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ORIGINAL ARTICLE

Glucose and lipid metabolism indexes and blood inflammatory biomarkers of patients with severe periodontitis: A cross-sectional study

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Abstract

Background: To investigate the relation of established glucose and lipid metabolism indexes and blood inflammatory biomarkers with severe periodontitis in systemically healthy patients.

Methods: Systemically healthy Stage III/IV periodontitis patients (case group) (n = 397), Stage II periodontitis patients (n = 36), and periodontally healthy subjects (control group) (n = 285) were recruited. A periodontal examination, complete blood cell examination, and blood biochemical examination were conducted for all participants. Full-mouth apical films were taken for the case group. Both the case and control groups were divided by age into younger (≤ 35 years) and elder subjects. Multiple logistic regression analysis and Pearson correlation analysis were conducted. A logistic least absolute shrinkage and selection operator (LASSO) model was constructed for the younger subgroups.

Results: Various glucose and lipid metabolism indexes and blood inflammatory biomarkers significantly differed between severe periodontitis patients and healthy controls, and the younger subgroups presented a greater degree of statistical differences than the elder ones. More pairs of periodontal parameters and blood indexes with significantly fair linear correlations were found in the younger patient subgroup. A logistic LASSO regression model containing eight blood indexes to assess a severe periodontitis outcome in younger subgroups showed satisfactory predictive ability.

Conclusion: The present study revealed various glucose and lipid metabolism indexes and blood inflammatory biomarkers significantly differ between severe periodontitis patients and healthy controls, especially in the younger subgroups. A LASSO regression model could be a viable option to assess severe periodontitis risk for younger patients.

KEYWORDS
biomarkers, glucose metabolism disorders, lipid metabolism disorders, periodontitis

Xiaoyuan Guan and Xiane Wang contributed equally to this work.

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1 | INTRODUCTION

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus,[1] which can lead to tooth drifting and tooth loss in severe cases.[2] Besides being characterized by local inflammation, periodontitis has also been demonstrated to have a systemic influence, and its relation with at least 57 systemic diseases and disabilities is now under research.[3]

Periodontitis-caused low-grade systemic inflammation may serve as one of several mechanisms connecting periodontitis and systemic comorbidities.[4] At the molecular level, elevated pro-inflammatory mediators[5–7] (such as C-reactive protein, IL-1, IL-6, and calprotectin) and certain blood constituents[8,9] (such as platelet, neutrophil, and neutrophil-to-lymphocyte ratio) were detected in the peripheral blood of patients with severe periodontitis compared to periodontally healthy individuals. At the disease level, previous studies observed that inflammatory diseases, including periodontal disease, are associated with the disturbance of patients' lipid and glycemic metabolism and further increase the risk of diabetes mellitus, obesity, and cardiometabolic disorders.[10,11]

However, former studies have mainly focused on index differences between periodontitis patients and periodontally healthy individuals. Thus it is still not known whether or not patients diagnosed with generalized Stage III/IV, Grade C periodontitis of different ages would reveal different degrees of glucose and lipid metabolic disorders. Also, most former studies investigated the index changes of individuals with systemic disease status. Differences in glucose and lipid metabolism indexes among severe periodontitis patients, moderate periodontitis patients, and periodontally healthy subjects who are systemically healthy are yet to be revealed. Therefore, we aimed to investigate the relation of established glucose and lipid metabolism indexes and blood inflammatory biomarkers with severe periodontitis in older versus younger systemically healthy individuals.

2 | MATERIALS AND METHODS

2.1 | Study population

This is a cross-sectional study designed to examine the association of glucose and lipid metabolism indexes and blood inflammatory biomarkers with severe periodontitis in the Chinese population. A total of 433 systemically healthy patients from the Department of Periodontology, Peking University School and Hospital of Stomatology (Beijing, China), aged 16–71 years, were enrolled from February 2010 to May 2017. Another 285 participants, serving as healthy controls, were recruited as volunteers from the staff and students of Peking University School and Hospital of Stomatology (Beijing, China). The study protocol was approved by the Ethics Committee of Peking University Health Science Center (IRB00001052-08010). All participants provided written informed consent, and data collection was performed following the principles outlined in the Declaration of Helsinki.

