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ORIGINAL ARTICLE

Increase in the level of oral neutrophils with gingival inflammation – A population survey

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Dental Plaque;
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Abstract *Objectives:* Host responses to oral inflammation include a continuous and substantive response with the influx of polymorphonuclear leukocytes (PMN). PMN, referred to as first responders, migrate rapidly from the circulatory system through the connective tissue to mitigate stimuli and localize in the saliva. This study examined the relationship between the well-established clinical indices of gingivitis and dental plaque and the PMN level.

Materials and Methods: This study enrolled adults aged 18–75 years, who provided voluntary informed consent. Oral rinse samples were collected from 159 participants to estimate the PMN levels prior to the full-mouth assessment for gingivitis and dental plaque using the respective clinical indices.

Results: The gingival index and dental plaque index scores were in the range of 0.098–2.71 and 0.73–4.78, respectively. Regardless of the age and gender, higher number of PMN was observed with higher gingival index and dental plaque index scores. Our analyses indicated a significant correlation between the oral PMN level and gingival index with a correlation coefficient of 0.66 ($p < 0.0001$). Similarly, the correlation between the PMN level and dental plaque index was statistically significant with a correlation coefficient of 0.57 ($p < 0.0001$). Regression analysis identified a significant relationship between the PMN level and clinical indices ($p < 0.0005$).

Conclusions: Increase in the PMN levels with increasing clinical scores (gingival and dental plaque indices) reflect the oral inflammatory burden, irrespective of age or gender. These observations warrant future studies on participants stratified by health status and research directed toward examining the effects of interventions.

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1. Introduction

The unique features of the human mouth include its relation to the external environment, distinctive anatomy, several mucosal interfaces, and resident microflora found as biofilms in distinct niches of the mouth (Arweiler et al., 2018, Chapple et al., 2015). Additionally, oral microorganisms can be readily found in the saliva that coat oral surfaces (Chan et al., 2015) and transmits the microflora intraorally (Lindenmuller and Lambrecht 2011).

Inflammatory signals in the mouth are derived from factors influencing the external environment, with diet and oral hygiene being the important contributors (Chapple et al., 2015, Twetman 2018, Mosaddad et al., 2017). Dietary residues in the mouth facilitate the growth and proliferation of indigenous microflora. Microbial proliferation is associated with the production of metabolic byproducts, such as acids (Twetman 2018), and those with immunogenic effects, including toxins, virulence factors, and cell wall components (Arweiler et al., 2018, Chapple et al., 2015, Mosaddad et al., 2017). Factors influencing the supragingival plaque biofilm also influence the composition of the subgingival microflora and support the nutritional networks of this complex microbial ecosystem (Takahashi 2017).

Oral hygiene measures for self-care are commonly practiced to reduce the dental plaque buildup and resultant gingivitis, with tooth brushing using a toothbrush and toothpaste being the most widespread approach (Barouch et al., 2019, Tartaglia et al., 2017). Despite the available evidence, informational programs, and outreach utilizing various educational channels to engage and implement adequate oral hygiene measures, common oral diseases remain prevalent (Arweiler et al., 2018, Chapple et al., 2015, Lindenmuller and Lambrecht 2011, Twetman 2018, Takahashi 2015). Various factors, including dexterity (Barouch et al., 2019) and oral health behaviors (Tartaglia et al., 2017, Knöfler et al., 2017), represent the modifiable factors influencing oral hygiene.

An important outcome of poor oral hygiene is gingival inflammation and the corresponding host response (Arweiler et al., 2018, Chapple et al., 2015). The critical feature of these host responses include accumulation of polymorphonuclear leukocytes (PMN) (Crawford et al., 2000, Jenne et al., 2018). These cells migrate from the systemic circulation through the connective tissues, providing a rapid host response, which is a notable characteristic of the PMN (Jenne et al., 2018). In addition to their established role in cellular responses, PMN have been shown to detach the bacteria from saliva-coated hydroxyapatite disks (Erad et al., 1989), which is influenced by the concentrations of serum and complement and is dependent on glycolytic metabolism.

Notwithstanding the advances in PMN biology and their role in the human mouth (Crawford et al., 2000, Nicu et al., 2018, Takubo et al., 1997, Seyedmajidi et al., 2015, Gasparoto et al., 2011), population surveys examining the relationship between PMN levels and common clinical indices for gingival inflammation and dental plaque are limited (Bhadbhade et al., 2012, Landzberg et al., 2015). Therefore, this study evaluated adult participants with varying levels of gingival inflammation and dental plaque index scores to determine their corresponding oral PMN levels.

