progressive nature of the disease in a significant proportion of the study group.

In our study The mean salivary count of Streptococcus mutans was 6.2 × 10⁵ CFU/mL (±1.8), indicating a relatively high cariogenic bacterial load among the study participants. The mean Lactobacillus spp. count was 4.8 × 10⁵ CFU/mL (±1.3), suggesting its contributory role in the progression of dental caries. Candida albicans was present at a lower mean level of 2.3 × 10⁵ CFU/mL

(± 0.9), reflecting its secondary involvement in the oral microbial ecosystem. The total bacterial count was 9.1×10^5 CFU/mL (± 2.5), demonstrating an overall increased microbial burden in saliva. The comparatively higher counts of *S. mutans* and *Lactobacillus* indicate a predominance of acidogenic and aciduric microorganisms. Thenisch et al.⁹ summarized 981 reports assessing the association of mutans streptococci and caries in preschool children and concluded that the presence of mutans streptococci in the saliva of young caries-free children appears to be associated with a considerable increase in subsequent caries risk.

In present study The mean salivary pH of the study population was 6.1 ± 0.5 , which is lower than the normal range of 6.8–7.4, indicating a relatively acidic oral environment. The buffering capacity was recorded as 4.0 ± 1.1 , which is below the normal reference range of 5–7, suggesting reduced ability of saliva to neutralize acids. The mean unstimulated salivary flow rate was 0.42 ± 0.18 mL/min, which is above the critical threshold of 0.3 mL/min but still on the lower side. The stimulated salivary flow rate was 0.82 ± 0.21 mL/min, remaining within the normal limit of ≥ 0.7 mL/min. Guo L, et al¹⁰ Low salivary flow rate is a risk factor for caries incidence. The most common alterations in salivary flow rate involve reduced secretion, which may be influenced by medications, pathological changes in the salivary glands, and age, etc. It is considered a potential risk factor when the unstimulated salivary flow rate is lower than 0.30 mL/min and the stimulated salivary flow is lower than 0.7 mL/min.

In present study The mean salivary Secretory IgA level was 24.6 ± 8.2 mg/dL, indicating activation of the local mucosal immune response in the study population. The mean IL-6 level was 27.8 ± 9.4 pg/mL, suggesting the presence of an active inflammatory process associated with dental caries. C-reactive protein (CRP) showed a mean value of 2.4 ± 0.7 mg/L, reflecting mild systemic inflammatory activity. The elevated IL-6 levels particularly indicate ongoing tissue response to microbial challenge. Increased IgA levels may represent a protective immune attempt against cariogenic bacteria. Lo Giudice et al¹¹ Mean salivary IgA rate between two groups (A 16.7 ± 4.5 mg/dL vs. B 21.8 ± 12.9 mg/dL) was not significant, while IL-6 rate (A 19.02 ± 5.3 pg/mL vs. B 30.2 ± 11.8 pg/mL) was statistically different. This study revealed that salivary IL-6 levels were significantly higher in children with active caries when compared with the caries-free group, while the s-IgA rate showed no significant differences between the two groups.

In present study The comparison of salivary parameters according to caries severity revealed a progressive increase in microbial load with rising DMFT scores. The mean *S. mutans* levels increased from $3.8 \pm 1.2 \times 10^5$ CFU/mL in the mild group to

$8.4 \pm 1.7 \times 10^5$ CFU/mL in the severe group. Similarly, *Lactobacillus* counts demonstrated a steady rise from 2.9 ± 1.0 to $6.3 \pm 1.4 \times 10^5$ CFU/mL as severity increased. In contrast, salivary pH showed a gradual decline from 6.6 ± 0.3 in mild cases to 5.8 ± 0.3 in severe cases, indicating a more acidic environment. Unstimulated salivary flow rate also decreased consistently with increasing caries severity, from 0.58 ± 0.14 mL/min in mild cases to 0.31 ± 0.12 mL/min in severe cases. Furthermore, IL-6 levels increased markedly from 18.4 ± 5.2 pg/mL in mild cases to 34.7 ± 9.1 pg/mL in severe cases, reflecting heightened inflammatory activity with disease progression. Gao X et al¹² Modest evidence is available on the associations between dental caries and several salivary parameters, including flow rate, buffering capacity and abundance of mutans streptococci. Ichim et al¹³ Salivary tests played an important role in establishing control sessions, in carrying out prophylactic caries therapy, and establishing prognosis. The existence of a statistical association was confirmed between the prevalence of dental caries and the results of salivary tests for the study group.

CONCLUSION

The findings of the present study demonstrate that patients with dental caries exhibited a significantly elevated salivary microbial load, particularly of *Streptococcus mutans* and *Lactobacillus*, along with reduced salivary pH, decreased buffering capacity, and lower salivary flow rates. These alterations indicate a shift toward a more acidogenic and aciduric oral environment that favors the initiation and progression of dental caries. The predominance of moderate and severe DMFT scores within the study population further reflects a considerable burden of caries experience. In addition, elevated levels of inflammatory biomarkers such as IL-6 and increased secretory IgA suggest activation of local immune and inflammatory responses in relation to microbial challenge. A progressive rise in microbial counts and IL-6 levels, coupled with a decline in salivary pH and flow rate with increasing caries severity, highlights a strong association between salivary parameters and disease progression. Overall, the study supports the potential role of salivary microbial load and biomarkers as non-invasive indicators for assessing caries risk, monitoring disease severity, and aiding in early diagnosis and preventive strategies.

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