# Comparative Evaluation of Clinical, Hematological and Systemic Inflammatory Markers in Smokers and Non-Smokers with Chronic Periodontitis

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## Abstract

**Context:** Systemic conditions, especially chronic infections, have a direct impact on the general health and well-being of an individual. Similarly, the long-standing inflammatory changes seen during periodontitis have been associated with the altered diabetic control, preterm, low birth weight infants, and cardiovascular disease. Being a low-grade infection, the signs may not be as severe as seen in other systemic conditions, but they definitely cannot be ignored. **Aims:** The present study was designed to compare clinical, hematological, and systemic inflammatory markers in patients with chronic periodontitis. **Subjects and Methods:** A total of 90 chronic periodontitis patients were selected for the present study from the outpatient department of the Department of Periodontology, and the various clinical and hematological parameters were then assessed. **Statistical Analysis Used:** Z-test was used to compare the probing depth, clinical attachment loss, hematological parameter, and interleukin-6 values between Group A and Group B. Mann–Whitney U-test was used to compare gingival index, plaque index, and bleeding on probing between Group A and Group B. **Results:** The results of the study were based on the comparison of the clinical, hematological, and systemic inflammatory markers in smokers and nonsmokers with chronic periodontitis and came out to be statistically highly significant. **Conclusions:** With the resurgence of emphasis on significance of oral diseases related to systemic health, the medical professionals also need to familiarize themselves with the oral cavity and the oral-systemic inter-relationships to treat or reduce the morbidity of the underlying medical condition. Furthermore, the oral health care professionals must reach out to the medical community and the general public to improve patient care through education and communication about the oral health-systemic health link.

**Keywords:** Chronic periodontitis, clinical, hematological, nonsmokers, smokers, systemic inflammatory markers

## Introduction

Periodontal diseases are initiated by consortia of oral bacteria that elicit local inflammatory responses that lead to bleeding on probing (BOP), loss of periodontal attachment, bone, and eventual tooth loss.[1] They have been linked to systemic conditions including heart disease, diabetes, obesity, and complex metabolic syndrome. The association between periodontal diseases and these systemic conditions seems to be due to a low-grade inflammatory burden that links them through a common pathophysiological mechanism. Conceivably, locally secreted cytokines and periodontal pathogens can enter the bloodstream and contribute to damage elsewhere in the body, and there has been a substantial evidence to support this hypothesis.[2]

These associations suggest that periodontal diseases have systemic effects. In addition, some studies have found that periodontal infection elicits systemic blood chemistry changes.[3] For thousands of years, blood has been regarded as the ultimate body fluid that could indicate the progression of a disease process and before that the presence of a pathological state. In the past decade, there has been a renewed interest to study the effect of periodontitis on changes in the cellular and molecular components of peripheral blood. The relationship of periodontitis with leukocytes, thrombocytes, C-reactive protein, interleukin-6 (IL-6), fibrinogen, erythrocyte sedimentation rate (ESR), Von Willebrand factor, and red blood cells has been investigated in a large number of studies.[4]

Anemia of chronic disease (ACD) has been described in the literature and seems to be one of the most common forms of anemia observed in clinical medicine. ACD is defined as the anemia occurring in chronic infections, chronic

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inflammatory processes, or tumor formation that is not due to dysfunction of bone marrow cells or other diseases, and occurring despite the presence of adequate iron stores and vitamins.[5] It is characterized by hypoferramia with adequate reticuloendothelial iron stores, normal to elevated ferritin concentration and is seen as a frequent complication of chronic inflammatory conditions. A characteristic finding of the disorders associated with ACD was the increased production of the cytokines that mediates the immune or inflammatory response such as Tumor necrosis factor α (TNF-α), IL-1, and interferons. All the processes involved in the development of ACD can be attributed to these cytokines including shortened red cell survival, blunted erythropoietin response to anemia, impaired erythroid colony formation in response to erythropoietin, and abnormal mobilization of the reticuloendothelial iron stores.[6]

Hutter *et al.*,[7] suggested that periodontitis has chronic and systemic effects and that periodontitis may tend toward anemia. Tumor necrosis factor α (TNF-α) and IL-6 are key cytokines in the initiation and maintenance of systemic inflammation which have been implicated in progression and severity of periodontitis.[8] These cytokines are also released by periodontal tissues in response to bacterial infection, which suggests that periodontitis like other chronic disease may cause ACD. In addition, higher serum levels of these cytokines have been observed in patients diagnosed with chronic periodontitis than in periodontally healthy individuals.[9]

Smoking and tobacco consumption, to add on, further comprise a major environmental factor for periodontal disease. Numerous functions of oral or peripheral neutrophil are negatively affected by smoking or nicotine exposure, including phagocytosis, as well as decreased production of superoxide, hydrogen peroxide, and protease inhibitors. Alterations in gingival crevicular fluid and cytokines in smokers, tipping the balance in favor of tissue breakdown, have been noted. Smoking as it represents a significant confounder in the inter-relationship between periodontal disease and its systemic squeal, therefore, needs to be investigated in specific population with regard to how it impacts this relationship. Considering the above factors, the need of the study was felt to evaluate the influence of smoking on clinical parameters, hematologic signs of ACD, and marker of systemic inflammation (IL-6) in patients with chronic periodontitis.