2. Materials and Methods

This study was conducted at the SDM Dental College and Hospital, Dharwad, India, after obtaining approval of the study protocol by the Ethical Board. This study enrolled 159 adults (age range, 18–70 years) based on certain inclusion and exclusion criteria. The inclusion criteria were (1) voluntary completion of the informed consent form prior to any study-related efforts and (2) age of 18–70 years with at least 20 natural teeth present, excluding third molars.

The exclusion criteria were (1) presence of orthodontic bands, partial removable dentures, periodontal disease, carious lesions requiring restorations, or oral ulcers; (2) impending or ongoing pregnancy; (3) lactation, systemic conditions, or ongoing care with either a medical or dental professional; and (4) participation in a clinical study in the past 30 days or history of medical or dental treatments in the past 3 months.

2.1. Clinical procedures

The enrolled individuals were interviewed regarding their medical and dental health and were scheduled to arrive at the dental clinic of the SDM Dental College and Hospital for examinations. Clinical examinations were conducted by a dentist to examine the teeth, palate, and soft tissue regions. A full-mouth assessment for dental plaque and gingivitis was performed using the Turesky Modification of the Quigley-Hein index (Turesky et al., 1970) and Loe–Silness index, respectively (Loe-Silness 1963). The participants were screened to include a broad range of dental plaque and gingival index scores. Eligible participants were provided with a commercially available fluoride toothpaste and toothbrush for use until their examination, which was scheduled one week after the screening visit. Following study enrollment, they were instructed to refrain from using any other oral hygiene measures. Clinical examinations were conducted by one calibrated examiner to reduce inter-examiner variability.

During their examination visit, participants were scheduled to arrive in the morning after refraining from food or oral hygiene measures prior to their visit. At the dental clinic, they were provided with 10 mL of sterile saline in a wide-mouth tube. The participants rinsed their mouths for 10 s with the saline provided. This oral rinse sample was expectorated, collected in appropriately marked sterile tubes, and transported to the laboratory for immediate estimation of PMN. The procedures used for PMN estimation were as described previously (Sreenivasan and Prasad 2019, Sreenivasan and Haraszthy 2021, Sreenivasan et al., 2021, Sreenivasan and Haraszthy 2022); this was followed by a full-mouth examination for determining the dental plaque index and gingival index scores.

2.2. Statistical analysis

The sample size estimations for this exploratory study were based on historical data. This study aimed to identify the parameters across the gingivitis spectrum for a convenience sample.

The formula given below was used for sample size estimation. It included previously identified correlation coefficient values (26) to detect differences between the intervals of the gingival index scale. The sample sizes were estimated for a two-sided assessment at 99 % power and an alpha error of 1. The following formula was used:

$$N = (Z1 - \alpha/2 + Z\beta)2/(r2/1 - r2)$$

An approximate sample size of 150 participants was estimated to identify the differences after accounting for attrition, age group availability, and prevalence of gingival index scores.

Descriptive statistics were used to summarize the demographic features and clinical parameters along with the results of the PMN evaluations. The PMN levels were transformed to $\log_{10}$ values for analysis. Correlations between the PMN levels and clinical outcomes, i.e. the dental plaque index or gingival index scores, were analyzed. The Pearson correlation coefficient was determined for each pair of evaluated parameters.

Regression analysis was used to examine the relationships between the PMN levels and clinical variables. The gingival index and PMN results were categorized as normal or diseased based on cutoff values of 1.0 and 5.25, respectively. The gingival index was the response variable, while the PMN level was the predictive variable for the binary logistic regression model. The corresponding analysis for normal or diseased dental plaque index and PMN results were based on cutoff values of 2.5 and 5.25, respectively. The dental plaque index score was the response variable, while the PMN level was the predictive variable.

