Section A-Research paper
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Evaluation of Platelet to Lymphocyte and Neutrophil to Lymphocyte Ratio in Chronic Gingivitis and Chronic Periodontitis individuals before and after Phase I Therapy

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ABSTRACT

Introduction: For a long time, haematological tests have been thought of as potential diagnostic and prognostic indicators in the development of Chronic Periodontitis. The relatively new markers Neutrophil to Lymphocyte Ratio (NLR) and Platelet to Lymphocyte Ratio (PLR), which are obtained from peripheral blood counts, may be used in conjunction with an exaggerated understanding of these markers to serve as a potential marker in the understanding of the relationship between Chronic Gingivitis, Chronic Periodontitis and Systemic Inflammatory response. There hasn't been much research done on these indicators in relation to Gingivitis and Periodontitis. **Materials and Methods:** 75 Patients were enrolled and 90 samples were collected. Group I-Controls (25 Patients) who were assessed at baseline and Post-treatment after 4 weeks, Group II-Chronic Gingivitis (25 Patients) and Group III Chronic Periodontitis (25 Patients). GI, PI, PPD, CAL, Neutrophil Count, Lymphocyte Count and Platelet counts were obtained and assessed at baseline and Post-treatment after 4 weeks. The ratio of neutrophils to lymphocytes, or NLR, and the ratio of platelets to lymphocytes, or PLR, were calculated. **Results:** The results varied significantly between Group A and Group C at baseline and post-treatment , however the results were insignificant between Group A and Group B at baseline and post-treatment. The ROC cut off values for NLR and PLR was found to be 1.843 and 99.5 respectively which may be used as prognostic values for Chronic Periodontitis. **Conclusion:** In order to bridge the link between periodontal and systemic diseases, NLR and PLR may be used as possible biomarkers of the SIR to CP.

Keyword: Chronic Periodontitis, Neutrophil, Lymphocyte, Platelet, Systemic Inflammatory response, Inflammatory markers.

INTRODUCTION

Chronic periodontitis is described as an infectious condition that leads to inflammation and loss of connective tissue attachment and alveolar bone in the supporting tissues of the teeth. Recent studies suggest that periodontitis not only affects the local integrity of the teeth but also increases systemic inflammatory burden.[1,2] The role of inflammatory cells, specifically lymphocytes and neutrophils, in the innate inflammatory response and adaptive immunity is well known.[3,4] Platelets are also highlighted as important mediators that contribute to local inflammation.[5] During periodontal infections, there may be an abundance of leukocytes and platelets, which are expected to decrease following periodontal therapy. [6,7,8] Given the chronic nature of periodontal disease, the presence of microbial plaque, and the strong local and systemic response to microbial assault, it is hypothesized that this infection can impact overall health and the progression of systemic diseases.[9-13] Changes in peripheral blood parameters have been linked to a systemic inflammatory response in periodontal diseases. Some of these parameters include red blood cells, hemoglobin, mean corpuscular volume, platelets, and white blood cells.

The study suggests that the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), along with other mentioned parameters, could be potential markers to assess the inflammatory response in chronic periodontitis patients and PLR are reported markers of systemic inflammatory response and have

been studied in various systemic diseases.[2] They can be easily calculated from a complete blood count with differential. Elevated NLR may indicate increased pro-inflammatory cytokines, while PLR can be a valuable marker for the systemic inflammatory response.[1,2]

Overall, NLR and PLR are considered to reflect the inflammatory response and may serve as prognostic indicators or predictors of systemic disorders. These markers may be associated with increased levels of pro-inflammatory mediators, contributing to a heightened inflammatory status.[14-24]

MATERIALS AND METHODS

75 participants were recruited in the study after obtaining the Institutional Ethical Clearance (EC/NEW/INST/2019/329). Group I- Systemically and Periodontally healthy controls (25 Patients) whose NLR and PLR was assessed at baseline only, Group II-Chronic Gingivitis (25 Patients) and Group III Chronic Periodontitis (25 Patients) whose NLR and PLR was assessed at baseline and 4 weeks post treatment.

