

## Quantitative Evaluation of Neutrophils in Different Stages of Periodontitis - A Histochemical Study

Koppuravuri Venkata Tirumala Susmitha<sup>1</sup>, Dr. Chaitanya Adurty<sup>2</sup>, Alladi Chelcy Bhushan<sup>3</sup>,  
Kondapaneni Tara<sup>4</sup>, Dr. Anumala Deepa<sup>5</sup>, Dr. Yamuna Marella<sup>6</sup>

<sup>1</sup>Undergraduate student, Department of periodontology, Sibar institute of dental sciences, Takkellapadu,  
Andhra Pradesh, India.

<sup>2</sup>Professor, Department of periodontology, Sibar institute of dental sciences, Takkellapadu, Andhra  
Pradesh, India.

<sup>3</sup>Undergraduate student, Department of periodontology, Sibar institute of dental sciences, Takkellapadu,  
Andhra Pradesh, India.

<sup>4</sup>Undergraduate student, Department of periodontology, Sibar institute of dental sciences, Takkellapadu,  
Andhra Pradesh, India.

<sup>5</sup>Professor, Department of periodontology, Sibar institute of dental sciences, Takkellapadu, Andhra  
Pradesh, India.

<sup>6</sup>Reader, Department of periodontology, Sibar institute of dental sciences, Takkellapadu, Andhra Pradesh,  
India.

### Abstract

**Introduction:** Neutrophils are key components of the innate immune response and play a critical role in the pathogenesis of periodontal disease. Their presence and activity can reflect the severity and progression of periodontitis. This study aimed to quantitatively evaluate neutrophil infiltration in gingival tissues across different stages of periodontitis using histochemical methods.

**Materials and Methods:** Under aseptic condition 5 ml venous blood samples were collected from patients diagnosed with Stage 1 to Stage 4 periodontitis based on clinical and radiographic criteria. Histochemical staining was performed to identify and count neutrophils. The neutrophil counts were statistically analyzed and compared across the stages.

**Results:** A statistically significant increase in neutrophil count was observed with advancing stages of periodontitis. Stage 1 tissues showed minimal neutrophil presence, whereas Stages 3 and 4 exhibited markedly elevated counts, indicating heightened inflammatory activity.

**Conclusion:** The study demonstrates a positive correlation between neutrophil count and the severity of periodontitis. These findings reinforce the role of neutrophils in disease progression and highlight their potential as indicators of periodontal inflammation and tissue destruction.

**Keywords:** Blood, Periodontitis, Probing Pocket Depth, Neutrophils

### **Introduction:**

Oral health is widely recognized as having a significant impact on an individual's overall well-being. Periodontal diseases, in particular, present a major public health concern globally. Periodontitis is a chronic inflammatory condition affecting the supporting structures of the teeth, triggered by specific pathogenic microorganisms in individuals with a predisposed immune response [1]. The localized infections typical of periodontitis are not confined to the oral cavity—they can exert systemic effects on both human and animal health. Similar to the localized immune-inflammatory response initiated in periodontal tissues, exposure to these microbial agents systemically can provoke widespread vascular responses [2].

A major advancement in the understanding of periodontal disease has come from the concept of host susceptibility [3]. This shift in perspective emphasizes that the progression of periodontal destruction is more closely associated with the host's immune response than with the mere presence of bacterial plaque. Both exaggerated and deficient immune responses to microbial insult have been observed in cases of periodontitis [4].

Neutrophils are the most prevalent immune cells found in periodontal tissues and oral fluids. These cells migrate from nearby capillaries toward the gingival sulcus in response to chemotactic signals [5]. Polymorphonuclear leukocytes (PMNs), primarily neutrophils, serve as key defenders against periodontal pathogens. Crevicular fluid analysis has shown that neutrophils dominate the leukocyte population. However, their role is twofold—they contribute to microbial clearance while also being implicated in tissue damage observed in periodontitis [6].

Impaired phagocytic activity of neutrophils has been linked to rapid alveolar bone loss, even in the presence of minimal local irritants. As such, quantifying neutrophil levels at various stages of periodontitis is essential for understanding the host's immune response and could aid in managing the hyperactivity of these cells during treatment [7].