All p-values < 0.05 were considered statistically significant. The present proof does not explain the analysis but has the results + Fig. 3. A statistical assessment classified subjects based on PMN and relationships to gingivitis to determine the sensitivity and specificity of this evaluation. This assessment evaluated the proportion of true positives and negatives to describe a decision rule to classify subjects as at risk for gingivitis. Subjects with gingivitis scores less than 1.0 were classified as without disease [categorized as "0"] and those with gingivitis scores greater than 1.0 were classified as with disease and categorized as "1". Correspondingly, PMN cutoffs were set at scores of 4.0, 4.5, 5.0, 5.5, 6.0 Log PMN. Thus, there are several possible outcomes from this analysis i.e. PMN above or below each cutoff point with GI above or below a score of 1.0.

3. Results

Table 1 summarizes the demographics of the study participants (n = 159; 98 females and 61 males; average age, 30.77 years). The age range of the study population was 19–61 years. The average age of the females was 31.77 years (range, –19–60 years) and that of the males was 29.11 years (range, 19–61 years).

Figs. 1A and 1B show the comparison between the PMN levels and the gingival index and dental plaque index outcomes, respectively, for both the sexes. Additionally, a correlation analysis was performed. The analysis revealed a positive linear relationship between the PMN levels and gingivitis scores, with a Pearson correlation coefficient of 0.661. The Pearson correlation coefficient for the association between the PMN levels and dental plaque index scores was 0.57. PMN levels significantly correlated with both the gingival index and dental plaque index scores ($p < 0.0001$). Table 2 shows the population stratified by increasing gingivitis scores by units of 0.5. Notably, this analysis also demonstrated an increase in the number of PMN with increasing gingival index and dental plaque index scores.

A regression analysis was conducted for participants classified as diseased with dental plaque scores ≥ 2.5 and gingival index scores > 1.0. The probability of being classified as having high plaque level or gingivitis as a function of PMN levels was evaluated. Fig. 2A shows the analysis of the gingivitis scores. The probability of gingivitis increased with every 1 log PMN score increment. Our analysis suggests that participants with log PMN score ≤ 4.5 had a low probability of acquiring a gingivitis score ≥ 1.5. The outcomes of the dental plaque analysis are shown in Fig. 2B. The probability of dental plaque increased with every 1 log PMN score increment. Moreover, participants with log PMN scores ≤ 4 had a very low probability of acquiring a dental plaque score ≥ 2.5. Regression analyses indicated a statistically significant relationship between the PMN levels and dental plaque or gingivitis ($p < 0.0005$).

Results from the sensitivity and specificity analysis is shown in Fig. 3. PMN scores in the population ranged from 4-6.7 with this examination seeking to describe a decision rule to classify subjects as at risk for gingivitis. Based on the above analysis, a PMN cutoff between 5.0 to 5.5 provided the best range for analysis.

4. Discussion

Gingivitis and periodontal disease are chronic conditions reported globally (Arweiler et al., 2018). External factors along with oral microorganisms and their metabolic by-products are modifiers in the transition from health to disease. Immunogenic factors associated with microbial proliferation and their influences on virulence have been reported in the literature (Twetman 2018). Recent investigations have emphasized new techniques that incorporate advances in genomics and complementary approaches to advance the biological basis of these conditions for further development of effective therapeutics and clinical management (Chapple et al., 2015).

This study was designed to examine the PMN levels in the human mouth and its association with the widely accepted clinical indices that examine dental plaque (Turesky et al., 1970) and gingivitis (Loe and Silness 1963). These indices have

Table 1 Demographic characteristics of the study population.

Parameters	Number of participants	Average age (years)	Standard deviation (years)	Minimum age (years)	Maximum age (years)
Entire population	159	30.774	8.873	19	61
Females	98	31.778	9.064	19	60
Males	61	29.117	8.361	19	61

