

# Evaluation of the correlation between dental caries and periodontitis - A clinico- immunological analysis

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## Abstract

**Background:** To study the correlation between dental caries and periodontitis.

**Aim:** The aim of the study was to evaluate the correlation between dental caries and periodontitis based on the clinical and immunological analysis. The null hypothesis was that there is no clinical and immunological correlation between dental caries and periodontitis.

**Settings and Design:** Fifty systemically healthy adults (mean age of 35 years) with decayed, missing, and filled teeth scores of more than 6 were assessed for periodontal health by recording their probing depth (PD) and bleeding on probing (BOP) values. Unstimulated whole saliva was collected, and enzyme-linked immunosorbent assay (ELISA) analysis was conducted for determining the immunoglobulin A (IgA) and immunoglobulin G (IgG) levels. The obtained clinical and immunological values were statistically analyzed.

**Results:** The results of the clinical examination revealed low PD and BOP scores, whereas the ELISA analysis showed significantly higher salivary IgA levels as compared to salivary IgG.

**Conclusions:** Based on the observations of this clinicoimmunological study, it was concluded that no correlation existed between dental caries and periodontal disease.

**Keywords:** Dental caries; enzyme-linked immunosorbent assay; immunoglobulin A; immunoglobulin G; periodontitis; whole saliva

## INTRODUCTION

The exact relation between dental caries and periodontitis has been subject to much controversy among the dental fraternity through the ages.<sup>[1]</sup> Although being the two most prevalent diseases occurring in the oral cavity, they show vast differences amidst them. Microbiologically, the presence of *Streptococcus mutans* in saliva is an indicator of caries activity, while evidence shows that *Porphyromonas gingivalis* is the key pathogen-promoting periodontal disease.

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The immune response to these disease processes is through the release of immunoglobulins. Secretory or salivary immunoglobulin A (IgA) is the most predominant immunoglobulin in saliva, and its levels are elevated in relation to caries. Being a mucosal guardian, it counters attack by *S. mutans* and prevents its adherence to the enamel.<sup>[2]</sup> Even the focus of caries vaccine has shifted to the stimulation of salivary IgA for producing increased immunity to the tooth structure.<sup>[3]</sup> However, research shows no correlation between IgA in saliva and periodontitis as they do not have access to bacteria deep within periodontal pockets. Moreover, periodontal pathogens can easily cleave salivary IgA.<sup>[4]</sup> Salivary and crevicular fluid titers of immunoglobulin G (IgG) are elevated in periodontitis as a response to periodontal pathogen *P. gingivalis* and increases

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with the severity of disease in serum and saliva (salivary form of which is derived from gingival crevicular fluid).<sup>[5]</sup>

Even though a great depth of knowledge on each separate disease is present, our basic understanding of the interrelation between the two diseases is still very minimal. Moreover, the available literature with regard to the correlation between these factors is also highly controversial.<sup>[1]</sup> The current study was an attempt to explore this interrelation. The aim of the study was to examine the clinical and immunological relation between dental caries and periodontitis.

## METHODOLOGY

This study was conducted following approval by the Institutional Ethical Committee, Rajah Muthiah Dental College and Hospital, Annamalai University, after obtaining written informed consent from the study participants. Inclusion criteria were fifty (32 males and 28 females) systemically healthy adults who visited our dental OP for oral examination and/or conservative dental treatment between May 2016 and June 2017, with age ranging from 20 to 50 years (mean age: 35 years) and patients with decayed, missing, and filled teeth score more than 6 [Table 1]. The exclusion criteria were patients who had received any kind of professional dental intervention during the previous year, patients with the habit of tobacco chewing or smoking, patients with diabetes, pregnant and lactating mothers, those undergoing radiation therapy, immunocompromised patients, patients with endo-perio lesions, patients with genetic abnormalities, and those whose periodontal status was bound to be affected due to any other external environmental factors.