One method to achieve this involves staining peripheral blood smears to visualize and count neutrophils. Leishman stain is particularly effective for this purpose. It enhances the contrast between the nucleus and cytoplasm of neutrophils, providing a distinct violet coloration of the nucleus and clear visualization of granules. This contrast facilitates accurate differential counting, unlike simpler stains such as methylene blue and eosin, which offer limited cytoplasmic-nuclear distinction. Therefore, Leishman stain will be used in the current study to allow for precise quantitative analysis of neutrophils [7].

The objective of this study is to quantitatively assess neutrophil counts across different stages of periodontitis, providing insight into the host immune response associated with disease progression.

## **Materials and Methods:**

### **Study Population**

Sample size was calculated by using G power 3.1.9.2 software version with Effect size – 0.5,  $\alpha$  error – 0.05, Power (1-  $\beta$ ) – 0.80 and sample size was estimated as Eighty Patients. A total of 80 patients (According to 2017 AAP Classification) who reported to the Department of Periodontics at Teaching Dental Institution, were selected for this study based on specific inclusion and exclusion criteria. The participants were divided into four groups, with each group consisting of 20 individuals. The study was approved by the institutional ethical committee (Pr. 2138/IEC/2021). The study included subjects with Age between 25 and 60 years with a minimum of 15 teeth present, Non-smokers, Systemically healthy individuals, No history of periodontal therapy within the last six months. Individuals who were smokers, immunocompromised patients, pregnant or lactating women, on long-term medication, who had received radiotherapy within the past 6 months were excluded from the study.

The procedure was explained to the patients, and a written and informed consent was obtained from patients who were willing to participate in the study.

### Method of Data Collection

Periodontal parameters such as Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD), and Clinical Attachment Level (CAL), modified Sulcular Bleeding Index (mSBI) were recorded. PD and CAL were measured at six sites around the tooth by using a periodontal probe. Under aseptic conditions about 5ml of venous blood was drawn from ante-cubital fossa using a syringe. Blood drawn was transferred into glass tubes coated with heparin. The smears were prepared with blood and allowed to dry. Blood smear will be stained with leishman stain and put one drop of cedarwood oil, observed under 100x magnification. The number of neutrophils in each particular field were counted. 95% Confidence Interval has been computed to find the significant features. All statistical parameters performed using a statistical software package (SPSS Version 26. NY). Statistical significance was accepted at  $p \leq 0.05$ .

### Results:

A total 80 patients were included in this study and were divided into four groups containing 20 each. The mean age of patients varied across the four stages of periodontitis as follows in Patients with Stage 1 periodontitis had a mean age of  $37.75 \pm 8.84$  years, Stage 2 had a mean of  $42.70 \pm 8.94$  years, Stage 3 patients had a mean age of  $41.35 \pm 11.00$  years and Stage 4 periodontitis had a mean age of  $47.08 \pm 7.77$  years (Table 1).

**Table 1: Mean values of age in different stages of periodontitis**

STAGES OF PERIODONTITIS	AGE
STAGE 1	$37.75 \pm 8.84$
STAGE 2	$42.70 \pm 8.94$
STAGE 3	$41.35 \pm 11.0$
STAGE 4	$47.08 \pm 7.77$