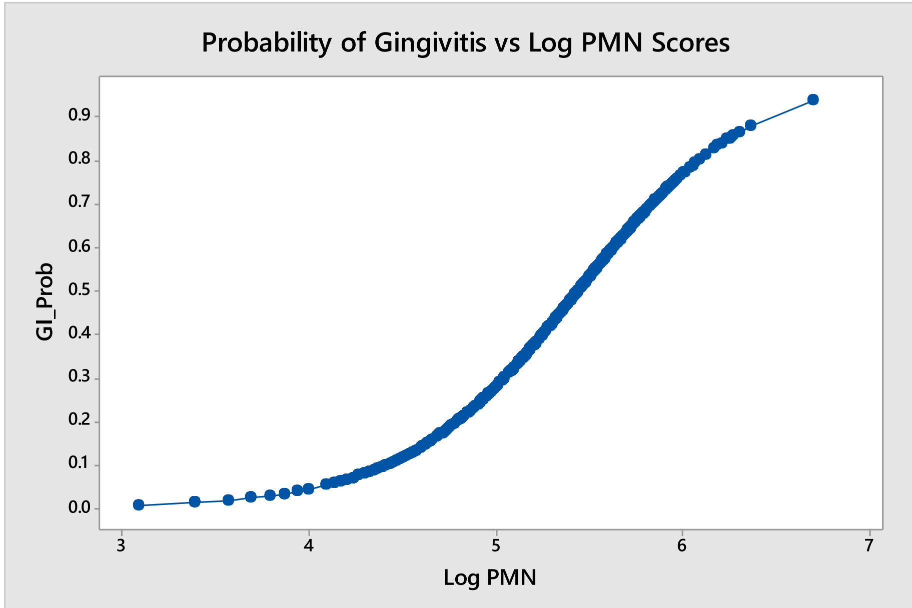

Fig. 1a Binary logistic regression equation for gingivitis.

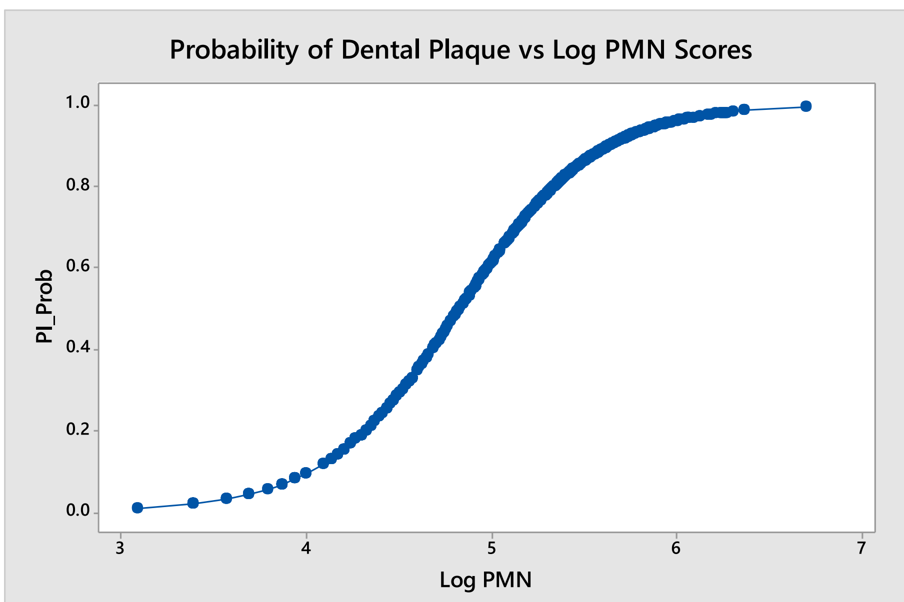

Fig. 1b Binary logistic regression equation for dental plaque index.

a long history and wide acceptance for determining the oral health status. Reports on the application of these indices in epidemiological surveys and investigations to document the effectiveness of interventions are readily available in the literature.

A substantial body of literature has examined the biology of PMN, its relation to disease, and markers and attributes appropriate for developing diagnostic tests. For instance, techniques quantifying neutrophils in nasal wash samples (Morris et al., 2017) and tear secretions (Postnikoff and Nichols 2017) and differentiation of airway inflammation by examining spu-

Table 2 Clinical outcomes and polymorphonuclear leukocytes levels of the participants based on clinical stratification.

Number of participants	Dental plaque index baseline score	Gingival index baseline score	Polymorphonuclear leukocytes
45	1.79 ± 0.49	0.29 ± 0.1	4.69 ± 0.33
11	1.99 ± 0.59	0.65 ± 0.11	4.98 ± 0.40
19	3.15 ± 0.66	1.30 ± 0.14	5.36 ± 0.38
38	3.35 ± 0.46	1.79 ± 0.14	5.60 ± 0.38
46	3.64 ± 0.47	2.29 ± 0.18	5.61 ± 0.45