### Clinical examination

Evaluation of the periodontal status of all fifty patients was done to assess two parameters: bleeding on probing (BOP) and probing depth (PD) [Figure 1]. Both parameters were recorded on all four surfaces of teeth with a periodontal probe (Community Periodontal Index for Treatment Needs [CPITN]). BOP was measured according to Lang *et al.* According to him, “the absence of BOP can serve as a predictor of periodontal stability.”<sup>[6]</sup> It was calculated and expressed in percentage using the formula:  $BOP = \frac{\text{number of bleeding sites}}{\text{number of sites evaluated}} \times 100$ . Local bleeding was defined as the percentage of sites with  $BOP < 30\%$  of all probed sites for each person. General BOP was defined as a percentage of 30% of sites or higher.<sup>[7]</sup>

**Table 1: Patient characteristics of the study population**

Number of subjects	Sex		Mean age (years)	Age range (years)
	Males	Females		
50	32	28	35±9.237	20-50

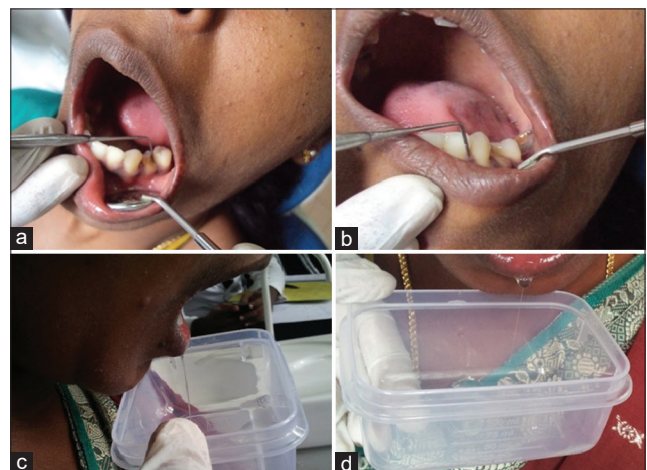
PD was used as a key indicator to determine the presence of periodontal inflammation, which was recorded and rounded off to the nearest millimeter.<sup>[8]</sup> According to the CPITN for PD, the following definition for periodontitis was used: PD: 0–3 mm as no/mild periodontitis, at least one pocket  $\geq 4$  mm and  $< 6$  mm as moderate, and with at least one pocket  $\geq 6$  mm as severe periodontitis.<sup>[8]</sup>

### Unstimulated whole saliva collection for immunological analysis

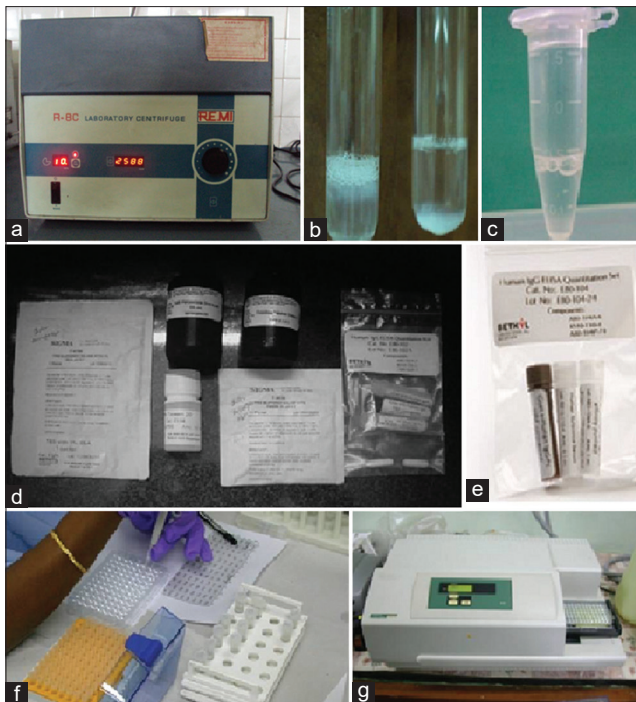
Proper guidelines were followed for the collection of unstimulated whole saliva.<sup>[9]</sup> To minimize possible food debris and stimulation of saliva, the patients were instructed not to eat or drink anything (except for water) 1 h before unstimulated saliva collection. All the samples were collected from 9 am to 11 am. Saliva was collected by draining method.<sup>[10]</sup> The patients were asked to sit upright calmly with head slightly tilted forward, and the eyes open. They were asked to keep their mouths open to allow the saliva to drip passively from the lower lip into the graduated sterile tubes 10 [Figure 1]. After collection, the samples were immediately frozen at 70°C until further analysis. At the time of analysis, the samples were thawed and centrifuged for 10 min at 2500 rpm to remove mucin and debris, and the clear supernatant which was obtained was submitted to enzyme-linked immunosorbent assay (ELISA) testing [Figure 2].

