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ORIGINAL ARTICLE

Clinical and diagnostic significance of blood leukocyte ratios in young patients with stage III grade C periodontitis

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ABSTRACT

Objectives: Blood leukocyte ratios have been recently proposed as simple, rapid, cheap and easily accessible biomarkers of systemic inflammation. However, little is known about the relationship of neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and lymphocyte-monocyte ratio (LMR) with periodontitis, which might not only serve as the potential biomarkers of systemic inflammation but also aid in diagnosis/screening of severe periodontitis. Hence, the purpose of this study was to evaluate the differences in the serum levels of these leukocyte ratios in healthy subjects and patients with generalized stage III Grade C periodontitis, and their applicability in identifying patients with the risk of developing severe periodontitis.

Material and methods: The subjects were categorized into case and control group. Clinical parameters including Plaque index (PI), modified Gingival Index (mGI), Mean ratio of bleeding sites, Probing Pocket depth (PPD) and the clinical attachment loss (CAL) were assessed in both the groups. Venous blood samples were collected from subjects from both groups for the biochemical analysis and blood leukocyte ratios- NLR, PLR and LMR were calculated. The values were then subjected to statistical analysis.

Results: The results showed significantly higher NLR and lower LMR values in patients with generalized stage III grade C periodontitis. Both the blood leukocyte ratios were moderately associated with increasing clinical parameters of periodontal disease. However, the values of PLR, although found to be higher in the periodontitis group, did not make a significant difference when compared with periodontally healthy subjects. The cut-off value of >2.15 for NLR and <7.16 for LMR fairly predicted the risk of severe periodontitis in young adults.

Conclusions: NLR and LMR can provide a new insight into the relationship between periodontitis and systemic diseases and can be of potential diagnostic value in identifying patients with severe periodontitis of younger age group.

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Introduction

Periodontitis, characterized by progressive destruction of the tooth- supporting structures, is a chronic inflammatory disease of multifactorial aetiology [1]. While the initiation of periodontitis is dependent on dysbiotic plaque biofilms [2], disease progression with increased severity is primarily the result of host response to such microbial biofilm accumulation mediated mainly by the neutrophils, monocytes/macrophages, and T and B lymphocytes [3]. Dysregulation in the immune response in periodontitis is believed to play a pivotal role in the pathogenesis of the disease [4].

Neutrophils being the most abundant among leukocytes are classical cells of inflammatory pathways that link the innate and adaptive arms of immune system and have often being considered as a double-edged sword in immune response [5]. The role of T and B lymphocytes in the pathogenesis of periodontitis has been well researched. Activation of various T lymphocytes, monocytes and macrophages leads to the production of a wide variety of pro-inflammatory cytokines that in a series of events result in progressive destruction of tooth supporting alveolar bone [3]. During the disease, peripheral blood monocytes have also found to reduce Th2- promoting cytokine IL-12 [6] Moreover, the role of platelets in inflammation and immune response has been consistently reported in several studies [7], and recently few studies have also identified that a possible relationship might exists between periodontitis and increased platelet count, partially mediated by leukocytes [7–9].

The diagnosis of periodontitis during its active stage and identifying patients at risk of developing severe form of periodontal disease constitute a predicament for clinicians and investigators [10]. Severe periodontal tissue destruction in young adults can lead to tooth loss at an early age that can affect quality of life. Hence, such individuals susceptible to develop severe periodontitis require prompt diagnosis thereby facilitating early intervention and monitoring [11]. Research in advanced diagnostic techniques for periodontal diseases has moved towards methods whereby identification and quantification of patients at periodontal risk, can be done by various diagnostic biomarkers found in serum, gingival crevicular fluid, or saliva samples [10]. Literature

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available in the context of periodontitis and inflammation has also indicated that patients with periodontitis exhibit elevated serum levels of various inflammatory markers indicating increased low-grade systemic inflammation, when compared to periodontally healthy population [10]. Although a number of novel disease specific biomarkers have been identified, most of them are expensive, require expertise and are time consuming [10].

