

Comparative Analysis of Change in pH, Oral Health Status, and the Count of *Streptococcus mutans* and *Lactobacillus* Species in the Oral Cavity in Patients with Gastroenteral Diseases Using Saliva: A Pilot Study

Mahima Seetaram¹, Vaidhegi Muralivel¹, Sangeeta Umesh Nayak², Suchitra Mala Shenoy³, Sudha Kuthethur⁴, Srikant Natarajan⁵, Suresh Shenoy⁶

¹Student, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India, ²Department of Periodontology, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India, ³Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India, ⁴Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India, ⁵Department of Oral Pathology and Microbiology, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India, ⁶Department of Gastroenterology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India

ABSTRACT

Background: Seventy million people are affected by gastrointestinal (GI) disturbances throughout the world. Oral cavity possesses various bacteria that remain as healthy commensals or turn pathogenic due to shift of balance with disturbances in health, which is reflected in the oral cavity too. Studies have shown a possible oro-systemic link. This study aimed at assessing the effect of GI disease on oral health comparing levels of pH, microbiological counts, and oral health status between test and control groups. **Materials and Methods:** This pilot study consisted of two groups: test group containing 14 participants (GI disease) and control group (healthy) containing 3 participants. Two saliva samples were collected per patient. One sample was inoculated onto Mitis Salivarius and Rugose agar plates at 37°C in the CO₂ incubator for 2 days. The second sample was used for recording pH. Parameters such as decayed, missing, and filled teeth, plaque index, gingival index, probing pocket depth, and clinical loss of attachment were also recorded. The results were analyzed using Statistical Package for Social Sciences (SPSS) version 11.5. Regression analysis was applied to predict the three-microbe culture based on the pH and GI disease. **Results:** The oral health parameters showed a higher number of missing teeth, higher bleeding on probing, higher values of plaque and gingival index, a higher amount of clinical loss of attachment, and acidic pH of saliva in the test group. Microbiological analysis showed more *Streptococcus mutans* in the control group (7,500–10,000 cfu/mL), with a mean of 8,833.33±1,258.31 cfu/mL; *S. salivarius* was more in the test group (2,000–25,000 cfu/mL) with a mean of 15,866.67±6,697.76 cfu/mL. *Candida* was seen only in the test group (2,166.67±2,549.51 cfu/mL) and absent in the control group. *Lactobacillus* was absent in both the groups. **Conclusion:** The present study suggests the relation between oral health and GI diseases. Hence, saliva could be used as an easy, non-invasive biomarker to analyze the gastroenteric status of the patient.

Address for correspondence: Dr. Sangeeta Umesh Nayak, Department of Periodontology, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India.
E-mail: Sangeeta.nayak@manipal.edu

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INTRODUCTION

Streptococcus mutans and *Lactobacillus* are the commonly found oral microbial species. Alteration in their count and levels due to various etiologies can lead to varied oral manifestations and presentations in the mouth. These bacteria are seen in varying levels in the oral cavity of different persons; also they are seen in diverse percentages in various parts of the oral cavity.^[1] Gastrointestinal (GI) diseases have various clinical presentations and also occur due to various mechanisms. They have different epidemiologies in populations. They are also of different types and subtypes, each having different etiology.^[2,3]

Gastrointestinal diseases might have manifestations as lesions of the jaws, oral, and perioral tissues.^[4] The oral lesions are similar to GI lesions in some cases, whereas the oral changes could be caused by systemic alterations due to GI disease, invariably due to those related to malabsorption.

The pH variation in the oral cavity can be seen due to various changes in the oral environment, of which one important causative agent changes in the oral microbial flora.^[5] The pH variation can also be seen

due to the consumption of various foodstuffs that can have a deleterious effect on the gastric mucosa and vice versa. Neutral pH in the saliva is said to be seen in healthy individuals who do permit the growth of many microorganisms at a particular temperature.^[6] Certain interactions that are seen among the microbes might have environmental mediated modifications wherein the microflora could take up resources or excrete their metabolites which affects the presence of themselves as well as other microflora.^[7] Variation in the pH can create loops of feedback that help or inhibit growth, thus determining the fate of the bacterial population.^[7]