Before the oral examination, every patient was quizzed about their past medical history and drug usage. Individuals with systemic diseases, smoking or use of any tobacco products, periodontal therapy within 1 year, antibiotics within the previous 3 months, or pregnancy were excluded.

An oral examination was conducted for every participant, and participants with less than 20 teeth (excluding third molars) were also excluded. Periodontal charts and radiographs (if necessary) were taken to determine their periodontal health status. Periodontal health was defined as (1) the percentage of a bleeding index (BI) ≥ two (i.e., bleeding on probing (+)) sites ≤ 10%, and (2) probing depth (PD) ≤ 3 mm.[12] To assess the severity and complexity of patients with periodontitis, we staged the periodontitis cases according to the 2017 World Workshop of the American Academy of Periodontology and the European Federation of Periodontology.[1,13] Stage I periodontitis was diagnosed with (1) interdental attachment loss (AL) between 1 and 2 mm at the site of greatest loss; (2) radiographic bone loss (BL) of <15%; (3) no tooth loss due to periodontitis; or (4) maximum PD ≤ 4 mm, or mostly horizontal BL. Stage II periodontitis was diagnosed by the following criteria: (1) interdental AL between 3 and 4 mm at the site of greatest loss; (2) radiographic BL between 15% and 33%; (3) no tooth loss due to periodontitis; or (4) maximum PD ≤ 5 mm or mostly horizontal BL. Stage III periodontitis was diagnosed by the following criteria: (1) interdental AL ≥ 5 mm at the site of greatest loss; (2) radiographic BL extending to mid-third of the root and beyond; (3) tooth loss due to periodontitis ≤4 teeth; or (4) in addition to Stage II, either PD ≥ 6 mm or vertical BL ≥ 3 mm, or Class II/III furcation involvement, or moderate ridge defect. Stage IV periodontitis was diagnosed by the following criteria: (1) interdental AL ≥ 5 mm at the site of greatest loss; (2) radiographic BL extending to mid-third of the root and beyond; (3) tooth loss due to periodontitis ≥5 teeth; or (4) in addition to Stage III, either masticatory dysfunction, or secondary occlusal trauma (tooth mobility degree ≥ 2), or severe ridge defect, or bite collapse, or teeth drifting and flaring. Then, patients diagnosed with Stage III/IV periodontitis (case group) were divided into two subgroups with respect to the age of 35 years: patients aged ≤ 35 years were classified into the younger patient group (YP group) and patients aged > 35 years into the elder patient

group (EP group). The control group was also divided into two subgroups, for the elimination of age as a confounding variable, with respect to the age of 35 in matching fashion (i.e., control group participants aged ≤ 35 years were classified as the younger control [YC] group, and those aged > 35 years as the elder control [EC] group).

2.2 | Clinical and radiological measurement

Full-mouth periodontal examinations including PD, AL, BI, and plaque index (PLI) were conducted for the eligible participants. PD and AL were recorded at six sites (mesiobuccal/mesiolabial, midbuccal/midlabial, distobuccal/distolabial, mesiolingual/mesiopalatal, midlingual/midpalatal, and distolingual/distopalatal) of each tooth using a UNC-15 probe*. The BI was scored on a scale of 0 to 5 using Mazza's method[14] and the most severe sites in the buccal/labial side and lingual/palatal surface were recorded. PLI for each tooth was determined on a scale of 0 to 3.[15]

Height (meters) and weight (kg) were measured for all participants, and body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2).

For patients with generalized Stage III/IV periodontitis, full-mouth radiographs were taken for the whole dentition. To assess the grade of every patient, we calculated the BL/age ratio of the worst site.[13]

2.3 | Blood examination

Fasting blood samples were collected from all participants in the morning by venipuncture, and each sample was divided into two parts: one with EDTA for blood cell analysis by hematology analyzers†; the other without EDTA for serum protein analyses by biochemical analyzers‡. Blood cell analysis consisted of white blood cell count (WBC), platelet count (PLT), mean platelet volume (MPV), neutrophil count (NEUT), and neutrophil percentage (NEUT%). Serum protein parameters contained total cholesterol (CHO), triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and fasting glucose (GLU).