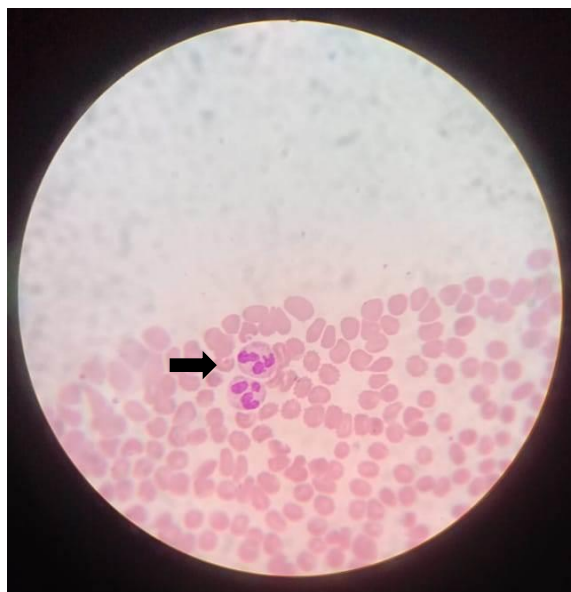
The mean neutrophil counts also demonstrated a progressive increase with the severity of the disease. In Stage 1, the mean neutrophil count was  $34.75 \pm 4.73$ , in Stage 2 it was  $37.05 \pm 6.23$ , in Stage 3 it rose to  $51.60 \pm 6.00$ , and in Stage 4, the highest count was observed at  $71.35 \pm 6.33$  (Table 2, Figures 1-4).

**Table 2: Mean values of neutrophils in different stages of periodontitis**

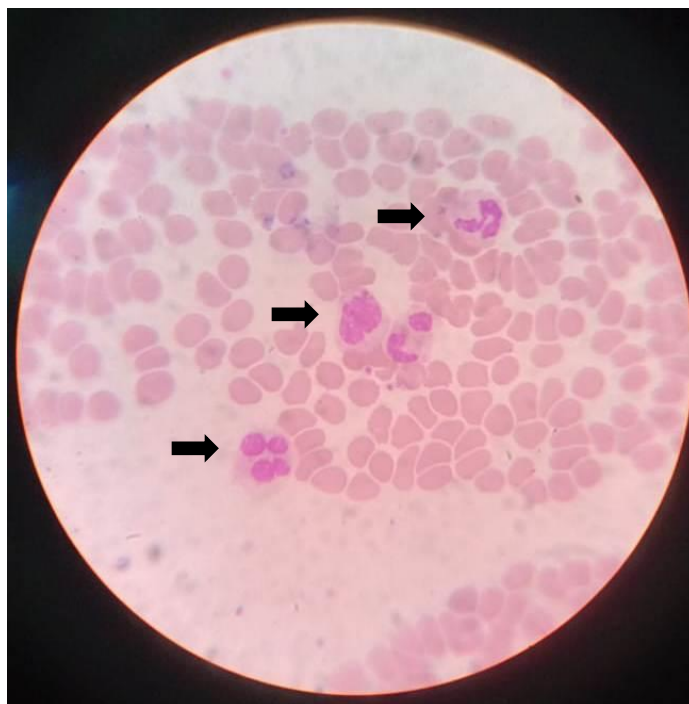
STAGES OF PERIODONTITIS	MEAN NUMBER OF NEUTROPHILS
STAGE 1	$34.75 \pm 4.73$
STAGE 2	$37.05 \pm 6.23$
STAGE 3	$51.60 \pm 6.0$
STAGE 4	$71.35 \pm 6.33$

**Figures:**

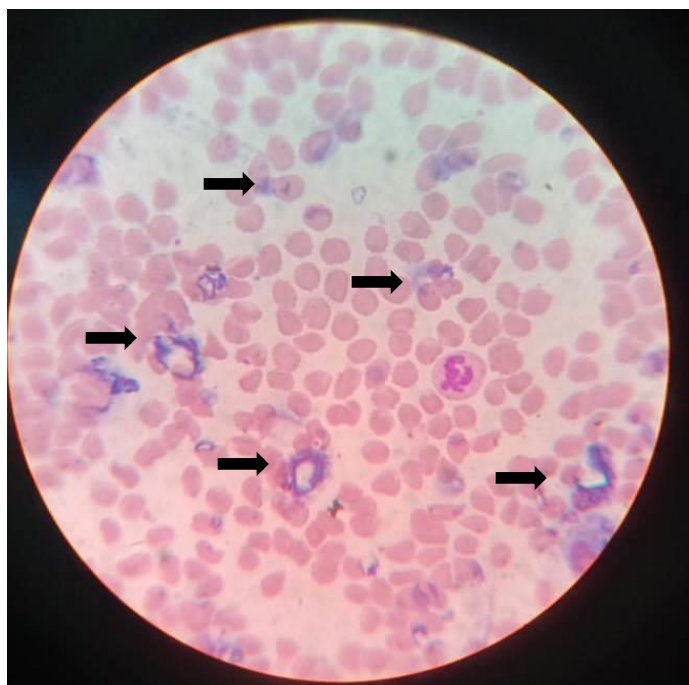
**FIGURE 1: Peripheral Smear Showing Neutrophils in Stage I Periodontitis**