## Subjects and Methods

A total of 90 patients were selected for the present study from the outpatient department of the Department of Periodontology. These patients were further divided into two groups with Group A having 45 nonsmokers and Group B having 45 smokers with moderate to severe chronic periodontitis [Figure1], with plaque highlighted with the help of disclosing solutions [Figure 2]. In the present study, smokers and nonsmokers were selected as per the criteria established by the Centre for Disease Control and Prevention.[10] Smokers were considered as "current smokers" if they had smoked 100 or more cigarettes over their lifetime and smokers at the time of interview. Nonsmokers were the ones who had not smoked 100 or more cigarettes in their lifetime.

The inclusion criteria for the study were:

- Patients who were willing to be a part of the study
- Male patients within age group of 30–65 years
- Patients with no other systemically illness
- Patients with body mass index within 18–28 kg/m$^2$
- Previously periodontally untreated patients
- Presence of at least 20 teeth and
- Patients with moderate to severe chronic generalized periodontitis including ≥30 % sites with probing depth (PD) ≥ 4 mm [Figure 3] and clinical attachment loss (CAL) ≥ 3 mm [Figure 4] (as per AAP criteria, 1999).

The exclusion criteria for the study were:

- Patients who were not willing to be a part of the study
- Chronically alcoholic patients
- Patients with history of use of vitamin or iron supplementation within last 6 months
- Patients on anti-inflammatory or antimicrobial therapy within last 6 months
- Patients with a history of trauma and/or recent tooth extractions within last 6 months.

A written informed consent was obtained from all. A detailed case history was then taken followed by a complete clinical examination. The following clinical parameters were recorded:

- Gingival index (GI), (Loe and Silness, 1963)
- Plaque index (PI), (Silness and Loe, 1964)
- BOP, (Ainamo and Bay, 1975)
- PD, (at six sites of teeth with UNC15 probe)
- CAL, (CEJ-Bottom of sulcus)

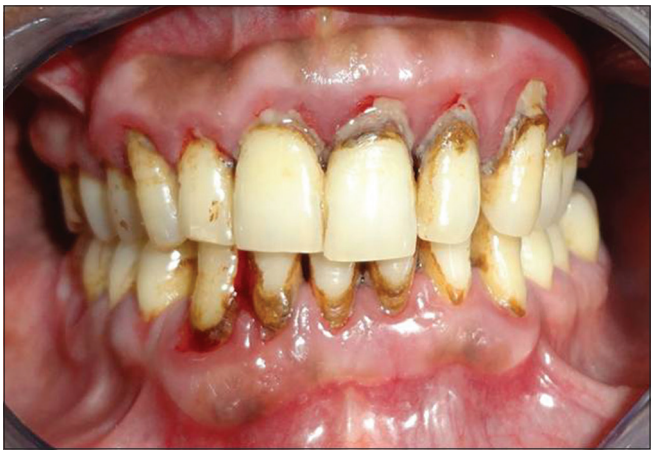

**Figure 1:** Chronic generalized periodontitis

- Percentage sites of pockets with CAL ≥ 3 mm and PD ≥ 4 mm (as per AAP criteria, 1999).

### Hematological analysis

Under aseptic conditions, 5 ml of venous blood sample was obtained between 8.30 and 11.00 am by venipuncture in the antecubital fossa [Figure 5] using 5 ml syringe. It was divided equally into two bulbs-one containing ethylenediaminetetraacetic acid (EDTA) and the other, a plain bulb [Figure 6]. The EDTA bulbs were then transported to the clinical laboratory for complete hemogram analysis. Plain tubes were immediately put on ice, centrifuged [Figure 7], and serum isolated [Figure 8] within 2 h. Serum samples were stored in Eppendorf tubes [Figure 9] in a storage vial box [Figure 10] containing a dry ice pack, before being transferred and stored in a freezer at −20°C till the time of evaluation.

Following parameters were assessed with the help of a standardized procedure:
Complete hemogram:

- Hemoglobin concentration (Hb %)
- Erythrocyte count (red blood cell [RBC] count)
- Hematocrit value (packed cell volume [PCV])
- ESR
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH) and
- MCH concentration (MCHC).

Sera assessment for IL-6 was done with the help of Diaclone Elisa Kit in the Department of Biochemistry [Figures 11-17].