S. mutans can produce large quantities of glucans including acid beyond the salivary buffering capability, thus giving the bacteria an advantage against non-cariogenic oral microflora even at low pH. The presence of *Candida albicans* species has shown to increase the adherence of *S. mutans*, hence favoring the action of both these microorganisms.^[8] It has been seen that a high level of sucrose causes interactions between *S. mutans* and *C. albicans*, thus increasing the microbial counts, and produces a biofilm that could have important clinical (oral) repercussions. *Lactobacillus* is said to produce lactic acid which is its product, thus lowering

A: mitis Salivarius agar plate (blue) and *S. salivarius* species
 Rugose agar plate (pale yellow) with *Candida* species.
 B: *S. Mutan* colonies
 C: *S. Salivarius* Colonies
 D: *Candida* Species

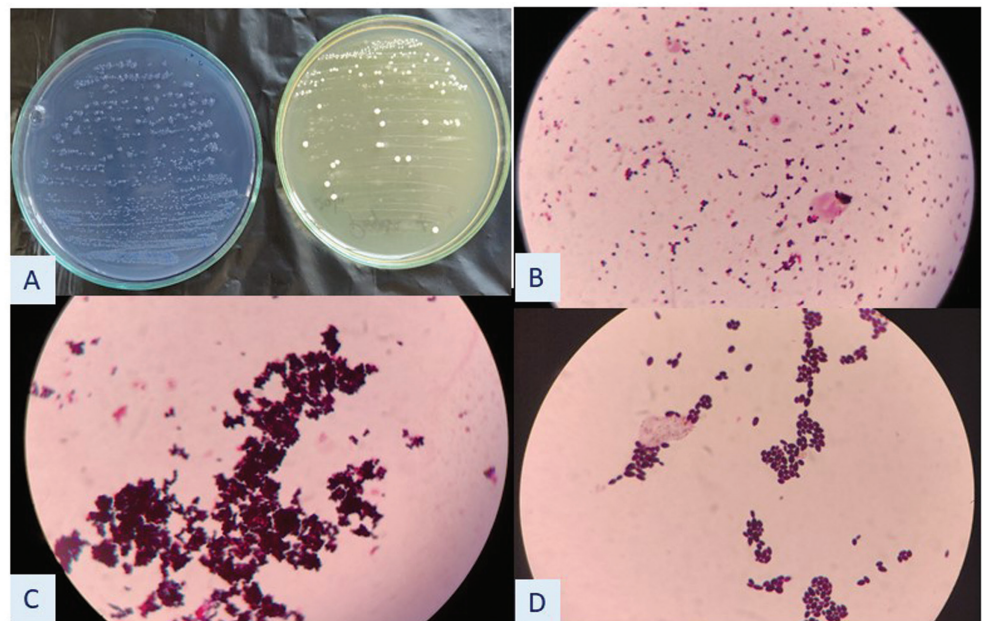


Figure 1: Microbial analysis of saliva sample

the overall pH. In general, *Lactobacillus* species also prefer lower pH.^[7] *S. mutans* is a cariogenic bacterium; the lowered pH prevents the growth of non-cariogenic streptococci. The presence of *Lactobacillus* species in a microbial biofilm can inhibit the formation of *S. mutans* and *C. albicans*.^[6]

The presence of *S. salivarius* prevents the adherence and filamentation of *C. albicans*. It mediates probiotic action against bacterial and few fungal species due to the presence of bacteriocin-like inhibitory substances.^[6]

The microbiome can turn into a foe if there is an imbalance in the host–microbe relationship. It was thought previously that oral microbes were transferred to the gut only in people with immunocompromised conditions and the acidic contents of the stomach would thus reduce the count of bacteria. Evidence proves that though the oral and gut microbiomes are distinct and different, there can be an interchange in the nidus of colonization. *Streptococcus* is one such species.^[9] Transient systemic bacteremia of the oral bacterial species is the most commonly observed after mastication, tooth brushing, dental procedures^[10] through swallowed saliva, nutrients, and drinks, wherein saliva production ranges from 0.75 to 1.5 L/day.^[11] It has been proved that in severe diseases oral bacteria may have been reported to be present in the intestine.^[11]

It is also proved along with poor periodontal status that a subset of oral microflora may colonize the gut when the gut bacteria are dysbiotic. Maintaining good oral hygiene and periodontal therapy along with probiotics may help in reducing oral bacteria-elicited gastrointestinal disorders.^[11]

According to a study, the mean counts of the oral microbial species were altered in patients with medical disorders and worse periodontal conditions.^[12]

Certain foodstuffs can cause a change in the balance of the oral microbial flora as well as harm the GI tract. The variation in the count of two of the major microbes of the oral cavity—*S. mutans* and *Lactobacillus* that thrive principally in the oral cavity and its relation with the change in the oral pH in patients presenting with GI diseases—was the main crux of this pilot study.