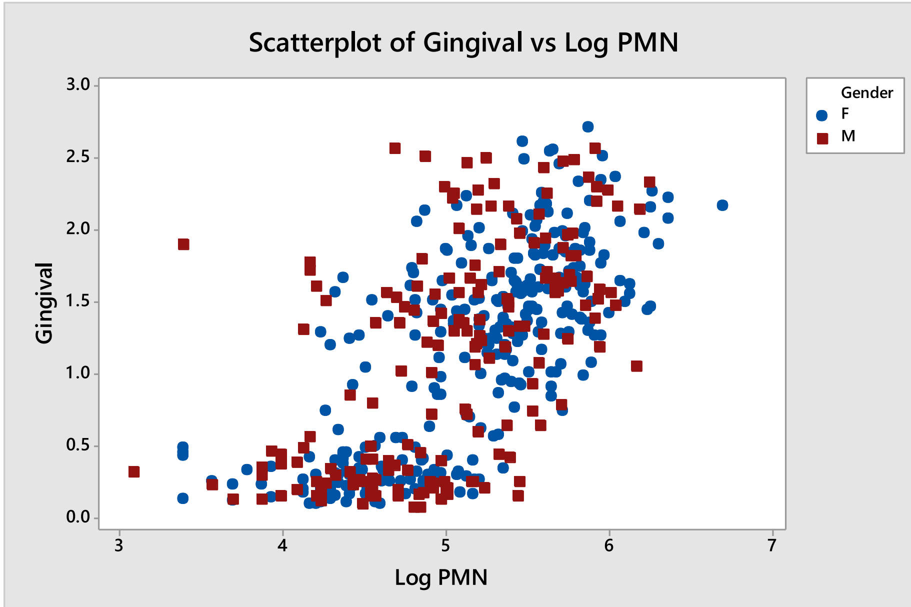

Fig. 2a Correlation between gingival index scores and polymorphonuclear leukocytes.

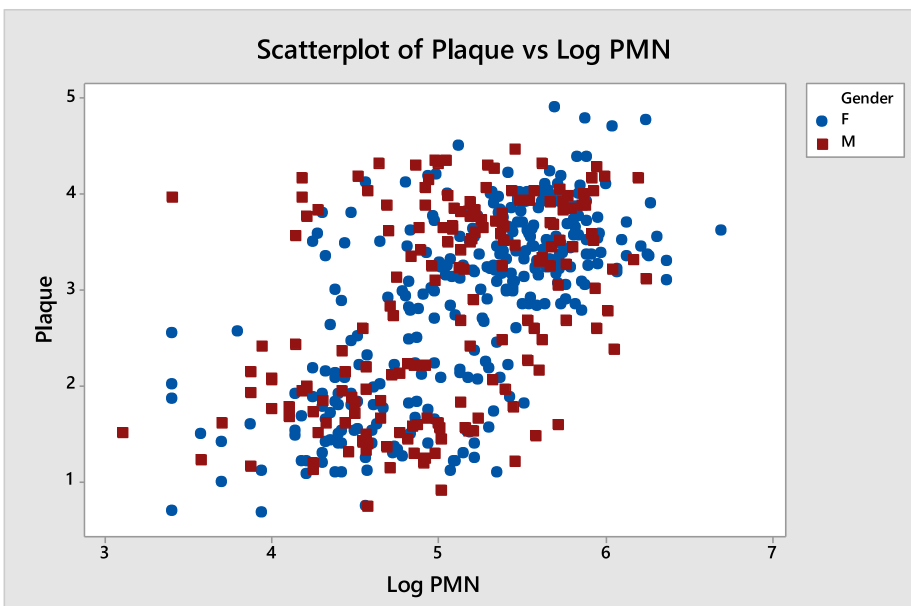

Fig. 2b Correlation between dental plaque index scores and polymorphonuclear leukocytes.

tum samples in asthma and chronic obstructive pulmonary disease have been reported (Gao et al., 2017). The central role of neutrophils in skin inflammation has led to investigations for developing point-of-care diagnostics for these samples (Ibarra-Silva et al., 2020, Langhorst et al., 2016). These efforts are directed at early detection of incipient disease, and they may offer advantages that augment the clinical management of patients. Notably, PMN-derived biomarkers have also been studied. The US Food and Drug Administration cleared PMN-derived biomarkers, such as lactoferrin and PMN-elastase (Langhorst et al., 2016), for assessing the clinical course of the disease.