Many contemporary studies have thoroughly investigated the role of serum total and differential leukocyte count in different chronic conditions including periodontitis [12–14]. Blood leukocyte ratios viz Neutrophil- lymphocyte ratio (NLR) and platelet- lymphocyte ratio (PLR) have already proved useful in diagnosis and prognosis of several chronic inflammatory diseases like diabetes mellitus, cardiovascular diseases, chronic lung disease and several types of cancer [15]. Similarly, utilization of the leukocyte-monocyte ratio (LMR) is being studied as a diagnostic factor. Its importance in determining prognosis in cardiovascular disorders and solid tumours is well established. [15]. These markers have been recently proposed as simple, rapid, cheap and easily accessible biomarkers of systemic inflammation and have been particularly helpful in low- and middle-income countries [15]. However, little is known and it is still unclear about the relationship of NLR, PLR and LMR with periodontitis, which might not only serve as the potential biomarkers of systemic inflammation but also aid in diagnosis/screening of severe periodontitis and predicting its prognosis.

Thus, considering the need to further investigate the role of NLR, PLR and LMR in young adults with periodontitis, the purpose of this study was to evaluate the differences in the serum levels of these leukocyte ratios in healthy subjects and patients with generalized stage III Grade C periodontitis, and their applicability in identifying patients with the risk of developing severe periodontitis.

Material and methods

Study design

The current study was a case-control, double-blind, single centred clinical study. Study sample, within the age group of 20-40 years, was recruited from the Outpatient Department of Periodontology, Government dental college and hospital, Raipur, Chhattisgarh, India. Data collection was done for a period of fifteen months starting from November 2019 till February 2021. The research protocol was approved by the Institutional Ethical Review Board of Government dental college and hospital, Raipur, India (ECR/6489/GDC/CG/2019) and written informed consent was obtained from all the participants.

The study participants were divided into two groups – the case group and the control group. Sample size required for the present study was done using MedCalc sample size calculator version 19.7.1. It was based on the previous study done by Lu *et al.* [16] by comparing the difference of means of the two groups, calculated at 80% power and alpha error of 0.05. The minimum sample size required for the case group was 105 and for control group came out to be 38. Thus, 115 participants in the case group and 50 in the control group were enrolled for the current study.

Categorization of subjects into case and control group was based on the classification criteria proposed by 2017 World Workshop Classification system for Periodontal and Peri-implant diseases and conditions [17]. The case group comprised systemically healthy subjects with generalized stage III grade C Periodontitis while the control group consisted of participants who were systemically healthy and also exhibited periodontal health.

Patients with history of use of tobacco in any form, diabetes mellitus, cardiovascular diseases, renal, haematological, inflammatory or any other known systemic disorders or those under any medication including anti- inflammatory drugs and nutritional supplements, pregnant and lactating women were excluded from the present study. Subjects who had undergone any periodontal therapy within the last six months were also excluded from the study.

Clinical examination

Complete medical and dental histories were taken from each participant prior to the periodontal examination. Using a mercury sphygmomanometer and following defined BP measurement techniques, trained and calibrated person measured arterial blood pressure (BP). There was only one measurement of resting blood pressure. If the blood pressure was more than 140/90 mm Hg, a second and third measurement was obtained. Chest X- rays were performed to exclude any lung disorders. The height of the participants was measured in metres, and weight in kilograms using a mechanical scale with precision. The Body Mass Index (BMI) was computed by dividing the subject's body weight (in kg) by their height squared (in metres).

A single, trained examiner and blinded about the study design (GMP) assessed both the groups for periodontal status. Clinical parameters recorded for both the groups were – plaque index (PI), Bleeding on Probing (BOP), modified gingival index (mGI), probing pocket depth (PPD) and clinical attachment level (CAL). The intraoral examination was done using a mouth mirror and a graduated periodontal probe[1] and the measurements were rounded off to the nearest millimetre. PI and mGI were recorded in all the participants for the entire dentition, excluding the third molars at four points namely – mesiobuccal papilla, buccal margin, distolingual papilla and the entire lingual margin as per the criteria given by Loe and Sillness [18] and Lobene et al. [19] respectively. BOP was recorded as presence or absence of gingival bleeding within 30 s of probing through the gingival sulcus of buccal and lingual surfaces of all teeth except third molars. It was computed as the ratio of number of sites with gingival bleeding to the number of sites examined. PPD and CAL were recorded at six points around each tooth except third molars. Full mouth radiographs were taken in all the patients using long cone technique.