MATERIALS AND METHODS

This pilot study was carried out in various facilities of Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education in association with the Departments of Gastroenterology, Microbiology, and Biochemistry of Kasturba Medical College and Hospital, Mangalore. The study protocol

was approved by the Institutional Ethics Committee of Manipal College of Dental Sciences, Mangalore. The protocol number was 18135.

Systemically healthy patients above the age of 18 years were included in the control group. Participants with diseases such as gastritis, gastroenteritis, chronic liver disease (CLD), chronic kidney disease (CKD), gastric erosions, *Helicobacter pylori* infections, gastroesophageal reflux disease (GERD), pancreatitis, esophageal candidiasis, and post-cholecystectomy and had not been treated with medications for the same were included in the test group. Patients with severe illness were excluded from the study. All the participants were explained about the purpose of the study, and written informed consent was signed prior to sample collection. The demographic details of the participants were collected. The socio-economic status and background were matched before including in the study.

In the control group, three unstimulated saliva samples of healthy subjects (without GI disturbances) were collected. The test group included 14 saliva samples of subjects with GI disease.

The GI disease status of the patient was analyzed by the experienced gastroenterologist. An intraoral examination was performed by a trained single examiner. The oral health status including the number of teeth present, wasting disease (erosion, abrasion, and attrition), DMFT (decay, missing, and filled teeth), soft tissue examination including that of the tongue, buccal mucosa, and floor of the mouth was done. Also bleeding on probing was recorded, and scores were given as per Mombelli's bleeding index.^[13] The plaque index was calculated using an explorer by examining all four surfaces, each of which were scored 0–3.^[14] The gingival index was used to assess the severity of gingivitis.^[15] All surfaces of all teeth were scored. Each of the four surfaces was scored 0–3 and the mean score was calculated per participant. The probing pocket depth was measured for all the four surfaces using William's periodontal probe, and the mean values were calculated. The clinical attachment loss was calculated using William's periodontal probe and the average was calculated. The distance between the cemento-enamel junction and the base of the sulcus was also recorded along with the gingival recession if present. In cases of recession, the amount of recession was added to the periodontal pocket depth for that respective tooth.

SAMPLE COLLECTION

The patient was asked to sit still for 2–3 min and unstimulated saliva was collected in a sterile vial

using the drooling method. Saliva was collected in two separate vials per patient. Then the samples were transported to the Department of Biochemistry and the Department of Microbiology for analysis.

BIOCHEMICAL ANALYSIS

The pH was tested using a pH meter. The pH meter consists of a rod-like structure made up of glass with a glass bulb containing the sensor at the bottom which is specifically designed to be selective to hydrogen ion concentration. The glass electrode was immersed into the saliva sample and held there for a sufficient time wherein the potential difference created was detected by a voltmeter which displayed the pH value on the screen. The pH meter was calibrated with a solution of known pH to obtain an accurate measurement of every saliva sample.

MICROBIOLOGICAL ANALYSIS

For the bacterial culture, the saliva samples were inoculated onto the media of Mitis Salivarius agar for *S. mutans* and Rugose agar for *Lactobacillus* using inoculation loops, which were dry heat-sterilized before every new sample was placed. All the plates were placed inside the McIntosh and Filde's anaerobic jar along with catalyst (Gaspak) to absorb the remaining oxygen from the jar. The plates were incubated at 37°C for up to 48 h and all the bacteria growing were identified [Figure 1].

STATISTICAL ANALYSIS

The collected data were entered on the Statistical Package for Social Sciences (SPSS) version 11.5. The results are thus expressed as proportions using appropriate tables, graphs, and figures. Regression analysis was used to predict the three-microbe culture based on the pH and GI disease.

RESULTS

Among the two groups, the test population consisted of people who majorly presented with diseases such as gastritis, gastroenteritis, CLD, CKD, gastric erosions, *H. pylori* infections, and GERD and had not been treated with medications for the same. The age of the participants ranged from 22 to 65 years.