## Results

The results of the study were based on the comparison of the clinical, hematological, and systemic inflammatory markers in smokers and nonsmokers with chronic periodontitis. All the measurements were subjected to statistical analysis with the help of SPSS version 19 software.

### Statistical analysis used

Z-test was used to compare the PD, CAL, hematological parameter, and IL-6 values between Group A and Group B. Mann–Whitney U-test was used to compare GI, PI, and BOP between Group A and Group B.

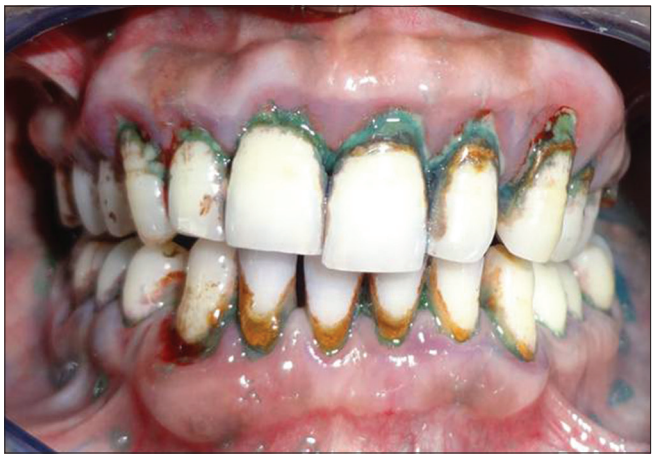

**Figure 2:** Chronic generalized periodontitis highlighted with disclosing solution

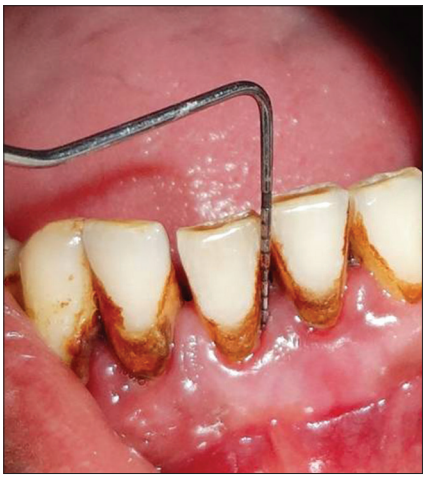

**Figure 3:** Measurement of probing depth

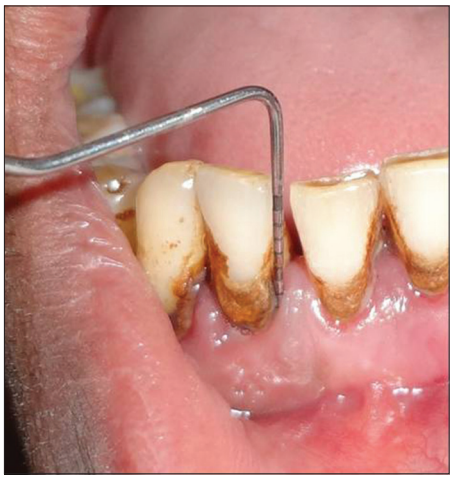

**Figure 4:** Measurement of clinical attachment level

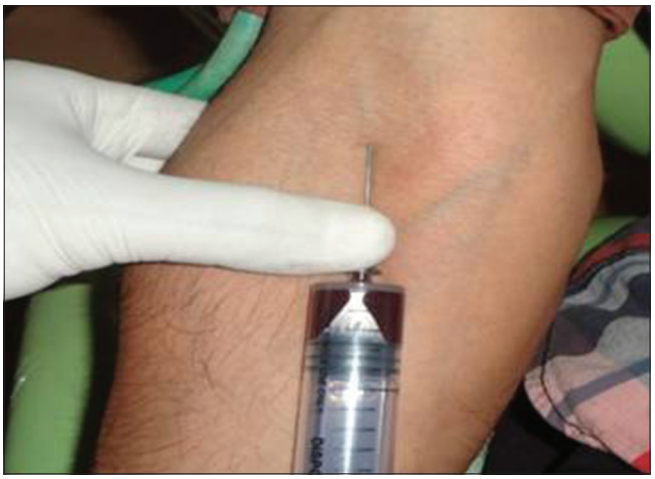

**Figure 5:** Collection of blood from antecubital vein

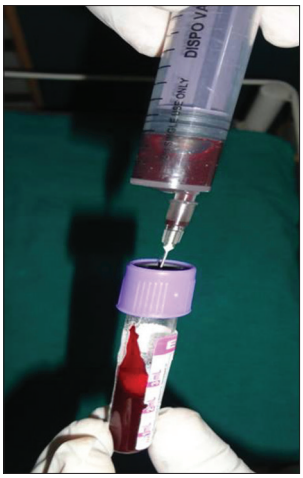

**Figure 6:** Blood being transferred in vial

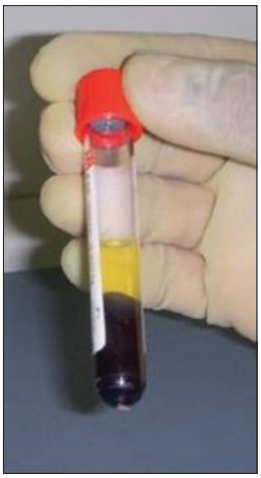

**Figure 7:** Centrifuged blood in vial

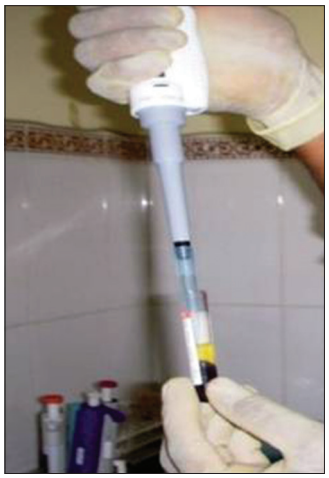

**Figure 8:** Separation of serum being carried out

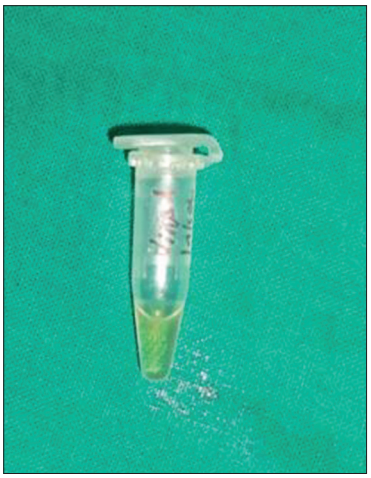

**Figure 9:** Separated serum in Eppendorf tube

**Figure 10:** All serum samples in storage box

**Figure 11:** Microplate washer

## Clinical results

Mean full mouth gingival score: Mean gingival score in smokers was 2.29 ± 0.24 and in nonsmokers was 2.09 ± 0.12. Comparison between smokers and nonsmokers showed statistically significant results as seen in Table 1 ($P < 0.0001$).