2.4 | Statistical analysis

Statistical analyses were performed in SPSS software§ and R Statistical Software‖. The characteristics of the YP and YC groups were analyzed using the Chi-square or *t*-test for significance of differences among frequencies and means, respectively. The same analyses were conducted for the YP group versus the EC group, and the YP group versus the EP group. A logistic regression model was used to estimate the odds ratios (ORs) and the 95% confidence intervals (CIs) of the risk of periodontitis outcome by comparing the level of glucose and lipid metabolism indexes and inflammatory biomarkers. In addition, the model was also adjusted for sex, using the enter method. The correlations of any two of the periodontal status variables (i.e., PD, BI, AL, and PLI) and all blood indexes were performed using Pearson's correlation coefficients. The strength of the relationship was quantified by the correlation coefficient.[16]

Since few significant correlations were found between the blood indexes and periodontitis outcomes in the EP and EC groups, we only constructed the prediction model for the YP and YC groups. As there was severe multicollinearity bias among BMI, WBC, and NEUT in this study, utilizing a least absolute shrinkage and selection operator (LASSO) model instead of a multivariable logistic regression model was preferable to predict the severe aggressive periodontitis outcome from the various blood indexes we studied. For obtaining the logistic LASSO estimator, we used the glmnet package in R. We randomly divided the mixed dataset (532 observations) of the YP and YC groups according to the ratio of 7:3 for the training and test datasets (372 and 160 observations, respectively). Then we fit the logistic LASSO regression using the training data set and assessed the predictive ability of the fitted models using the test data. For the logistic LASSO regression, we used cross-validation to select the penalty coefficient λ. The predicted outcomes were visualized by box plot, and the Wilcoxon signed-rank test was also applied to investigate the statistical significance of the difference between the two groups. Moreover, we calculated the area under the receiver operating characteristic curve (AUC) for the test data as measures of the predictive performance of the fitted models.

Results of all hypothesis tests with P values of <0.05 (two-sided) were considered statistically significant.

* Hu-Friedy, Chicago, IL.
† Sysmex, Kobe, Japan.
‡ HITACHI, Tokyo, Japan.
§ IBM-SPSS, Armonk, NY.
‖ X&Y Solutions, Inc., Boston, MA.

TABLE 1 Demographic characteristics, periodontal parameters, glucose and lipid metabolism indexes, and inflammatory biomarkers (mean ± SD) of the control, moderate periodontitis, and case (severe periodontitis) groups