A comparison of the age between the two groups shows that age is higher in the test group when compared with the control group, which was statistically significant with a *P*-value of less than 0.001. The analysis of DMFT showed varied results. Decayed teeth (D) and filled teeth (F) were found higher in the control group than in the test group but it was statistically insignificant. Missing teeth (M) were higher in the test group than in the control group and statistically insignificant. Bleeding on probing, gingival index, and plaque index was higher in the test group and statistically significant. Probing pocket depth and clinical loss of attachment were higher in the test group than in the control group but only clinical attachment loss showed statistically significant value [Table 1 and Graphs 1 and 2].

The pH value was higher in the control group than in the test group and the difference was statistically significant. While the pH in control population ranged between 7.01 and 7.11, the pH in test population ranged between 5.97 and 7.64 [Table 1 and Graph 3].

The *S. mutans* count ranged from 7,500 to 10,000 cfu/mL, whereas that of *S. salivarius* ranged from 3,000 to 4,200 cfu/mL with no *Lactobacillus/Candida* species, in the control group.

Table 1: DMFT, periodontal and microbial parameters among control and test groups

	Control group (n=3)	Test group (n=14)	P-value
	Mean ± SD	Mean ± SD	
D	3.67±6.35	1.64±1.78	0.637
M	0.67±1.16	5.64±7.58	0.286
F	4.67±4.04	1.07±1.82	0.261
Bleeding on probing	0±0	1.86±0.66	<0.0018*
PI	1±0.23	1.84±0.43	0.006
GI	0.37±0.64	1.6±0.45	0.001*
Probing pocket	1.28±0.25	1.75±0.7	0.281
CAL	0.33±0.58	3.89±1.16	<0.001*
pH	7.08±0.05	6.5±0.55	0.002*
<i>S. mutans</i> (cfu/mL)	8,833.33±1,258.31	5,255.56±3,427.87	0.116
<i>S. salivarius</i> (cfu/mL)	3,733.33±642.91	15,866.67±6,697.76	0.013
<i>Candida</i> (cfu/mL)	0±0	2,166.67±2,549.51	0.034

D = decayed, M = missing, F = filled; PI = plaque index; GI = gingival index; CAL = clinical attachment loss; cfu = colony-forming units, **P*-value <0.001 statistically significant

In the test group, an inverse relationship was found between *S. mutans* and *S. salivarius*. *S. mutans* ranged from 0 to 11,000 cfu/mL, whereas *S. salivarius* ranged from 2,000 to 25,000 cfu/mL. *Candida* species were found to range between 0 and 7500 cfu/mL.

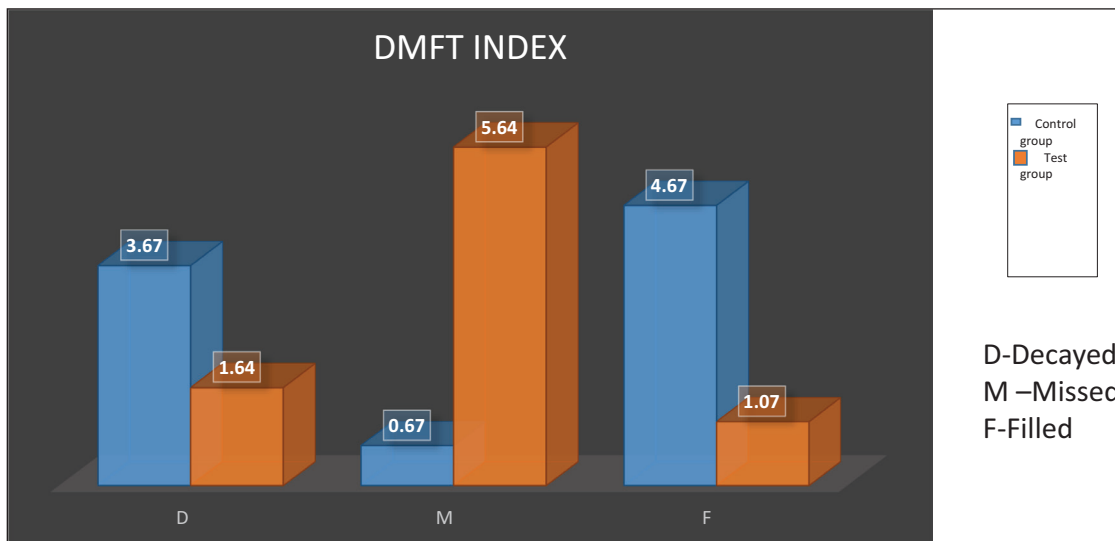
S. mutans (cfu/mL) were higher in the control group and were statistically insignificant. *S. salivarius* (cfu/mL) is higher in the test group and was statistically significant with a *P*-value of 0.013. *Candida* (cfu/mL) was higher in the test group and was statistically significant with a *P*-value of 0.034 [Table 1 and Graph 4].