Calibration exercise prior to the commencement of baseline examinations was performed for variables PD and CAL on randomly selected quadrant in ten patients with severe

periodontitis and who were not part of the study. The re-examination was done twice at the same visit by the examiner. Intra-class correlation for PD was 0.92 and 0.90 for CAL yielded adequate intra-examiner reliability.

Biochemical analysis

Fasting venous blood sample of 10 ml was collected between 8:30 and 9:30 am from each patient under complete aseptic precautions by venipuncture from the antecubital fossa and transferred into sterile EDTA tubes by experienced, qualified personnel. The test tubes were placed in an ice carrier box and transported to the laboratory for the assay. Complete blood cell analyses of blood samples were assessed by fully automated bidirectional analyser (6 Part Differential SYSMEX XN − 1000, Sysmex Corporation, Kobe, Japan). Both NLR and PLR were calculated as ratios of the absolute values of neutrophils and total platelet count to absolute values of lymphocytes, respectively while LMR was calculated as ratio of absolute lymphocyte count to absolute monocyte count. Routine blood investigations apart from complete blood cell picture– Hba1c, lipid profile, Liver function tests (LFT), renal function tests (RFT) and thyroid function tests (TFT) were also performed to rule out any systemic abnormality.

After the collection of blood samples for biochemical analysis, non-surgical periodontal therapy like scaling and root planing was done for periodontitis patients. Patients were advised to follow oral hygiene instructions and were recalled at regular intervals for review and surgical periodontal therapy was performed in necessary cases.

Statistical analysis

Statistical analysis of the collected data was done using statistical package for social sciences (SPSS) version 22.0 (IBM Corporation, New York, NY). NLR, PLR and LMR were considered as primary outcome variables. Periodontal parameters were considered as primary explanatory variables. Descriptive statistics – mean and standard deviation for continuous variables and counts (n) and percentages (%) for categorical variables were calculated. All quantitative variables were checked for normal distribution within each category of explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro- wilk test was also conducted to assess normal distribution. Data that did not follow normal distribution were calculated and compared between the two groups using Mann-Whitney U-test while data that exhibited normalcy were compared using unpaired t-test. Comparison of categorical variables between the two groups was performed using Fisher's exact test. Association between quantitative explanatory and outcome variables was assessed by calculating Spearman correlation coefficient. Receiver Operative Curve (ROC) analysis was used to evaluate the NLR, PLR, and LMR in predicting periodontal status. The area under the ROC curve, as well as its 95 percent confidence interval (CI) and p value, were calculated. The screening test's sensitivity, specificity, predictive values, and diagnostic accuracy were provided, together with the cut-off values and associated 95 percent CI. Logistic regression analysis was performed to assess the role of various risk predictors. Odds ratio at 95% CI was calculated. A value of $p < .05$ was considered to be significant.

Results

Of 115 participants with generalized stage III grade C periodontitis, 3 participants had abnormally high Hba1c levels, 1 had persistent high systolic and diastolic BP measurements, 2 had abnormal chest x-ray suggesting of SARS-CoV-2 infection which they later confirmed to have recently recovered from the infection and 2 were found to have hypercholesterolaemia. Hence, these participants were excluded from the study. Similarly, of 50 healthy controls, 10 patients were excluded from the current study (4 had high abnormal Hba1c levels, 3 had hypercholesterolaemia, 1 recently recovered from SARS-CoV-2 infection and 2 had high TSH levels). Hence, a total of 148 patients, 108 subjects in the periodontitis case group and 40 in the healthy control group were included for further analyses.