INCLUSION CRITERIA

Age between 25 to 60 years of age. CP defined as the presence of at least 20 natural teeth with generalized (i.e., >30% of the sites examined) probing pocket depths (PPD) of ≥4 mm and clinical attachment level (CAL) of ≥2 mm (stent as reference); positive for bleeding on probing, Score 2 of Gingival Index (Loe and Sillness,1963); radiographic evidence of bone loss.

EXCLUSION CRITERIA

Patients with systemic diseases; known allergies; tobacco users; pregnant, lactating women, women in menopause; patients with immunosuppressed conditions such as systemic lupus erythematosus and rheumatoid arthritis; periodontal therapy in the last 6 months; antibiotic and/or anti-inflammatory drug regimen before the study; and teeth with calculus or cervical caries or without a clinical tooth crown (CAL not measured).

A medical and dental history was recorded, and the participants were subjected to a periodontal examination. On the basis of their plaque index (PI),[34] gingival index (GI),[35] PPD, CAL, and radiographic (long cone, paralleling technique) evidence of bone loss, the participants were grouped as twenty five healthy individuals (H), twenty five Chronic Gingivitis (CG) patients and and twenty five Chronic Periodontitis (CP) patients. PPD and CAL assessments were conducted with a UNC-15 periodontal probe (Hu-Friedy® Manufacturing Inc., Chicago, IL, USA). Measurements were made at six different sites of each tooth present: mesiobuccal, midbuccal, distobuccal, midlingual, distolingual, and lingual. The CP patients were grouped as CP-BL (CP at baseline) and CP-PT (CP posttreatment). The mean value of the measurements was taken into consideration for each patient. Blood was drawn and collected from the antecubital fossa of the arm using a 21 gauge syringe by a hematology laboratory staff into a vacutainer incrementally in small volumes. The WBCs and PLT were estimated using pocH-100i automated hematology analyzer (Sysmex Corporation, Kobe, Japan), and the differential count was calculated.

Calculation of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio NLR was calculated as total neutrophil count/absolute lymphocyte count, and PLR was calculated as total PLT count/ absolute lymphocyte count, i.e., At baseline, oral hygiene instructions were given to each participant. The CG and CP patients received SRP with the use of an ultrasonic scaler (Electromedical Systems EMS, Nyon, Switzerland), manual instruments (Hu-Friedy® Manufacturing Inc., Chicago, IL, USA) under local anesthesia when required, over two appointments 1 week apart. During each visit, the patients received oral hygiene instructions. They were prescribed analgesics. Antibiotics or anti-inflammatory medications weren't given to any of the patients. All the parameters were recorded for groups after 1 month.

STATISTICAL ANALYSIS

Means and standard deviation were used to represent descriptive statistics. Comparison of the study variables between the three groups was done using One Way ANOVA test followed by post hoc Bonferroni test for pairwise comparison. Before and after treatment comparison of study variables was done using paired t test. In the above tests, p value less than or equal to 0.05 was considered to be statistically significant.

RESULTS

Table no.1: Age distribution of study participants

Age, in years	Group I	Group II	Group III
Mean	38.00	27.72	39.64
Standard deviation	4.73	3.84	5.92