Variables	Periodontal healthy individuals (control group) ($n = 285$)			Stage II (moderate) periodontitis patients ($n = 36$)	Stage III/IV; (severe) periodontitis patients (case group) ($n = 397$)		
	YC group ($n = 222$)	EC group ($n = 63$)	Total		YP group ($n = 310$)	EP group ($n = 87$)	Total
Age (years)	27.14 ± 3.84	44.82 ± 7.66	31.05 ± 8.85	44.25 ± 12.87	27.94 ± 4.766	47.59 ± 9.47‡	32.11 ± 9.95
Sex (males/females)	68/154	14/49	82/203	14/22	127/183*	40/47†‡	167/230§
PD (mm)	1.45 ± 0.40	2.21 ± 0.81	1.62 ± 0.60	3.31 ± 0.70‖	4.89 ± 0.96*	4.29 ± 0.70†‡	4.69 ± 0.98§
BI	1.21 ± 0.31	1.70 ± 0.66	1.31 ± 0.46	2.72 ± 0.86‖	3.48 ± 0.52*	3.17 ± 0.48†‡	3.37 ± 0.57§
AL (mm)	0.08 ± 0.37	0.92 ± 0.59	0.27 ± 0.42	2.68 ± 0.79‖	4.45 ± 1.44*	4.11 ± 1.09†	4.29 ± 1.45§
PLI	0.22 ± 0.30	0.72 ± 0.62	0.53 ± 0.26	1.52 ± 0.32‖	2.43 ± 0.51*	2.41 ± 0.46†	2.43 ± 0.50§
BL/Age					3.05 ± 0.83	1.64 ± 0.46‡	2.85 ± 0.92
BMI (kg/m^2)	21.44 ± 2.64	23.06 ± 2.77	21.79 ± 2.75	22.80 ± 3.34	21.77 ± 3.41	23.46 ± 2.72‡	22.24 ± 3.31
CHO (mmol/L)	4.39 ± 0.77	4.99 ± 0.85	4.52 ± 0.82	4.81 ± 0.88	4.18 ± 0.85*	4.77 ± 0.89‡	4.31 ± 0.90§
TG (mmol/L)	0.88 ± 0.47	1.18 ± 0.60	0.95 ± 0.52	1.24 ± 0.79	0.99 ± 0.54*	1.63 ± 1.14†‡	1.13 ± 0.77§
HDL (mmol/L)	1.53 ± 0.35	1.48 ± 0.31	1.52 ± 0.34	1.47 ± 0.31	1.47 ± 0.32	1.34 ± 0.29†‡	1.46 ± 0.32§
LDL (mmol/L)	2.46 ± 0.63	3.05 ± 0.74	2.59 ± 0.70	2.81 ± 0.78	2.41 ± 0.82	2.97 ± 0.79‡	2.51 ± 0.83
GLU (mmol/L)	4.82 ± 0.41	5.19 ± 0.52	4.90 ± 0.46	5.36 ± 0.47	5.21 ± 0.55*	5.40 ± 0.58†‡	5.26 ± 0.56§
WBC (×10^9/L)	5.77 ± 1.39	5.85 ± 1.57	5.78 ± 1.43	6.00 ± 1.57	6.58 ± 1.84*	6.01 ± 1.61‡	6.45 ± 1.80§
PLT (×10^9/L)	241.88 ± 56.74	236.19 ± 51.66	240.62 ± 55.62	236.85 ± 59.05	238.87 ± 57.14	241.88 ± 56.69	238.92 ± 56.42
MPV (fL)	9.10 ± 1.16	9.49 ± 1.16	9.19 ± 1.17	9.54 ± 3.51	9.70 ± 1.50*	10.10 ± 1.22†	9.78 ± 1.46§
NEUT (×10^9/L)	3.29 ± 1.15	3.49 ± 1.36	3.34 ± 1.20	3.65 ± 1.30	4.27 ± 1.60*	3.69 ± 1.39‡	4.15 ± 1.57§
NEUT% (%)	56.36 ± 8.37	58.72 ± 8.45	56.88 ± 8.43	58.75 ± 8.85	63.73 ± 8.82*	61.11 ± 8.56‡	63.15 ± 8.80§

Note: Continuous data are presented as mean ± SD and tested by *t*-test; categorical data are presented as ratio and tested by Chi-square. Indications of statistical significance noted for the case group and its subgroups are for comparisons with the corresponding control group or subgroups, while those noted for the moderate periodontitis patients are for comparisons with the EC group.

Abbreviations: AL, attachment loss; BI, bleeding index; BL, bone loss; BMI, body mass index; CHO, total cholesterol; EC group, elder control group; EP group, elder patient group; GLU, fasting glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MPV, mean platelet volume; NEUT%, neutrophil percentage; NEUT, neutrophil count; PD, probing depth; PLI, plaque index; PLT, platelet count; TG, triglycerides; WBC, white blood cell count; YC group, younger control group; YP group, younger patient group.

*Statistical significance level between the YP group and the YC group: $P < 0.05$. †Statistical significance level between the EP group and the EC group: $P < 0.05$. ‡Statistical significance level between the YP group and the EP group: $P < 0.05$. §Statistical significance level between the case group and the control group: $P < 0.05$. ‖Statistical significance level between the Stage II periodontitis patients and the EC group: P < 0.05.

3 | RESULTS

In the present study, 718 individuals were included in the analysis. Among all subjects, 397 (55.3%) were diagnosed with Stage III/IV periodontitis (case group), 36 (5.0%) with Stage II periodontitis, and 285 (39.7%) with periodontal health (control group). In the case group, there were 310 (78.1%) YPs and 87 (21.9%) EPs. In the control group, there were 222 (77.9%) YCs and 63 (22.1%) ECs.