The participant characteristics such as age, gender and BMI of the study population indicate no significant difference between the periodontitis cases and healthy controls. All periodontal parameters viz., PI (2.21 ± 0.38 vs 0.07 ± 0.05), mGI (2.01 ± 0.35 vs 0.03 ± 0.03), BOP (0.93 ± 0.08 vs 0.07 ± 0.06), PPD (5.11 ± 0.61 vs 1.81 ± 0.47) and CAL (5.22 ± 0.77 vs 0.00 ± 0.00) were significantly higher in periodontitis group compared to healthy controls ($p < .0001$). The neutrophil count (5.84 ± 1.91 vs 4.98 ± 1.67, $p = .01$) and NLR (2.84 ± 1.18 vs 2.10 ± 1.08, $p = .0001$) were significantly higher in periodontitis patients than in periodontally healthy patients. However, periodontitis patients reported lower lymphocyte count (2.12 ± 0.85 vs 2.43 ± 0.64, $p = .03$) and LMR values that reached statistical significance, when compared with periodontally healthy subjects (7.26 ± 4.94 vs 9.31 ± 4.88, $p = .004$). Values of platelet count, monocyte count and PLR although higher in periodontitis group, did not show any significant difference compared to healthy group (Table 1).

The data pertaining to correlation of blood cell ratios – NLR, PLR and LMR with periodontal parameters is presented in Table 2. There was a moderate positive correlation between NLR and PPD ($r = 0.62$), CAL (0.57), BOP (0.49), mGI (0.45) and PI (0.48) which was statistically significant ($p < .001$). Conversely, LMR exhibited a significant negative correlation with PPD (−0.36) and CAL (−0.31) ($p < .001$) while no significant correlation was reported between PLR and any of the periodontal parameters.

ROC analyses yielded cut off values of > 2.15 for NLR and ≤ 7.16 for LMR in predicting the risk of stage III-IV grade C periodontitis (Figures 1 and 2). Based on these cut off values, the odds of having stage III Grade C periodontitis was 11.4356 (95% CI, 4.8080-27.1986, $p < .0001$) with every 0.1 increase in NLR and 4.9327 (95% CI, 2.2612–10.7604, $p = .0001$) with each 0.1 decrease in LMR. Logistic regression analysis with age, male gender and BMI as explanatory variables and NLR and LMR values as dependent variables,

demonstrated only age as the significant predictor of differences in NLR AND LMR values in periodontitis group and healthy group (Table 3).

Both NLR and LMR had a fair predictive validity as indicated by the Area under the curve (AUC), which was 0.743 (95% CI 0.627 to 0.779, $p < .0001$) and 0.654 (95% CI 0.529 to 0.691, $p = .003$) respectively. The NLR had sensitivity of 76.85% (95% CI 67.75% to 84.42%) and specificity was 77.5% (95% CI 61.54% to 89.16%) in predicting stage III-IV Grade C periodontitis and the total diagnostic accuracy was 77.02% (95% CI 69.40% to 83.53%). The diagnostic accuracy of LMR was 69.59% (95% CI 61.50% – 76.88%) with a sensitivity of 70.37% and specificity of 67.5% (Table 4).

Discussion

Critical loss of periodontal tissues isn't always a natural consequence of ageing. Young adults also develop severe periodontal destruction due to genetically predisposed altered host response [20]. Due to the shorter time frame within which the destruction takes place in young individuals, the prognosis gets worse [21]. The quality of life in such individuals is affected drastically due to premature loss of tooth requiring the need for early diagnosis and intervention [11]. The present study was thus conducted in young adults with stage III grade C periodontitis in order to estimate the levels of blood leukocyte ratios – NLR, PLR and LMR values and to assess their usefulness in predicting the risk of developing severe periodontitis.