**Figure 12:** Microplate reader

**Figure 13:** Coated walls for interleukin-6

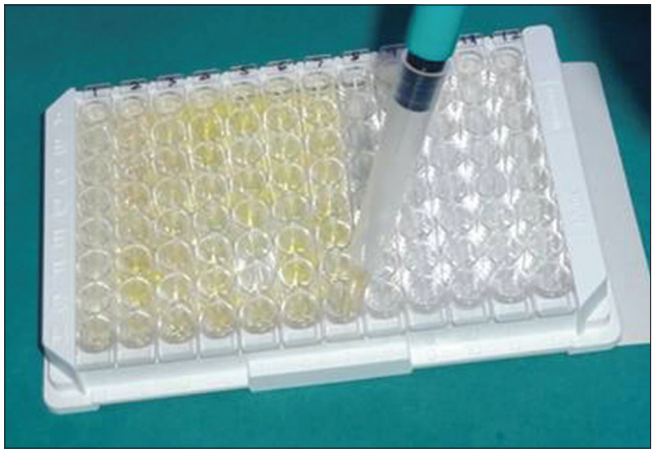

**Figure 14:** Samples added to coated walls for interleukin-6

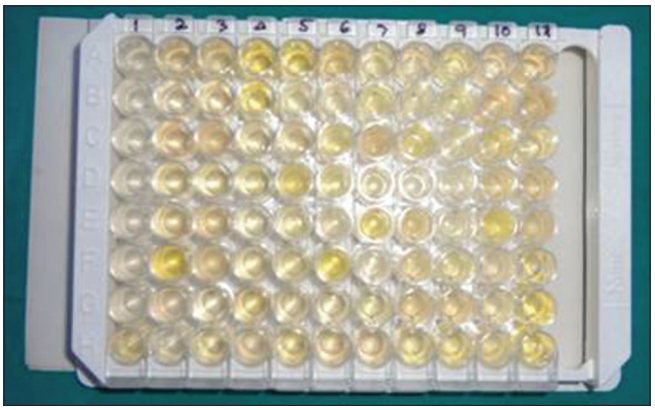

**Figure 15:** Samples during incubation

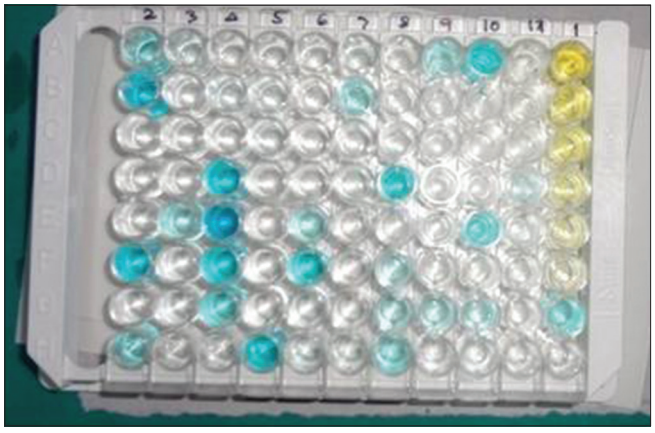

**Figure 16:** Samples after incubation and completion of procedure


**Figure 17:** Readings on microplate reader

*Mean full mouth bleeding on probing score*

Mean BOP score in smokers was 0.88 ± 0.06 and in nonsmokers was 0.87 ± 0.07. Comparison between these two groups showed a $P > 0.05$ which was statistically not significant [Table 1].

*Mean full mouth plaque score*

Mean plaque score in smokers was 2.18 ± 0.18 and in non-smokers was 1.18 ± 0.14. Comparison between these two groups showed a $P < 0.0001$ which was statistically significant [Table 1].

Mean full mouth PD score: Mean PD score in smokers was 5.99 ± 0.33 and in nonsmokers was 4.98 ± 0.35. Comparison between these two groups showed a $P < 0.0001$ which was statistically significant [Table 2].

*Mean full mouth clinical attachment level score*

Mean clinical attachment level (CAL) score in smokers was 8.41 ± 0.35 and in nonsmokers was 7.02 ± 0.52. Comparison between these two groups showed a $P < 0.0001$ which was statistically significant [Table 2].

Percentage of sites with pockets with PD ≥ 4 mm (as per AAP criteria, 1999): Percentage of sites with pockets with PD ≥ 4 mm (as per AAP criteria, 1999) was calculated for both the groups.

Percentage of sites with pockets with probing depth (PD) ≥ 4 mm

$$= \frac{\text{Total no. of sites with pockets with } (\text{PD}) \geq 4 \text{ mm}}{\text{Total no. of sites examined}} \times 100$$

Mean % of sites with pockets with PD ≥ 4 mm (as per AAP criteria, 1999) in smokers was 72.28 ± 7.80 and in nonsmokers was 53.46 ± 3.56. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant [Table 3].

Percentage of sites with pockets with CAL ≥ 3 mm (as per AAP criteria, 1999): Percentage of sites with pockets with CAL ≥ 3 mm (as per AAP criteria, 1999) was calculated for both the groups.