Linear regression analysis shows that *S. mutans* and *S. salivarius* colony counts can be significantly predicted based on the presence or absence of GI disease and pH. *S. mutans* shows an inverse correlation; however, *S. salivarius* shows a positive correlation, i.e., as the pH values increase, *S. salivarius* increases (*P* = 0.115), but *S. mutans* decreases (*P* = 0.013) [Table 2].

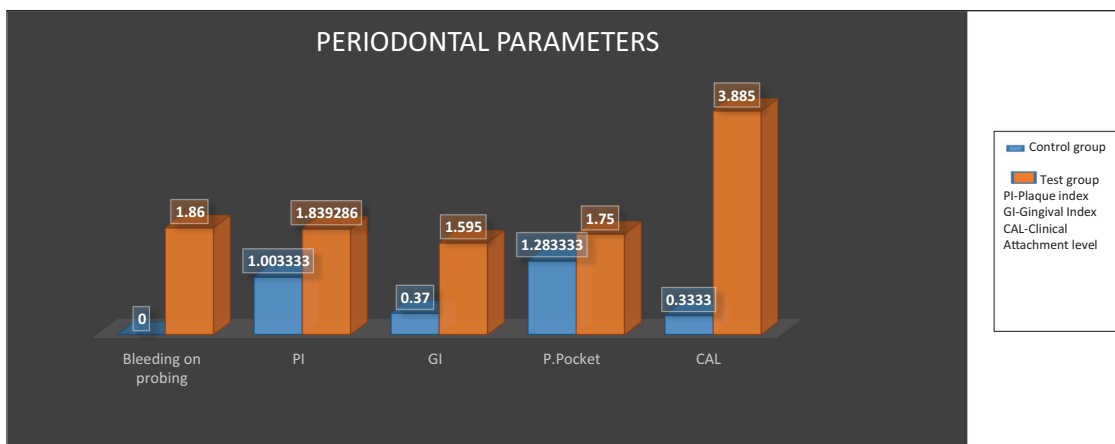
DISCUSSION

The present study included 17 participants with the age range of 22–65 years. The study aimed at assessing the effect of GI disease on oral health and comparing it with healthy individual using saliva pH, microbiological counts, and oral health status.

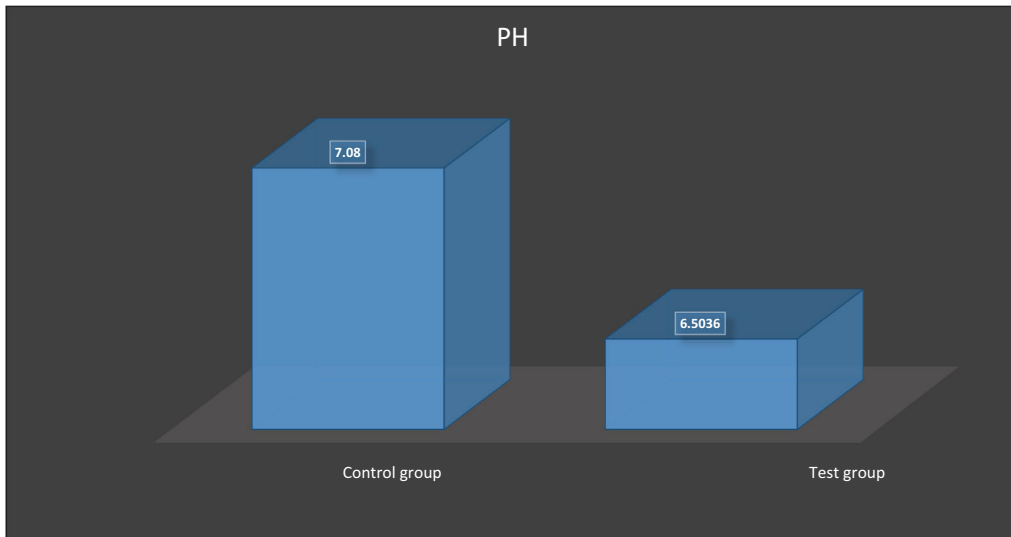
A symbiotic relationship between the residing oral microbes and host is important for maintaining oral homeostasis, whereas the progression of periodontitis mostly involves the modification of the sub-gingival microbes.^[16] Periodontal disease is a chronic infection of the gums characterized by a loss of attachment between the tooth and bone along with loss of bone itself.^[17] It may have negative consequences on the quality of life of the affected individuals, such as tooth loss, financial and social problems, and poor alimentation.^[18] It is generally considered that the periodontal plaque has multifactorial etiology.^[19] Data show that some specific