Age, gender and BMI are some of the variables that can act as potential confounders and affect the data interpretation. In the present study, age, gender and BMI values were comparable between both the groups and did not attain significance. Evidence has shown that smoking status, alcoholism, presence of systemic diseases and use of various medications may affect the blood cell ratios [22], hence such patients were excluded from the current study. The length of

Table 1. Comparison of general status, periodontal parameters and blood cell ratios between periodontitis case group and healthy control group.

Variable	Periodontitis group ($n = 108$) Mean ± SD/*n* (%)	Healthy group ($n = 40$) Mean ± SD/*n*(%)	*P*-value
Age in years	30.67 ± 4.89	31.50 ± 5.31	0.3718
Gender **			
M	59 (54.63%)	21 (52.5%)	0.8541
F	49 (45.37%)	19 (47.5%)	
BMI (kg/m^2)	22.25 ± 1.55	21.81 ± 1.30	0.8694
PI	2.21 ± 0.38	0.07 ± 0.05	<0.0001*
mGI	2.01 ± 0.35	0.03 ± 0.03	<0.0001*
BOP	0.93 ± 0.08	0.07 ± 0.06	<0.0001*
PPD	5.11 ± 0.61	1.81 ± 0.47	<0.0001*
CAL	5.22 ± 0.77	0.00 ± 0.00	<0.0001*
NEUT (x 10^9/l)	5.84 ± 1.91	4.98 ± 1.67	0.01*
LYM (x 10^9/l)	2.12 ± 0.85	2.43 ± 0.64	0.0312*
MON (x 10^9/l)	0.32 ± 0.18	0.30 ± 0.24	0.5857
PLT (x 10^9/l)	298.69 ± 84.17	286.28 ± 82.69	0.4248
NLR	2.84 ± 1.18	2.10 ± 1.08	<0.0001*
PLR	143.98 ± 59.00	134.16 ± 38.53	0.5740
LMR	7.26 ± 4.94	9.31 ± 4.88	0.0040*

SD: standard deviation; n: frequency; M: Male; F: Female; BMI: body mass index; PI: Plaque Index; mGI: modified gingival index; BOP: bleeding on probing; PPD: probing pocket depth; CAL: clinical attachment level; NEUT: absolute neutrophil count; LYM: absolute lymphocyte count; MON: absolute monocyte count; PLT: platelet count; NLR: neutrophil lymphocyte ratio; PLR: Platelet lymphocyte ratio; LMR: lymphocyte ratio.
*$p < .05$ is significant.
**Fisher exact test.

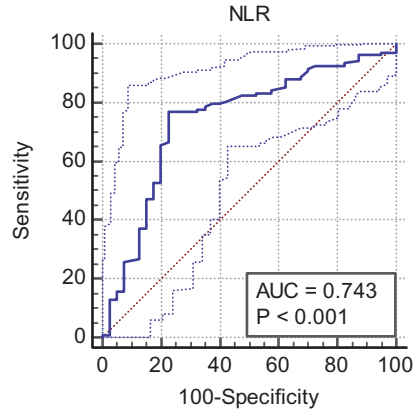

Figure 1. Receiver operating curve (ROC) analysis of NLR for stage III grade C periodontitis. Blue line is the ROC of NLR; segmented lines are 95% confidence intervals. Area under the curve (AUC) = 0.743.

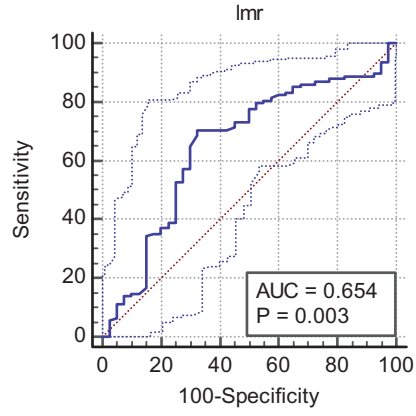

Figure 2. Receiver operating curve (ROC) analysis of LMR for stage III grade C periodontitis. Blue line is the ROC of LMR; segmented lines are 95% confidence intervals. Area under the curve (AUC) = 0.654.

Table 2. Correlation between different blood cell indices with periodontal parameters in patients with stage III grade C Periodontitis.