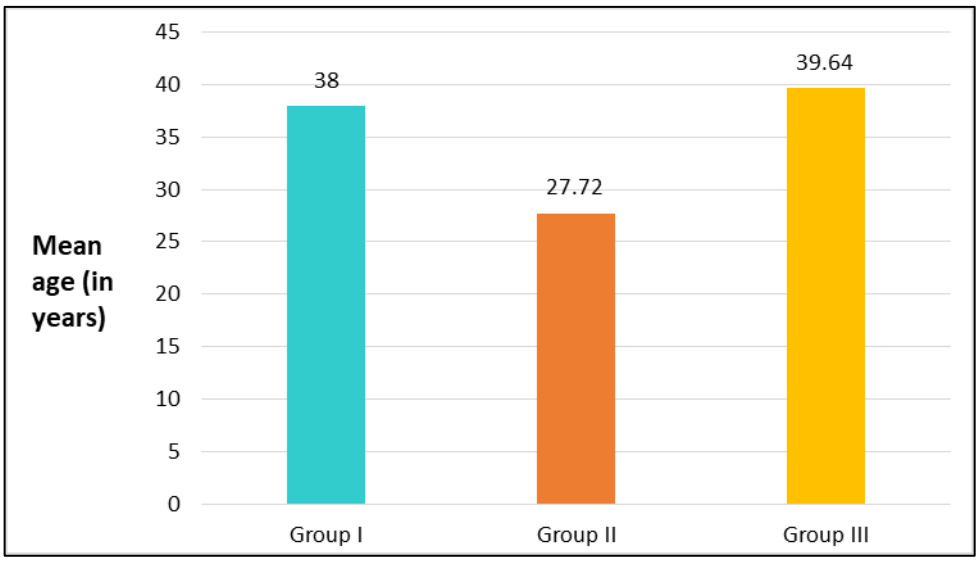


Figure no.1: Age distribution of study participants

Table no.2: Gender distribution of study participants

No. of participants	Group I	Group II	Group III
Males	12	13	12
Females	13	12	13

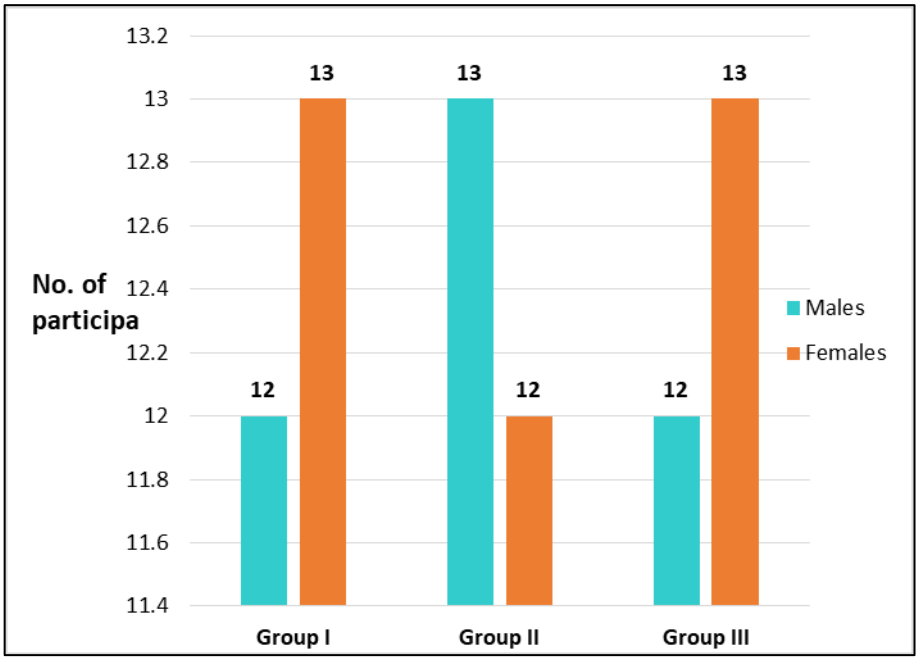


Figure no.2: Gender distribution of study participants

Table 3: Mean±standard deviation of the variables in the healthy group	
PlI	0.62 ± 0.30
GI	0.584±0.35
PPD	0
CAL	0
Neutrophils	3638.52±1282.236
Lymphocytes	2889.44±870.30
Platelets	237114±78891.91
NLR	1.29 ± 0.40
PLR	81.65 ± 7.21

PlI – Plaque Index; GI – Gingival Index; PPD – Probing pocket depths; CAL – Clinical attachment level; NLR – Neutrophil-to-lymphocyte ratio; PLR – Platelet-to-Lymphocyte Ratio; H – Health