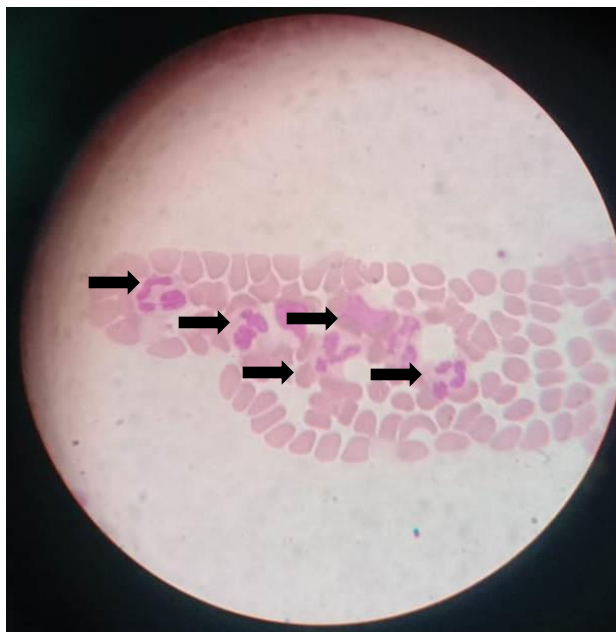


**FIGURE 2: Peripheral Smear Showing Neutrophils in Stage II Periodontitis**



**FIGURE 3: Peripheral Smear Showing Neutrophils in Stage III Periodontitis**



**FIGURE 4: Peripheral Smear Showing Neutrophils in Stage IV Periodontitis**

The mean PD increased progressively with the severity of periodontitis. Stage 1 patients exhibited a mean PD of  $2.38 \pm 0.46$  mm, while Stage 2 showed  $3.92 \pm 0.56$  mm. In Stage 3, the mean PD rose to  $5.72 \pm 0.53$  mm, and the highest value was observed in Stage 4, reaching  $6.82 \pm 0.81$  mm. A similar trend was noted for CAL, with Stage 1 presenting  $2.74 \pm 0.56$  mm and Stage 2 increasing to  $3.98 \pm 0.54$  mm. Stage 3 patients had a mean CAL of  $5.88 \pm 0.52$  mm, and Stage 4 patients exhibited the most severe loss at  $6.75 \pm 0.83$  mm. The PI values also increased across the stages. Stage 1 had a mean of  $0.61 \pm 0.35$ , which rose to  $1.05 \pm 0.43$  in Stage 2,  $1.62 \pm 0.39$  in Stage 3, and peaked at  $2.35 \pm 0.36$  in Stage 4. The GI followed a comparable pattern. Stage 1 presented with  $0.61 \pm 0.34$ , followed by  $1.07 \pm 0.44$  in Stage 2,  $1.74 \pm 0.38$  in Stage 3, and  $2.39 \pm 0.29$  in Stage 4. The bleeding index also reflected the progression of the disease. Stage 1 showed  $0.77 \pm 0.41$ , increasing to  $0.90 \pm 0.45$  in Stage 2,  $1.80 \pm 0.45$  in Stage 3, and reaching  $2.35 \pm 0.38$  in Stage 4 (Table 3).