Percentage of sites with pockets with clinical attachment loss (CAL) ≥ 3 mm

$$= \frac{\text{Total no. of sites with pockets with } (\text{CAL}) \geq 3 \text{ mm}}{\text{Total no. of sites examined}} \times 100$$

Mean % of sites with pockets with CAL ≥ 3 mm (as per AAP criteria, 1999) in smokers was 56.85 ± 8.49 and in nonsmokers was 47.75 ± 7.27. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant [Table 3].

## Hematological results

*Mean interleukin-6 level*

Mean IL-6 level in smokers was 15.47 ± 4.43 and in nonsmokers was 5.38 ± 0.49. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant [Table 4].

*Mean hemoglobin level*

Mean Hb level in smokers was 11.9 ± 0.91 and in nonsmokers was 13.67 ± 0.53. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant [Table 5].

**Table 1: Comparison of GI, BOP, and PI in smoker and nonsmoker groups**

| Clinical parameters | Mean±SD (*n*=45) | | *Z* | *P* |
|---|---|---|---|---|
| | Smokers | Nonsmokers | | |
| GI | 2.29±0.24 | 2.09±0.12 | 3.95 | <0.0001 |
| BOP | 0.88±0.06 | 0.87±0.07 | 0.42 | >0.05 |
| PI | 2.18±0.18 | 1.81±0.14 | 7.02 | <0.0001 |

GI: Gingival index; BOP: Bleeding on probing; PI: Plaque index; SD: Standard deviation

**Table 2: Comparison of PD and CAL in smoker and nonsmoker groups**

| Clinical parameters | Mean±SD (*n*=45) | | *Z* | *P* |
|---|---|---|---|---|
| | Smokers | Nonsmokers | | |
| PD (mm) | 5.99±0.33 | 4.98±0.35 | 14.13 | <0.0001 |
| CAL (mm) | 8.41±0.35 | 7.02±0.52 | 14.86 | <0.0001 |

PD: Probing depth; CAL: Clinical attachment loss; SD: Standard deviation

**Table 3: Comparison of percentage of sites with PD and CAL as per AAP criteria, 1999 in smoker and nonsmoker groups**

| Clinical parameters | Mean±SD (*n*=45) | | *Z* | *P* |
|---|---|---|---|---|
| | Smokers | Nonsmokers | | |
| Percentage of sites with PD ≥4 mm | 72.28±7.80 | 53.46±3.56 | 14.72 | <0.0001 |
| Percentage of sites with CAL ≥3 mm | 56.85±8.49 | 47.75±7.27 | 5.46 | <0.0001 |