As presented in Table 1, PD, BI, AL, and PLI in the case group are all significantly higher than in the control group ($P < 0.001$ for every index). When conducting subgroup analyses for younger and elder subjects, we found that both the YP group and EP group had significantly higher periodontal parameters (i.e., PD, BI, AL, and PLI; $P < 0.001$ for every index) when compared with those of the respective control groups, while PD and BI for the YP group were higher than for the EP group ($P < 0.001$ for both indexes). It is also striking that the BL/age ratio in the YP group was significantly higher than in the EP group (3.05 ± 0.83 vs. 1.64 ± 0.46, $P < 0.001$). The case group had significantly higher TG and GLU ($P < 0.001$ for both indexes), and lower CHO and HDL ($P = 0.002$, $P = 0.011$, respectively) than the control group. Subgroup analysis results showed that the YP group had significantly lower CHO than the YC group (4.18 ± 0.85 mmol/L vs. 4.39 ± 0.77 mmol/L, $P = 0.005$), whereas higher TG and GLU were observed in the YP group than in the YC group (0.99 ± 0.54 mmol/L vs. 0.88 ± 0.47 mmol/L, $P = 0.014$, and 5.21 ± 0.55 mmol/L vs. 4.82 ± 0.41 mmol/L, $P < 0.001$, respectively). While for the EP group, significantly higher TG and GLU, and lower HDL, were shown compared to the EC group (1.63 ± 1.14 mmol/L vs. 1.18 ± 0.60 mmol/L, $P = 0.002$; 5.40 ± 0.58 mmol/L vs. 5.19 ± 0.52 mmol/L, $P = 0.014$;