Table 4: Comparison of baseline and posttreatment haematological and clinical parameters in Chronic Periodontitis group at baseline and posttreatment by dependent t- test			
Variables	Time points	Mean±SD	P value (Paired t test)
Platelet	BL	375744 ± 96734.54	0.0001
	PT	218365.09± 47221.92	
Lymphocyte	BL	3273.48 ± 957.14	0.0001
	PT	2447.68 ± 543.30	
Neutrophil	BL	7831.76 ± 1494.37	0.0001
	PT	3233.7 ± 3200.12	
NLR	BL	2.64 ± 1.27	0.0001
	PT	1.25 ± 0.34	
PLR	BL	117.68 ± 18.23	0.0001
	PT	81.64 ± 10.68	
PII	BL	1.90 ± 0.31	0.0001
	PT	0.86 ± 0.15	
GI	BL	2.17 ± 0.37	0.0001
	PT	1.15 ± 0.13	
PPD	BL	7.21 ± 0.38	0.0001
	PT	5.82 ± 0.34	
CAL	BL	8.05 ± 0.45	0.0001
	PT	6.28 ± 0.56	

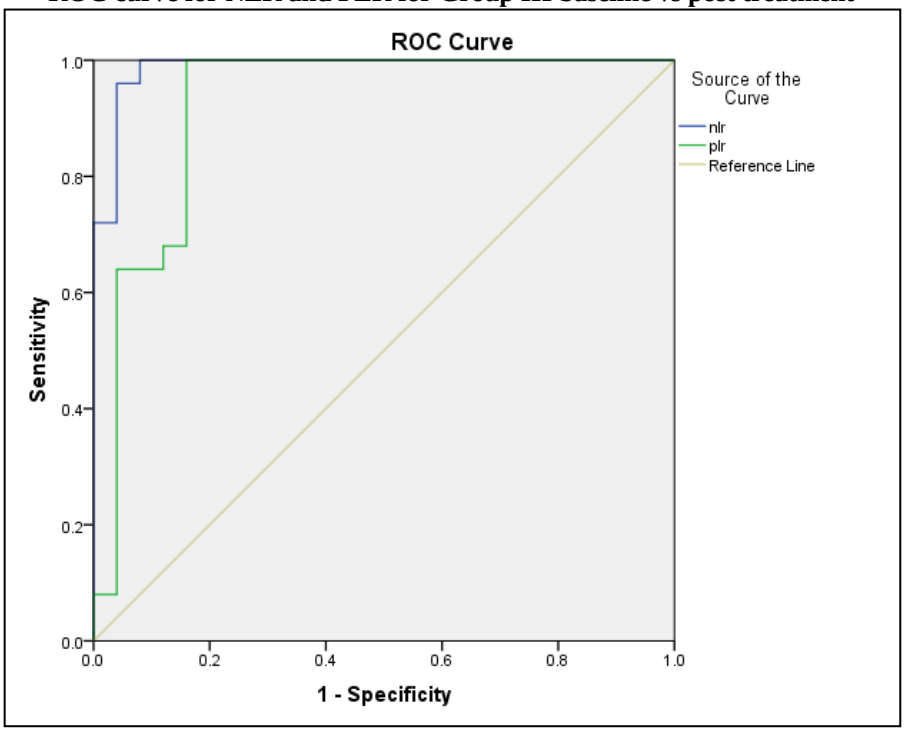
*P≤0.05. NLR – Neutrophil-to-lymphocyte ratio; PLR – Platelet-to-lymphocyte ratio; SD – Standard deviation; 5. PlI – Plaque Index; GI – Gingival Index; PPD – Probing pocket depth; CAL – Clinical attachment loss; BL – Baseline; PT – Posttreatment

Table 5: Comparison of baseline and posttreatment haematological and clinical parameters in Chronic Gingivitis group at baseline and posttreatment by dependent t- test			
Variables	Time points	Mean±SD	P value (Paired t test)
Platelet	BL	213378.9 ± 66621.22	1
	PT	213378.9 ± 66621.22	
Lymphocyte	BL	2671.84 ± 791.72	1
	PT	2597.88 ± 777.31	
Neutrophil	BL	3379.92 ± 1146.662	1
	PT	2910.2 ± 2886.36	
NLR	BL	1.27 ± 0.28	0.141
	PT	1.25 ± 0.29	
PLR	BL	83.08 ± 10.34	0.773
	PT	82.55 ± 11.01	
PII	BL	1.74 ± 0.32	<0.001*
	PT	0.86 ± 0.15	