PD: Probing depth; CAL: Clinical attachment loss; SD: Standard deviation; AAP: American Academy of Periodontology

**Table 4: Comparison of IL-6 levels in smoker and nonsmoker groups**

| Parameters | Mean±SD (*n*=45) | | *Z* | *P* |
|---|---|---|---|---|
| | Smoker | Nonsmoker | | |
| IL-6 pg/mL | 15.47±4.43 | 5.38±0.49 | 15.19 | <0.0001 |

IL-6: Interleukin-6; SD: Standard deviation

**Table 5: Comparison of hematological parameters in smoker and nonsmoker groups**

| Hematological parameters | Mean±SD (*n*=45) | | *Z* | *P* |
|---|---|---|---|---|
| | Smokers | Nonsmokers | | |
| Hb | 11.9±0.91 | 13.67±0.53 | 11.21 | <0.0001 |
| PCV | 35.44±1.45 | 40.54±1.50 | 16.34 | <0.0001 |
| RBC | 3.98±0.18 | 4.62±0.27 | 13.43 | <0.0001 |
| ESR | 17.76±2.38 | 10.6±1.51 | 16.99 | <0.0001 |
| MCV | 85.41±5.34 | 85.32±5.68 | 0.08 | >0.05 |
| MCH | 29.38±2.71 | 28.12±2.78 | 2.17 | <0.05 |
| MCHC | 34.44±1.58 | 34.49±1.95 | 0.14 | >0.05 |

SD: Standard deviation; Hb: Hemoglobin; PCV: Packed cell volume; RBC: Red blood cell; ESR: Erythrocyte sedimentation rate; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration

*Mean packed cell volume*

Mean PCV level in smokers was 35.44 ± 1.45 and in nonsmokers was 40.54 ± 1.50. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant [Table 5].

*Mean red blood cell count*

Mean RBC count in smokers was 3.98 ± 0.18 and in nonsmokers was 4.62 ± 0.27. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant [Table 5].

*Mean erythrocyte sedimentation rate*

Mean ESR level in smokers was 17.76 ± 2.38 and in nonsmokers was 10.6 ± 1.51. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant [Table 5].

*Mean corpuscular volume*

Mean MCV level in smokers was 85.41 ± 5.34 and in nonsmokers was 85.32 ± 5.68. Comparison between these two groups showed a $P > 0.05$ which was statistically insignificant [Table 5].

*Mean corpuscular hemoglobin*

Mean MCH level in smokers was 29.38 ± 2.71 and in nonsmokers was 28.12 ± 2.78. Comparison between these two groups showed a $P < 0.05$ which was statistically significant [Table 5].

*Mean corpuscular hemoglobin concentration*

Mean MCHC level in smokers was 34.44 ± 1.58 and in nonsmokers was 34.49 ± 1.95. Comparison between these two groups showed a $P > 0.05$ which was statistically insignificant [Table 5].

## Discussion

Mouth is the mirror of health and disease, as an assessable model for the study of other tissues or organs and as a potential source of pathology affecting other systems and organs. The concept of periodontal diseases as localized entities affecting only the teeth and supporting apparatus has been revised, as it has been seen that rather being confined to the periodontium, periodontal diseases have wide-ranging systemic effects. Periodontal disease has a potential relationship with several other systemic conditions such as cardiovascular diseases, diabetes mellitus, adverse pregnancy outcomes, obesity, and stroke.[10-15] Substantial scientific data indicate that the localized infections characteristic of periodontitis can have a significant impact on the systemic health of both humans and animals.[3] Just as the periodontal tissues mount an immune-inflammatory response to bacteria and their products, the systemic challenge with these agents also induces a major vascular response. This host response may offer explanatory mechanisms for the interactions between periodontal infection and a variety of other potential systemic disorders.[16] One of the lesser documented associations has been the inter-relationship between periodontal disease and anemia.