Variables	Mean PPD r (*p* Value*)	Mean CAL r (*p* Value*)	Mean BOP r (*p* Value*)	Mean mGI r (*p* Value*)	Mean PI r (*p* Value*)
NLR	0.62 (<.001)	0.57 (<.001)	0.49 (<.001)	0.45 (<.001)	0.48 (<.001)
PLR	0.18 (.033)	0.14 (.093)	0.16 (.058)	0.16 (.056)	0.16 (.048)
LMR	−0.33 (<.001)	−0.31 (<.001)	−0.12 (.160)	−0.16 (.059)	−0.15 (.065)

PI: Plaque Index; mGI: modified gingival index; BOP: bleeding on probing; PPD: probing pocket depth; CAL: clinical attachment level; NLR: neutrophil lymphocyte ratio; PLR: Platelet lymphocyte ratio; LMR: lymphocyte ratio.
*: $p < .05$ is significant.

Table 3. Logistic regression analyses of NLR and LMR for assessment of predictors.

	BLOOD CELL RATIOS			
	NLR		LMR	
PARAMETERS	Odds Ratio (95% CI)	*p* Value	Odds Ratio (95% CI)	*p* Value
AGE	1.0830 (1.0090 to 1.1624)	.02*	1.1116 (1.0341 to 1.1949)	.004*
MALE GENDER	1.2715 (0.6356 to 2.5436)	.49	1.3827 (0.6885 to 2.7766)	.36
BMI	1.0422 (0.8283 to 1.3113)	.72	0.9372 (0.7446 to 1.1797)	.58

BMI: Body Mass Index; NLR: neutrophil lymphocyte ratio; LMR: lymphocyte monocyte ratio. Age; male gender and BMI are explanatory variables and NLR and LMR are dependent variables.
*$p < .05$ is significant. Only age came out as the significant predictor.

Table 4. Predictive validity of NLR and LMR for stage III grade C periodontitis.

PARAMETER Value (95% CI)	BLOOD CELL RATIOS NLR	 LMR
Sensitivity	76.85% (67.75%–84.42%)	70.37% (60.81%–78.77%)
Specificity	77.50% (61.54%–89.16%)	67.50% (50.87%–81.42%)
Positive Likelihood Ratio	2.27 (1.43–3.61)	2.16 (1.36–3.44)
Negative Likelihood Ratio	0.38 (0.26–0.56)	0.43 (0.30–0.63)
Disease prevalence	72.97% (65.06%–79.93%)	72.97% (65.06%–79.93%)
Positive Predictive Value	86.02% (79.52%–90.69%)	85.39% (78.62%–90.28%)
Negative Predictive Value	49.09% (39.63%–58.61%)	45.76% (37.01%–54.77%)
Accuracy	77.02% (69.40%–83.53%)	69.59% (61.50%–76.88%)

NLR: neutrophil lymphocyte ratio; LMR: lymphocyte monocyte ratio; CI: Confidence interval.

time between blood collection and analysis is considered crucial as the composition of blood cells could be altered or destroyed depending on the time elapsed between the two processes [23]. To minimize such inaccuracy, the blood cell analyses were immediately performed after the sample collection in the present study.

The major findings of the current study revealed significantly higher NLR and lower LMR values in patients with generalized stage III grade C periodontitis. Both the blood leukocyte ratios were moderately associated with increasing clinical parameters of periodontal disease, hence, can be considered as potential biomarkers of periodontal inflammation as well as severity of the disease. Also, the risk of developing severe periodontitis increased with the elevated NLR values and decreased LMR values. However, the values of PLR, although found to be higher in the periodontitis group, did not make a significant difference when compared with periodontally healthy subjects.