### Enzyme-linked immunosorbent assay test for salivary immunoglobulin A and immunoglobulin G levels

The ELISA protocol was performed, and each test was performed in duplicate [Figure 2]. A volume of 100  $\mu$ l of diluted coating antibody (that is antibody IgA/IgG with carbonate–bicarbonate) was coated on each well of the plate. The plates were first incubated at room



**Figure 1:** From top left; (a) evaluation of probing depth, (b) assessment of bleeding on probing, (c) head position while collecting saliva sample, (d) unstimulated saliva sample collection



**Figure 2:** From top left; (a) centrifuge machine, (b) thawed and centrifuged samples, (c) clear supernatant obtained, (d) and (e) enzyme-linked immunosorbent assay kit, (f) enzyme-linked immunosorbent assay plate reader (Span Autoreader 3011, Span Diagnostics Ltd, Surat), (g) enzyme-linked immunosorbent assay protocol

temperature (20°C–25°C) for 1 h and were washed five times. Following this, 200  $\mu$ l of blocking solution (50 mM Tris[hydroxymethyl] aminomethane, 0.14 M NaCl, 1% bovine serum albumin, and pH 8.0) was added to each well and again incubated at room temperature for 30 min, after which plates were washed five times. Then, 100  $\mu$ l of saliva sample was added to each well and incubated at room temperature for 1 h, after which plates were washed five times. One hundred microliters of diluted horseradish peroxidase (HRP) detection antibody (antibody anti-IgA conjugated with HRP) was then added to each well incubated at room temperature for 1 h and washed five times. Finally, 100  $\mu$ l of tetramethylbenzidine peroxidase enzyme-substrate solution was added to each well. Enzymatic color reaction was allowed to develop at room temperature (20°C–25°C) in the dark for 15 min. This enzyme-substrate reaction had changed the solution blue. After 15 min, the reaction was stopped by adding 100  $\mu$ l of ELISA Stop Solution of 0.18 M sulfuric acid. The solutions were next mixed by tapping the plates gently. Subsequently, they changed from blue to yellow in color. The underside of wells was wiped with a lint-free tissue, and the absorbance was measured on an ELISA plate reader (Span Autoreader 3011, Span Diagnostics Ltd., Surat) set at 450 nm [Figure 2]. The values of all the samples were recorded in this manner. The obtained periodontal scores and ELISA values were statistically analyzed.

**Table 2: Periodontal characteristics and salivary immunological levels of the study population**

Clinical parameters	Values obtained
Probing depth (mm)	2.30 $\pm$ 0.22
Bleeding on probing (%)	22 $\pm$ 18
Salivary IgA ( $\mu$ g/ml)	92.93 $\pm$ 5.25
Salivary IgG ( $\mu$ g/ml)	2.13 $\pm$ 0.57

All values are expressed as the mean $\pm$ SD. SD: Standard deviation

## RESULTS

The mean PD and BOP scores are enumerated in Table 2. These results signify that PD was <3 mm indicating no/mild periodontitis in the study population.<sup>[8]</sup> Mean BOP values were <30% indicating only localized bleeding.<sup>[7]</sup> Mean salivary IgA and IgG values are listed in Table 2. The results of ELISA analysis showed a significantly higher release of salivary IgA as compared to IgG.

## DISCUSSION

This study assessed salivary immunoglobulin levels of IgA and IgG using ELISA, in addition to clinical evaluation of periodontal status in patients with dental caries, to derive at a correlation between dental caries and periodontitis.