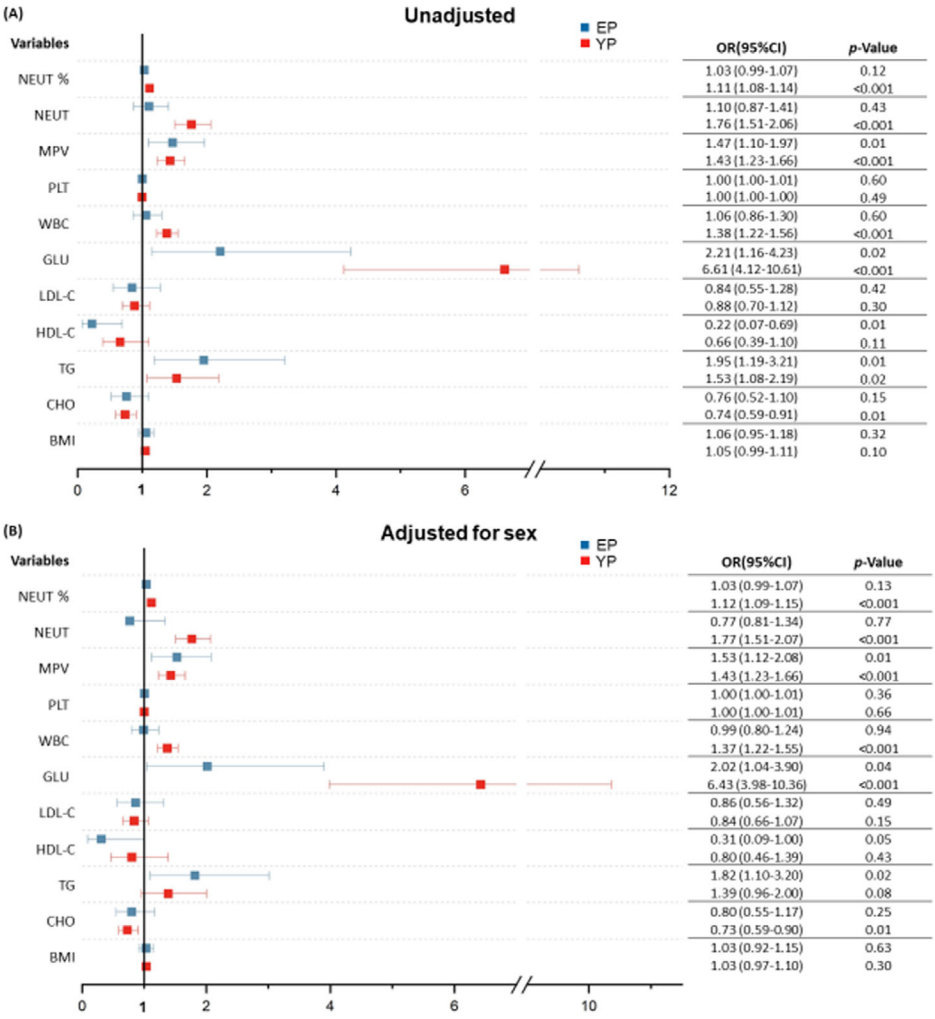

FIGURE 1 Univariate and multivariable logistic regression models, (**A**) unadjusted and (**B**) adjusted for sex, of the glucose and lipid metabolism indexes and inflammatory biomarkers for younger (YP; ≤ 35 years) and older (EP; > 35 years) periodontitis patient groups. Colored squares in the graphs signify median odds ratios (ORs), and the left and right bars signify the 50th to 25th and 75th to 50th quartiles, respectively, with red for comparisons between the YP group and same-aged controls, and blue for similar comparisons for the EP group. Data in the charts on the right side are presented as ORs and 95% confidence intervals (CIs) using multiple logistic regression analysis, with *P*-values given accordingly. BMI, body mass index; CHO, total cholesterol; GLU, fasting glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MPV, mean platelet volume; NEUT, neutrophil count; NEUT%, neutrophil percentage; PLT, platelet count; TG, triglycerides; WBC, white blood cell count

1.34 ± 0.29 vs. 1.48 ± 0.31 mmol/L, $P = 0.008$, respectively). Additionally, all the glucose and lipid metabolism indexes were significantly lower in the YP group than in the EP group except for HDL. Significantly higher WBC, MPV, NEUT, and NEUT% were observed in the case group than in the control group ($P < 0.001$ for all indexes). Subgroup analysis results revealed that there were significantly higher levels of WBC, MPV, NEUT, and NEUT% in the YP group than the YC group ([6.58 ± 1.84] × 10^9/L vs. [5.77 ± 1.39] × 10^9/L, 9.70 ± 1.50 fL vs. 9.10 ± 1.16 fL, [4.27 ± 1.60] × 10^9/L vs. [3.29 ± 1.15] × 10^9/L, 63.73 ± 8.82% vs. 56.36 ± 8.37%, respectively, $P < 0.001$ for every index), while only significantly greater MPV was found in the EP group than the EC group (10.10 ± 1.22 fL versus 9.49 ± 1.16 fL, $P = 0.001$). Besides, the YP group presented significantly higher WBC, NEUT, and NEUT% than the EP group. With a similar mean age to the EC group ($P = 0.81$), Stage II periodontitis patients showed significantly higher PD, BI, AL, and PLI ($P < 0.001$ for all indexes) than the EC group. However, there was no difference in any glucose and lipid metabolism indexes or blood inflammatory biomarkers.

Figure 1A shows the unadjusted OR (95% CI) data for the glucose and lipid metabolism indexes and blood inflammatory biomarkers in the YP and EP groups, with the YC and EC groups serving as controls, respectively. For the YP group (in red), a significantly higher prevalence of TG, GLU, WBC, MPV, NEUT, and NEUT% and a lower