The results of the present study concerning elevated NLR and insignificant difference in PLR values in patients with and without periodontitis were consistent with the previous studies done by Lu et al. [16] and Acharya et al. [24]. As opposed to this, Torrungruang et al. [25] in their study reported a significant decrease in PLR values in severe periodontitis patients. This could be due to the differences in the study design as well as differences in the absolute blood cell count in both the studies, when each PLR component was analysed individually. The individual NLR and PLR values in periodontitis patients were higher in the present study compared to studies done on Chinese generalized aggressive periodontitis patients [16] and chronic periodontitis patients of Asian Indian race [24] suggesting a possible racial as well as regional variation within the same race. To the best of our knowledge there is hardly any available literature addressing LMR values in periodontitis patients making it difficult to draw any comparisons with the present study.

There are no widely accepted cut-off values for blood leukocyte ratios – NLR, PLR and LMR and studies have reported that the average values differ in different racial backgrounds. While in Korean healthy adults of age group 20-40 years the mean NLR ranged between 1.74 and 1.77 [22], the average NLR in healthy Caucasians was found to be 2.15 [26]. In non-Hispanics of African lineage NLR value is 1.76 [26] and in Chinese young individuals belonging to Hans' race it is averaged at 1.84 [16]. In the present study the mean value of NLR in systemically and periodontally healthy young adults were 2.10, higher than the previously mentioned studies but similar to the studies done on healthy Indian population [24,27]. The reported PLR value in the present study was in line with the studies consummated on Korean [23], Chinese [16] and African population [28] but surprisingly was higher when compared to other Indian studies [24,27]. The possible reason could be either due to regional variation or the younger age group included in the current study. Very few studies addressing the reference values of LMR have been done and have demonstrated the cut off values lower than that reported in the present study. Reference values ranging from 2-3 for preoperative LMR were considered a 'high risk' group with various solid tumours and lymphomas [23,29,30]. Normal or reference values from a healthy Asian Indian population are yet to be documented. The cut-off value of > 2.15 for NLR and < 7.16 for LMR fairly predicted the risk of severe periodontitis in young adults in the current study. These cut – off values were different from the study accomplished on Chinese young adults and the predictive validity of the leukocyte ratios was also found to be better in the present study [16]. The difference in cut- off

points suggests that further studies should be undertaken in other racial groups too.

Studies done on South Korean population [23] and in Chinese Han population [31] have exhibited positive correlation between NLR and age, gender and BMI in systemically healthy adults. In an Indian study [27], no differences were noted in blood leukocyte ratios with respect to age. While gender and BMI did not emerge as risk predictors in the current study, the study did showed a significant relationship between increasing age and NLR and LMR values, indicating a possible age-dependent effect. Hence, the possible influence of age should be considered in reflecting the blood cell ratios as predictors of development of severe form of periodontitis.

Ample evidence from the published literature has indicated that elevated NLR might predict systemic inflammation and hence, might prove to be a new potential link between periodontitis and various systemic diseases. For instance, a recent systematic review on NLR and cardiovascular disease risk indicated cut –off values of 1.80-2.60 for coronary artery disease (CAD), 2.19-5.70 for acute coronary syndrome, and 3.0-3.17 for cerebrovascular stroke [32]. Increased NLR has also been indicative of poor glycemic control and risk of type 2 diabetes mellitus with a cut-off point ranging from 2.44- 4.34 in different studies [33–35]. Moreover, NLR has also been a valuable prognostic indicator in various types of cancer [36], obstructive sleep apnoea [37], worsening renal function [38] and lung disorders [39]. In the present study, the NLR value was >2 in about two-thirds of periodontitis patients. Hence, it can be inferred from the findings that the elevated NLR, if persistent in the long run, in untreated periodontitis patients might be a plausible gateway to the development of various systemic diseases, in future.

LMR functions as an inflammatory complex in the same way that NLR does [40]. It is a unique systemic inflammatory marker that combines two separate measures of inflammation. It has recently been discovered to be a possible prognostic factor in a variety of cancers [41–43]. Also, few recent reports have demonstrated decreased LMR in patients with rheumatoid arthritis [44, 45]. Additionally, a study attempting to find out the association between LMR and severity of CAD revealed that the risk of developing severe atherosclerosis is capped at LMR value of <5.06 [40]. Periodontitis being a prototype of low grade systemic inflammation has also been associated with a number of systemic diseases including CAD [46], rheumatoid arthritis [47] and certain tumours [48] through various biomarkers. Hence, decreased LMR in periodontitis patients, as in the present study, might emerge as another prospective biomarker in connecting the link between periodontitis and systemic diseases. However, these findings also imply probable influence of systemic diseases on NLR and LMR values which should be kept in mind in predicting periodontitis.