GI	BL	1.80 ± 0.04	<0.001*
	PT	0.84 ± 0.31	

p> 0.001 (statistically insignificant) NLR – Neutrophil to lymphocyte ratio; PLR – Platelet to lymphocyte ratio; SD – Standard deviation; 5. PlI – Plaque Index; GI – Gingival Index;; BL – Baseline; PT – Posttreatment

ROC curve for NLR and PLR for Group III baseline vs post treatment



Area under the curve (AUC)

Test result variables	Area (%)
NLR	98.7
PLR	92.2

Table no6: Cut off score for NLR and PLR

Test variable	Cut off score	Sensitivity	Specificity
NLR	1.843	0.960	0.920
PLR	99.595	0.960	0.840

ROC cutoff value for NLR is 1.84 and that for PLR is 99.59.

The mean age of the participants in Group I was 38 years ± 4.73 years, in Group II it was 27.72 ± 3.84, and in Group III it was 39.64 ± 5.92 and each group comprised of 15 Males and 15 females (Table 1 & 2) (Figure 1 &2). The experimental and control groups considerably differed in terms of NLR and PLR, with the latter group having a lower NLR and PLR than the former. In the Experimental group III both NLR and PLR are significantly reduced after treatment when assessed after 4 weeks but there was no change in values in Group II (Table 4 & 5). After phase 1 therapy, 4 weeks may be a justifiable time frame to achieve reduction in gingival inflammation and, consequently, systemic inflammation reduction i.e reduction in TLC and platelet counts.[25] The ROC cut off value in the CP Group for NLR (Sensitivity-0.960 and Specificity-0.920) was 1.85 and that for PLR (Sensitivity-0.960 and Specificity-0.840) was 99.56. (Table 6) Hence these values can be used as prognostic values for Chronic Periodontitis.

DISCUSSION

It is well recognised that changes in peripheral blood parameters can help determine a disease's prognosis in many cases. The study highlights the importance of peripheral blood parameters in determining the prognosis of diseases, including periodontal conditions. NLR and PLR are considered reliable indicators

of the inflammatory response, reflecting innate immune mechanisms involved in periodontal destruction. Higher NLR and PLR values suggest a more severe inflammatory response.[26] Comparing the current study with existing research is challenging due to limited available literature on NLR and PLR as systemic indicators of chronic gingivitis (CG) and chronic periodontitis (CP) before and after scaling and root planing (SRP). One study evaluated these parameters in dogs with periodontal disease but did not find a correlation with periodontitis.[27] Another study examined NLR and PLR in CP and diabetes found that PLR decreased with increasing glycemic status and more severe periodontitis, while NLR increased with severe periodontitis.[28] However, both NLR and PLR have been studied as systemic inflammatory indicators in various disorders, including carcinomas and Mediterranean fever.[29] The average NLR in healthy Caucasians is reported to be higher compared to other races, indicating a potential racial predilection.[30] In the current study, PLR was lower in the healthy group compared to the periodontitis group, suggesting an increase in PLR during local inflammatory diseases. It is worth noting that NLR and PLR were statistically insignificant between all groups post treatment, indicating a significant decrease due to therapeutic intervention. The ROC cutoff values for NLR (1.84) and PLR (99.59) suggest that these measurements can be useful as prognostic markers for CP. Overall, while there is limited research specifically focusing on NLR and PLR in CG and CP before and after SRP, the findings of this study suggest the potential utility of these parameters in assessing the inflammatory response and predicting the prognosis of CP. Further research is needed to validate and expand upon these findings.

Limitations

1. The inadequate sample size can also prove to be insufficient for drawing conclusions.
2. Graduating the follow-up session could potentially influence the outcomes.

CONCLUSION

To better understand the potential effects of CP on systemic health, we propose that NLR (whose elevation could disturb the balance between pro and anti inflammatory mediators in disease) and PLR (a marker of systemic inflammation) be included as potential parameters in studies exploring the oral-systemic axis. In combination with other inflammatory mediators, NLR and PLR may be helpful in stratifying CP patients and will likely establish a grading or scoring system similar to the Glasgow prognostic score (used in carcinomas) to predict incidence and treatment results.

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