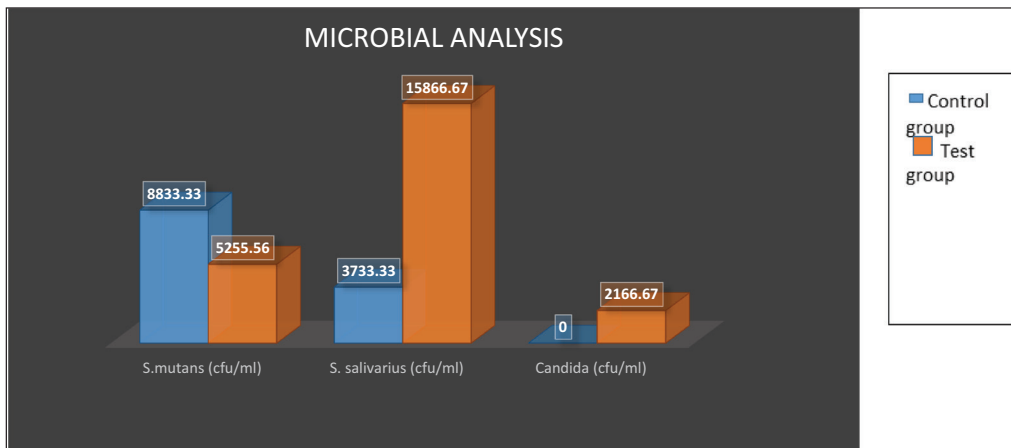
Graph 1: DMFT index details in control and test (study) groups



Graph 2: Periodontal parameters of control and test groups



Graph 3: pH details of control and test groups



Graph 4: Microbial analysis of control and test groups

Table 2: Linear regression analysis to predict *S. mutans*, *S. salivarius*, and *Candida* using GI and pH

Prediction of (dependent variable)	R	Std. error of the estimate	Equation	Significance of GI	Significance of pH
<i>S. mutans</i> (cfu/mL)	0.789	2,297.295	<i>S. mutans</i> (cfu/mL)=52,709.116–7,158.35 (gastroenteral disease) –6,197.144 (pH)	0.005	0.013
<i>S. salivarius</i> (cfu/mL)	0.782	5,465.129	<i>S. salivarius</i> (cfu/mL)= –55,620.863 + 16,977.053 (gastroenteral disease)+8,383.361 (pH)	0.005	0.115
<i>Candida</i> (cfu/mL)	0.412	2,402.461	<i>Candida</i> (cfu/mL)=1,441.522 + 2,049.028 (gastroenteral disease)–203.605 (pH)	0.335	0.925

Gram-negative microorganisms in the sub-gingival plaque play a fundamental role in the initiation and progression of periodontitis.^[20]

Oral cavity possesses various classes of bacteria which may remain as healthy commensals or turn pathogenic due to the shift of balance due to various disturbances in health which is reflected in the mouth too. A complex interaction of the oral microbial species and its relation to pH and the oral environment

with periodontal involvement could give a small insight into the various GI presentations that a patient could exhibit. The present study aimed at finding the effect of gastrointestinal diseases and their impact on the oral cavity.