Saliva, which was used to assess the immunoglobulin levels, is a valuable diagnostic fluid that can be collected in a simple and noninvasive method for assessing locally and systemically derived markers of both periodontal disease and dental caries.<sup>[11]</sup> It contains glandular secretions not only from the major and minor salivary glands but also secretions of nonglandular nature like the gingival crevicular fluid, thus providing a very rich source of host-derived proteins such as immunoglobulins and enzymes.

The collection of whole saliva can be done by various methods presently available including draining, spitting, suction, and swab method.<sup>[12]</sup> Saliva collected in this study by draining method was found to be without any stimulation and more reliable, according to Yamuna and Muthu.<sup>[9]</sup> According to studies conducted on salivary flow rates by Yamuna et al., he reported “higher salivary flow rate values in the standing position and lower values in the lying position as compared with the flow rate in the sitting position.”<sup>[9]</sup> Thus, it is ideal to collect saliva, while the subject is sitting upright with the head slightly tilted forward and the eyes open as it was done in the current study.<sup>[10]</sup>

Antibody titer defined the severity of the disease with greater accuracy than did levels of microorganisms because assessing antibody levels proves to be highly specific, and even transient alterations can be detected to reveal the true nature of the infectious load.<sup>[13]</sup> Antibody titer recorded through salivary immunoglobulin levels as done

in the current study provides a marker for the severity and progression of different diseases.

ELISA test, which was used for immunological analysis, is based on the principle of simultaneous binding of human immunoglobulin to two antibodies, one monoclonal immobilized on microwell plates, and the other polyclonal conjugates with HRP. Bound/free separation is performed by a simple solid-phase washing after the incubation period. The enzyme in the bound-fraction reacts with the substrate (hydrogen peroxide) and develops a blue color that changes into yellow when the stop solution (sulfuric acid) is added. The color intensity is proportional to the IgA or IgG concentration in the sample.

The results of this clinicoimmunological study reveal that healthy patients with high caries index had very low BOP and PD scores, while their salivary immunoglobulin profile showed elevated IgA and no increase in IgG levels. These results point to a negative or inverse correlation between the two diseases.

These findings are in agreement with a previously done clinicobacteriological study,<sup>[14]</sup> which assessed the clinical parameters of the two diseases along with salivary levels of *P. gingivalis* and *S. mutans* using real-time polymerase chain reaction which concluded that an inverse relationship existed between caries and periodontitis. Studies corroborate an inverse relation between dental caries and aggressive periodontitis in patients with Down's syndrome.<sup>[15]</sup>

Two main hypotheses may be put forth for this inverse relation: (1) occurrence of bacterial antagonistic interactions wherein one species could inhibit the other by the production of antimicrobial compounds, which may be nonprotein metabolites such as organic acids and hydrogen peroxide or proteinaceous compounds such as bacteriocins<sup>[16]</sup> and (2) pH change which occurs when acidogenic *S. mutans* make conditions uninhabitable for *P. gingivalis*. Experimental studies indicate that *S. mutans* has the ability to produce large amounts of acids, which consequently decrease the pH in the oral cavity below 4, while *P. gingivalis* cannot survive stably at a pH below 6.5, and their growth is only reproducible over a pH range of 6.7–8.3.<sup>[17]</sup>

The prevalence of a disease in the oral cavity is to a large extent decided by the nature of the organisms that can colonize the oral cavity and the conditions which make this colonization favorable.<sup>[18]</sup> Factors ranging from immune response to the disease process, genetic predisposition of the individual, nature and pH of saliva, etc., may influence this. With respect to caries and periodontitis, most of these

factors are in a state of contradiction with one another, thus making an individual more susceptible to either one of the diseases but not the other.

The clinical and immunological examination of healthy patients prone to dental caries in the current study revealed good periodontal health, higher IgA, and lower IgG titers in their saliva. Within the limitations of the study, it can be concluded that periodontal disease and dental caries are two separate entities which are entirely independent of each other with no correlation. However, further studies are needed with a larger population size and additional variables to reach more explicit results.

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### Conflicts of interest

There are no conflicts of interest.

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