ACD is an immune driven process in which cytokines result in decreased erythropoietin production, impaired proliferation of erythroid progenitor cells, and disturbed iron homeostasis.[17,18] This normocytic and normochromic anemia has been described in many chronic diseases such as rheumatoid arthritis, renal failure, bacterial and parasitic infections, and chronic periodontitis, among others. Long-standing chronic inflammation can lead to anemia. Similarly, such chronic diseases as bacterial, fungal, and parasitic infections have also been reported to show signs of anemia. Anemia seen in chronic diseases is collectively termed as "ACD."[19,20] The association of anemia and periodontitis has been explored since the early 20$^{th}$ century. Earlier reports have suggested anemia to be a cause, and not a consequence, of destructive periodontitis. Lainson[21] was one of the first authors to implicate anemia as a systemic cause of periodontitis. Chawla *et al*.[22] suggested that anemia is an important factor in the etiology or pathogenesis of periodontal disease. Seigel[23] reported a depression in the number of erythrocytes apparently secondary to the presence of periodontal disease. Hutter *et al*.[7] evaluated the blood parameters in patients with chronic periodontitis and concluded that these patients show signs of anemia. The literature regarding the effect of a chronic periodontal inflammatory burden on ACD is sparse, especially in Asian countries with a high incidence of anemia.

Cigarette smoking is the major preventable risk factor in the incidence as well as a progression of periodontal disease[24,25] and also impacts red blood cells. Females were excluded as they undergo physiological blood loss and cyclic hormonal imbalance, which is responsible for an altered and exaggerated response to local factors. Gokhale *et al.*,[26] reported that in India, anemia is more prevalent in females due to poor nutrition, increased menstrual losses, high incidence of tropical and intestinal infections, and other miscellaneous factors. Furthermore, adiposity has well-recognized effects on the systemic host response. Therefore, in this study, the BMI measures were also analyzed. Nishida *et al.*,[27] suggested that the immunological disorders or inflammation might be the reason that obese smokers tend to exhibit escalating poor periodontal status relative to nonobese and nonsmoking individuals. Hence, obese patients were excluded from the study.

In the present study, mean plaque score in smokers was 2.18 ± 0.18 and in nonsmokers was 1.18 ± 0.14 which is in agreement with the studies conducted by Linden *et al.*,[28] Preber *et al.*,[29] and Zambon *et al.*,[30] who interpreted the effect of cigarette smoking on the periodontium to be indirect

and due to inadequate levels of oral hygiene and increased plaque accumulation among smokers relative to nonsmokers. The mean gingival score in smokers was 2.29 ± 0.24 and in nonsmokers was 2.09 ± 0.12 which was again in accordance with the study conducted by Haber *et al*.[31] The statistically insignificant mean BOP score in smokers of 0.88 ± 0.06 and in nonsmokers of 0.87 ± 0.07 was congruent with the reports regarding the disputed effects of nicotine on blood flow with some observers claiming a reduced[32] while a few others, an increased[33] or largely unchanged[34] blood flow. Mean PD score in smokers came out to be 5.99 ± 0.33 while in nonsmokers, came out to be 4.98 ± 0.35. Comparison between these two groups showed a $P < 0.0001$ which was statistically significant. Mean CAL score also came out to be statistically significant with values of 8.41 ± 0.35 in smokers while 7.02 ± 0.52 in nonsmokers. In the present study, PD and CAL values were higher in smokers than in nonsmokers in accordance with the previous literature.[28,30,35-38] A significant positive correlation has been shown between smoking and CAL values.[35,39] The reason of increased PD and CAL values in smokers may depend on the accumulation of dental plaque and poor oral hygiene.[29,30] Mean % of sites with pockets with PD ≥ 4 mm (as per AAP criteria, 1999) in smokers was 72.28 ± 7.80 and in nonsmokers was 53.46 ± 3.56 while the mean % of sites with pockets with CAL ≥ 3 mm (as per AAP criteria, 1999) in smokers was 56.85 ± 8.49 and in nonsmokers was 47.75 ± 7.27. Comparison between these two groups showed a $P < 0.0001$ which was statistically highly significant. These findings were in support of smoking's role in increased severity of periodontal disease.[24,25]

Smoking affects the immune system and microflora of the patient leading to deeper PDs[40,41] and greater clinical attachment[42] and bone loss.[43,44] Neutrophil functions such as phagocytosis,[45] superoxide production,[46] and protease inhibitor production[47] are hampered by exposure to nicotine. In addition, tobacco products may modify the production of cytokines. Smoking has a greater impact on the release of cytokines from neutrophils than periodontal disease. Smoking also affects erythrocytes and other blood parameters.[48] According to a study by Erdemir *et al*.,[49] smokers with chronic periodontitis had a lower number of erythrocytes, a lower value of Hb, and lower hematocrit and iron compared to nonsmokers with chronic periodontitis. Our findings supported this observation of the previous studies as indicated by the lower mean Hb level in smokers of 11.9 ± 0.91 and in nonsmokers of 13.67 ± 0.53. Similarly, the mean RBC count in smokers came out to be 3.98 ± 0.18 while in nonsmokers came out to be 4.62 ± 0.27. Mean PCV level in smokers was 35.44 ± 1.45 and in nonsmokers was 40.54 ± 1.50, while mean MCV level in smokers was 85.41 ± 5.34 and in nonsmokers was 85.32 ± 5.68. A mean MCH level in smokers of 29.38 ± 2.71 and in nonsmokers of 28.12 ± 2.78 while a mean MCHC level in smokers of 34.44 ± 1.58 and in nonsmokers of 34.49 ± 1.95 were also in agreement with the symptoms of ACD. MCV levels are the main determinants of the some kinds of anemia. A depressed level of MCV (microcytosis) relates anemia to iron deficiency while an elevated level of MCV (macrocytosis) relates anemia to vitamin deficiency.[50,51] In our study, MCV levels were between the reference values, as mostly seen in ACD and called as normocytosis.