Saliva acts as a biomarker and it has various diagnostic markers in it. The collection of the saliva sample is non-invasive and easy when compared with blood or other body fluids which act as a biomarker in the diagnosis

of the disease. The changes which occur in saliva may favor the progression of the disease.

pH is a single environmental parameter that influences the presence and growth of various microbes. It exhibits that a complex set of interactions can mediate through the same environmental parameter.^[7] Poor oral hygiene can alter the pH of the saliva in the oral cavity. The pH of the test group participants was acidic, whereas the pH of the control group participants was above 7.

S. mutans was higher in the control group and no relation was found between the count of *S. mutans* and gingival inflammation; this was in agreement with an earlier study.^[21,22] Bacteria belonging to the genus of *Streptococcus* have gained a lot of attention for various reasons along with their potential to cause periodontitis.^[23]

Whether colonization by these bacteria requires a shift in levels of microorganisms cannot be excluded, but the presence of good oral hygiene practice and periodontal therapy may help in the reduction of oral bacteria load, which is one of the etiological factors of gastrointestinal diseases. GI and modified bleeding index were higher in the test group than in the control group. Gingival bleeding is the first sign of inflammation.

When seen in the results, *S. mutans* and *S. salivarius* show inverse relation. *Candida* is seen only in the test group, which also shows considerable acidic pH and fair-to-poor periodontal parameters. These parameters differed in the study and test population.

Hence, the above parameters could be used to support the study by assessing GI disease status of a patient, the difference in levels of pH, the difference in the count of *S. mutans* and *Lactobacillus* (along with *S. salivarius* and *Candida* species) between the test and control population, analyzing oral health status, and establishing a relationship of the same.

There is ample literature on the relationship between changes in the oral environment and gastroenteric diseases.^[4,8,9] An early study by Daley and Armstrong^[4] reported that oral manifestations of GI diseases may be useful in the development of a differential diagnosis for patients with GI complaints, and oral tissues may offer an easy biopsy site to diagnose many such conditions, for example, Crohn's disease. They also stated that the severity or probability of progression of a disease can be monitored by the presence or extent of oral manifestations, and the success of the management of GI diseases may be reflected in terms

of the response of oral tissues. Increased incidence of wasting diseases due to the acidic pH of saliva in conditions such as gastric reflux was also found.^[4] Schmidt *et al.* used DNA shotgun sequencing data obtained from the saliva and stools of 470 people to track oral-fecal strain transmission. They found that of the 125 microbial species, 74 species were frequently found in both the mouth and the gut. They showed evidence of oral-gut transmission in all individuals which included a number of bacteria that are found in the mouth, such as *Streptococcus*, *Veillonella*, *Actinomyces*, and *Haemophilus*.^[24] Samples taken from colorectal cancer patients were compared with healthy individuals; patients diagnosed with cancer had higher transmission rates of bacteria from the oral cavity to the gut.^[10]

Further studies have confirmed associations between GI symptoms and oral conditions. A series of human and animal studies conducted by Olsen and Yamazaki^[11] showed that *Porphyromonas gingivalis*, one of the causative agents of periodontitis, may influence the gut microbiota causing dysbiosis. It was also reported that *Aggregatibacter actinomycetemcomitans* may alter the gut microbiome.

The main strengths of the study are the coverage of all relevant GI diseases, minimal time consumption, easy availability of equipment, and the simplicity and credibility of the tests performed. The main limitations of this study were the sample size, sample collection, age ratio, and geographical limitations.^[25]

CONCLUSION

Based on the present study, the results obtained from the biochemical and microbiological analysis of saliva from patients diagnosed with various gastroenteric diseases showed more amount of bacteria of *S. salivarius* and *Candida* species, acidic pH, poor oral health, and deteriorated periodontal health, thus substantiating the relationship between the oral microflora and gastrointestinal system.

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CONFLICTS OF INTEREST

No potential conflict of interest was reported by the authors.

AUTHORS' CONTRIBUTIONS

Mahima Seetaram, Vaidhegi Muralivel, Sangeeta Nayak, and Srikant N. have provided contributions in processing of the data. Suchitra Shenoy, Sangeeta Nayak, Sudha K., and Suresh Shenoy have provided contributions in the planning stages of the study. All of them have provided contributions in reviewing the literature and writing the article.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

This study was reviewed and approved by the institutional ethics and review board, Manipal College of Dental Sciences, Mangalore.

PATIENT DECLARATION OF CONSENT

Not applicable.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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