**Table 3: Mean values of Clinical Parameters and neutrophil count in different stages of periodontitis**

	Stage 1 Periodontitis	Stage 2 Periodontitis	Stage 3 Periodontitis	Stage4 Periodontitis
Neutrophil count	34.75±4.73	37.05±6.23	51.60±6.0	71.35±6.33
PPD	2.38±0.46	3.92±0.56	5.72±0.53	6.82±0.81
Neutrophil count	34.75±4.73	37.05±6.23	51.60±6.0	71.35±6.33
CAL	2.74±0.56	3.98±0.54	5.88±0.52	6.75±0.83
Neutrophil count	34.75±4.73	37.05±6.23	51.60±6.0	71.35±6.33
PI	0.61±0.35	1.05±0.43	1.62±0.39	2.35±0.36
Neutrophil count	34.75±4.73	37.05±6.23	51.60±6.0	71.35±6.33
GI	0.61±0.34	1.07±0.44	1.74±0.38	2.39±0.29
Neutrophil count	34.75±4.73	37.05±6.23	51.60±6.0	71.35±6.33
mSBI	0.77±0.41	0.90±0.45	1.80±0.45	2.35±0.38

**Discussion:**

Chronic periodontitis is a common inflammatory disease characterized by progressive destruction of the supporting structures of the teeth. The clinical features include gingival inflammation, pocket formation, clinical attachment loss, and alveolar bone resorption. It is generally associated with dental plaque and calculus, with disease progression being episodic and potentially rapid in certain individuals [8].

Recent evidence has highlighted the impact of periodontitis on systemic inflammatory markers, including alterations in circulating leukocyte subpopulations such as neutrophils. Neutrophils, the most abundant polymorphonuclear leukocytes, play a pivotal role in innate immunity and have long been recognized as biomarkers of acute and chronic inflammation [9].

In the present study, peripheral blood neutrophil counts were evaluated across four stages of

periodontitis based on the 2017 classification. A progressive increase in mean neutrophil count was observed from Stage 1 ( $34.75 \pm 4.73$ ) to Stage 4 ( $71.35 \pm 6.33$ ), suggesting a correlation between disease severity and systemic inflammatory burden. This trend aligns with previous findings who reported elevated neutrophil activity and increased levels of reactive oxygen species in patients with periodontitis [10].

In contrary with a study which evaluated neutrophil levels in gingival crevicular fluid (GCF), the current study employed peripheral blood smears stained using Leishman-Giemsa combination to assess systemic changes in neutrophil counts. This method was chosen to avoid the limitations associated with GCF collection, including small sample volume and contamination risk [11].

Clinical parameters such as probing depth (PD), clinical attachment loss (CAL), plaque index (PI), gingival index (GI), and modified sulcular bleeding index (mSBI) were also found to increase progressively with disease severity. These clinical findings are consistent with the literature, supporting

the notion that worsening periodontal status is accompanied by increased inflammatory markers and neutrophil recruitment [12].

The positive correlation between neutrophil counts and clinical parameters, although statistically non-significant, supports the hypothesis that peripheral neutrophil levels may reflect the inflammatory status of periodontal disease. Similar observations were made by previous studies noted altered neutrophil counts and function in periodontitis patient [13,14]. Kweider et al also emphasized the systemic implications of periodontal inflammation, reporting elevated leukocyte counts in affected individuals [15].

The study findings also support the growing consensus that chronic periodontitis may contribute to systemic inflammatory load, with potential implications for cardiovascular health. Dorn et al. and other researchers have suggested that periodontal pathogens may translocate to distant sites, linking oral infections to systemic diseases such as atherosclerosis [16].

### **Limitations:**

The primary limitation of this study is its relatively small sample size which may limit the generalizability of findings. Future longitudinal studies with larger cohorts and pre- and post-treatment comparisons are warranted to better understand the diagnostic and prognostic value of peripheral neutrophil counts in periodontal disease.

**Conclusion:**

This study highlights a clear and progressive increase in neutrophil count corresponding with the severity of periodontitis, as assessed through histochemical analysis. The findings suggest that neutrophils play a significant role in the host immune response and inflammation associated with periodontal disease progression. Elevated neutrophil levels in advanced stages may reflect their involvement in both protective and destructive processes within periodontal tissues. These results underscore the importance of neutrophil activity as a potential biomarker for disease severity and progression. Further research is warranted to explore the functional characteristics of neutrophils in periodontitis and their potential as therapeutic targets in periodontal treatment strategies.

**Conflict of Interest:** NIL**Funding:**

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