Tobacco components may also modify the production of cytokines or inflammatory mediators. An imbalance in cytokine production seems to occur in smokers. Elevated concentrations of IL-6 as observed in the present study with a mean IL-6 level in smokers of 15.47 ± 4.43 while in nonsmokers of 5.38 ± 0.49 were in accordance with the previous studies which revealed an elevated IL-6 level in the plasma of smokers,[51] as well as in the alveolar cells of healthy donors stimulated by tobacco glycoprotein.[47] Nicotine also, one of the most deleterious products of cigarette, has been shown to increase the release of IL-6 by cultured murine osteoblasts.[52,53] Giannopoulou *et al*.[54] indicated that smoking interferes with cytokine production. It has also been reported that the release of cytokines from peripheral neutrophils and various parameters of inflammation in plasma seem to be affected more by cigarette smoking than periodontal disease.[55]

It has been proposed that hepcidin is a primary factor in the pathogenesis of the ACD, a cytokine-mediated anemia commonly encountered in clinical practice and characterized by hypoferremia with adequate reticuloendothelial iron stores.[56] Previous studies indicated that IL-6 mediates an increase in hepcidin levels and consequent hypoferremia during inflammation.[57] This also suggested that hepcidin could be the pathogenic mediator of ACD. Kemna *et al*.[58] showed the importance of IL-6-hepcidin axis in the development of hypoferremia in inflammation and highlighted the rapid responsiveness of this iron regulatory system. Nemeth *et al*.[59] found that patients with ACD due to inflammatory disorders or infections had markedly increased excretion of urinary hepcidin. *In vitro* stimulation of fresh human hepatocytes with a panel of cytokines showed strong induction of hepcidin mRNA by IL-6, but not by TNF-α and other potential cytokines, indicating that IL-6 may be the mediator of hepcidin induction by inflammation. Although there are not many studies about the relationship between hepcidin and periodontal diseases or the effect of smoking on hepcidin, it is well-known that pro-inflammatory cytokines and mediators are significantly elevated with gingival inflammation during the destructive phase of periodontitis.[60-65]

To brief, our findings imply that smokers with chronic periodontitis manifest an increased systemic inflammatory burden as evidenced by higher IL-6 levels when compared to nonsmoker subjects with chronic periodontitis owing to dual effects of smoking and periodontal inflammation on the cytokine response. This is congruent with hematological parameters suggestive of ACD in this group. Taken together,

the present study demonstrates a positive association of concurrent periodontal disease and smoking status on ACD. Future studies are needed to verify the causal link by use of larger sample sizes, prospective study designs, and investigation of hepcidin levels. Potential drawbacks of the study were that no female patients were included in the study; all parameters were recorded at baseline with no evaluation of change in level was done post periodontal therapy and that the data obtained were specific to individuals suffering from chronic generalized periodontitis and were not compared with healthy individuals.

## Conclusion

The following conclusions were arrived at from this study:

- Smoking increases severity of periodontal disease
- Chronic generalized periodontitis being a long standing infection resulted in greater depression in the values of hematological parameters. The reduction of values was more for smokers than for nonsmokers
- Highly significant values were observed with respect to Hb, PCV, RBC, ESR, IL-6, PD, CAL, GI, PI, percentage of PD, and percentage of CAL in the smokers with chronic generalized periodontitis than the nonsmokers
- Statistically significant changes in the values of MCH while nonsignificant changes in the values of MCV and MCHC indicated that the decreased red cell counts were not due to any vitamin or mineral deficiency, but secondary to the inflammatory changes induced by periodontal disease
- No significant changes were observed with respect to BOP.

The emerging field of periodontal medicine offers new insights into the concept of the oral cavity as the different systems in the body are interconnected with each other. If this notion is found to be accurate, we need to assume a larger responsibility apart from diagnosing and treating the periodontal infections, and also educate the public about the importance of oral health in the overall systemic well-being of the individual. With the resurgence of emphasis on significance of oral diseases related to systemic health, the medical professionals also need to familiarize themselves with the oral cavity and the oral-systemic inter-relationships to treat or reduce the morbidity of the underlying medical condition. Furthermore, the oral health care professionals must reach out to the medical community and the general public to improve patient care through education and communication about the oral health-systemic health link.

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