Neutrophils, monocytes and lymphocytes play a crucial role in innate and acquired immunity [40]. Several previous studies have evaluated the association between periodontitis and the peripheral blood leukocyte and its subtypes [14,49–51]. The present study supported the findings of the previous studies that demonstrated increased peripheral blood neutrophil count in patients with periodontitis. While neutrophils are considered the first line of defence against microbial infections, an increase in the numbers of neutrophils in the peripheral blood of patients with periodontitis is natural to observe [14]. Lymphocytes are important regulators of the inflammatory response [40,52]. A low lymphocyte count indicates a slowed immunological response [40,52] and has been associated with periodontal tissue destruction [53]. While most studies failed to report significant difference in the lymphocyte counts between periodontitis patients and healthy controls [49,50,53–55], the present study exhibited significantly lowered lymphocyte count in periodontitis patients. Regarding monocyte count, although one study reported a significant increase in monocyte count among patients with periodontitis [56], such a difference was not observed in the current study, and this finding was similar to that reported by other authors [50,54,57]. Concerning, the platelet level, the results of the present study was in line with other foregoing studies that reported no prominent differences in the platelet count between periodontitis patients and periodontally healthy patients [16,58], suggesting that platelet activation and function play a more significant role in the pathogenesis of periodontitis rather than the absolute platelet counts [16,59]. Such variable results with regard to association of periodontitis and different leukocyte subtypes make the applicability of individual leukocyte counts in predicting periodontitis open to doubt.

The NLR and LMR are a combination of two independent markers of inflammation that reflect two conversely and related immune pathways [40]. Since these leukocyte ratios indicate the balance between neutrophils (in case of NLR)/ monocytes (in case of LMR) and lymphocyte levels and are less affected by conditions that could change the individual cell count, they have been considered as more stable and reliable biomarkers in several previous studies [16,60,61], and the same was demonstrated in the present study too.

Clinical periodontal examination remains the benchmark for diagnosing and monitoring periodontal diseases. Blood leukocyte ratios – NLR and LMR might prove to be helpful adjuncts in screening among large population and identification and quantification of individuals at risk of developing severe periodontal diseases and also in monitoring such patients. These tests are not only cheap and reliable; they are easier to obtain than other blood biomarkers that have limited use in daily clinical practice due to technical complications.

There are certain limitations to the study. The present study was a retrospective, single centred study suggesting multi-centred, prospective research work in populations belonging to other racial groups, in future. The influence of possible seasonal variation and menopause in the levels of blood leukocyte ratios was not accounted in the present study. Whether a change is seen in the values of blood leukocyte ratios after periodontal intervention needs further investigation. However, one major strength of the study is that it is the first study that determined the predictive value of LMR in young patients with severe periodontitis.

Conclusion

Within the limitations of the study, the present study revealed elevated NLR and decreased LMR in young patients with severe periodontitis. Furthermore, the two leukocyte ratios can be considered as novel inflammatory biomarkers that could provide a new insight into the relationship between periodontitis and systemic diseases. The study also demonstrated promising diagnostic potential of NLR and LMR that requires further validation through prospective studies.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Ethics approval

All procedures performed in the study were in accordance with the ethical standards of the institutional ethical committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Disclosure statement

Supriya Mishra declares that she has no conflict of interest. Gazala MP declares that she has no conflict of interest. Waheda Rahman declares that she has no conflict of interest.

No potential conflict of interest was reported by the author(s).

Author contributions

SM: Concept and design of the work, drafting the manuscript, data analysis, G MP: data collection, WR: manuscript review and editing. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [Mishra S], upon reasonable request.

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