Neutrophils in the saliva have been reported in previous investigations (Nicu et al., 2018, Takubo et al., 1997, Bhadbhade at l., 2012), and they were found in denture wearers (Gasparoto et al., 2011), children (Seyedmajidi et al., 2015), and clinically healthy individuals in their saliva, gingival crevice, and periodontal pockets samples (Crawford et al., 2000). In addition to determining the neutrophil count, several investigations have evaluated neutrophil-derived markers to

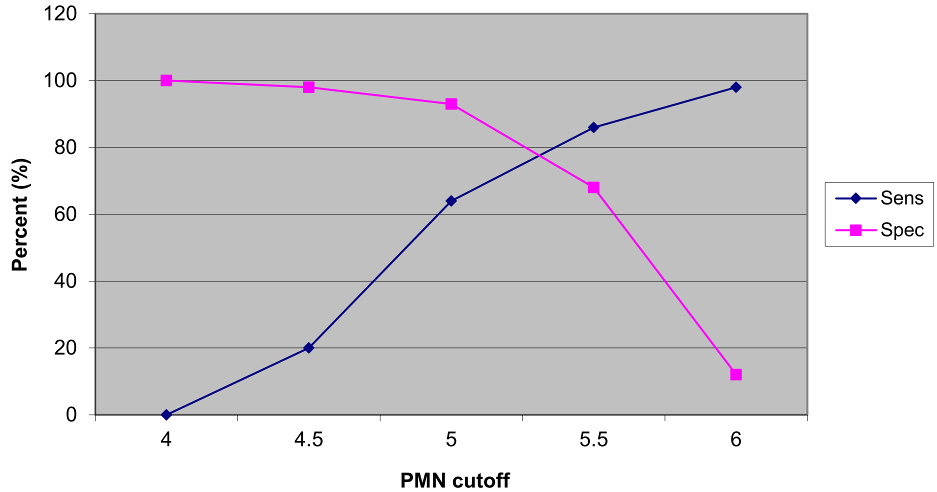

Fig. 3 Sensitivity and specificity analysis of the polymorphonuclear leukocytes results.

aid in clinical decisions (Ibarra-Silva et al., 2020, Langhorst et al., 2016, Tamimi et al., 2018, Marcaccini et al., 2010, Tan et al., 2020). The relative ease of salivary sampling (Marcaccini et al., 2010, Tan et al., 2020) with its many intrinsic advantages has increased the focus on this approach for both diagnostic and research initiatives (Tan et al., 2020, Hofman 2001) supporting biomarker discovery for high-frequency monitoring (Hofman 2001, Castagnola et al., 2017, Francosi et al., 2019).

In this investigation, an oral rinse sample was collected from the participants, representing a non-invasive sample obtained with limited preparatory steps (Sreenivasan and Prasad 2019, Sreenivasan and Haraszthy 2021, Sreenivasan et al., 2021, Sreenivasan and Haraszthy 2022). This procedure allows standardization of the collection steps and improves patient compliance with the study. Furthermore, the sample volume was appropriate for laboratory analyses in triplicate. The ability to transport these samples for subsequent analyses offers substantial flexibility for conducting studies in remote areas with limited analytical facilities. These findings are consistent with the increasingly accepted need to enroll diverse populations and to facilitate participation. Future areas of investigation include the establishment of procedures that feature robust sample storage protocols for greater flexibility in the study design.

Outcomes of relevance in this investigation included the correlation of the PMN levels with the clinical indices measuring gingivitis and dental plaque. A strong correlation was observed with the gingival index scores, regardless of the age and sex. Furthermore, the stepwise increase in the PMN levels with every 0.5-unit increase in the gingival index scores for this population highlights the utility of PMN as an appropriate marker for oral inflammation. Notable features of this cross-sectional population survey include its relatively large cohort and wide age range. Statistical outcomes determined the probability of using PMN levels to identify those at a risk of gingivitis, and a decision rule offers important guidance on these relationships. Utilizing this rule, it was evident that PMN levels < 4.5 were not associated with high clinical index scores. These outcomes corroborated the results of the regression analyses of the entire population irrespective of the age and sex.

5. Conclusion

Oral PMN levels increased with decreasing oral health status, which was determined by the clinical indices for dental plaque and gingivitis in a large cohort of adult participants. These associations were observed irrespective of age or sex. Future studies are warranted to evaluate the effects of interventions, enhance education and awareness, and manage outcomes in this regard.

Ethical approval

This study was approved by the Ethical Board of the SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India.

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