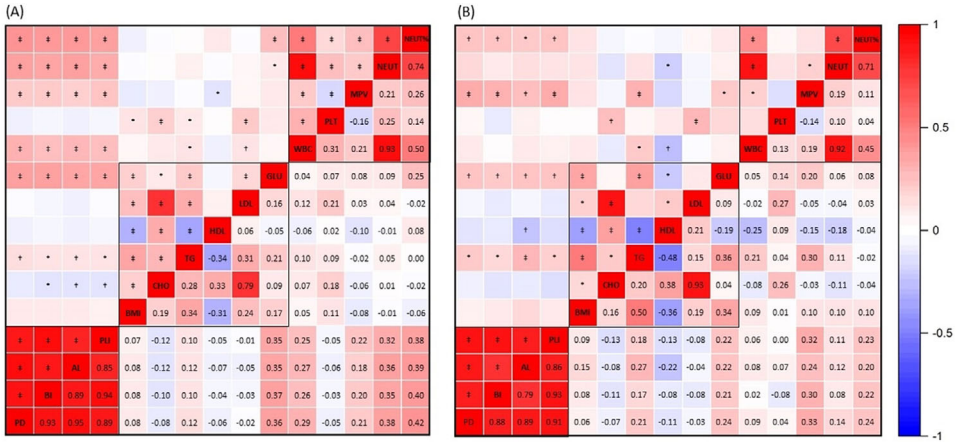

FIGURE 2 Pearson correlations among periodontal parameters and blood indexes, for (**A**) younger (YP; ≤ 35 years) and (**B**) older (EP; > 35 years) patient groups. Red colors signify positive and blue colors signify negative correlations, with deeper shades indicating stronger correlations. AL, attachment loss; BI, bleeding index; BMI, body mass index; CHO, total cholesterol; GLU, fasting glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MPV, mean platelet volume; NEUT, neutrophil count; NEUT%, neutrophil percentage; PD, probing depth; PLI, plaque index; PLT, platelet count; TG, triglycerides; WBC, white blood cell count. *correlation significant at the 0.05 level; †correlation significant at the 0.01 level; ‡correlation significant at the 0.001 level

prevalence of CHO are seen as compared to the YC group. For the EP group, there were fewer indexes, including TG, GLU, and MPV, for which the OR (95% CI) values were significantly higher compared to the EC group, while the OR (95% CI) of HDL was significantly lower. In Figure 1B, the multivariate logistic models adjusted for sex also indicated similar OR (95% CI) data for the indexes except for TG for the YP group and HDL for the EP group, for which the differences were not statistically significant compared with their respective control groups.

Figure 2 illustrates the Pearson's correlation results for every pair of variables analyzed in this study, including the periodontal parameters and all blood indexes mentioned above, in the YP and EP groups (Figure 2A and Figure 2B, respectively). When we focus on the association between periodontal parameters and the blood indexes, with increasing PD, BI, AL, and PLI, significantly fair (≥ 0.25) positive linear correlations with GLU, WBC, NEUT, and NEUT% were detected for the YP group ($P < 0.001$; Figure 2A). Turning now to the EP group, only four pairs of the indexes are found to be similarly correlated (i.e., TG and AL, MPV and PLI, MPV and PD, MPV and BI) with a significantly fair r value ($P < 0.001$; Figure 2B). Some other blood indexes may exhibit positive correlations with periodontal status in both groups, but the coefficients are small enough to be negligible.

If we now turn to the association between glucose and lipid metabolism indexes and blood inflammatory biomarkers, a significantly fair linear correlation was found for the YP group between GLU and NEUT%, ($P < 0.001$; Figure 2A). Figure 2B displays the significantly fair correlations for the EP group, between LDL and PLT, CHO and PLT, TG and MPV ($P = 0.002$, $P < 0.001$, $P < 0.001$, respectively). There were also significant but negligible associations seen for both groups between glucose and lipid metabolism indexes and blood inflammatory biomarkers (not listed here).

For the YP group, we found that when penalty parameter λ in the LASSO regression model increased to 0.025, only eight variables, which are probably the most contributing factors to periodontitis, remained in the model (i.e., the coefficients of three variables, HDL, LDL, and WBC, approached zero more quickly; thus these three variables were removed from the model). Specifically, when the value of λ was taken as 0.025, BMI, CHO, TG, GLU, PLT, MPV, NEUT, and NEUT% confer the largest signal in the model (Figure 3). Among all the contributing variables, GLU ($\beta = 1.63$), TG ($\beta = 0.33$), NEUT ($\beta = 0.19$), NEUT% ($\beta = 0.06$), and BMI ($\beta = 0.01$) were positively associated with severe periodontitis, while PLT ($\beta = -0.01$) and CHO ($\beta = -0.34$) were inversely associated.

Figure 4A,B displays the predictive performance of the LASSO regression model. Figure 4A demonstrates that the model could significantly discriminate periodontitis from the control in the validation set ($P < 0.001$). Figure 4B presents the receiver operating characteristic curve for the test data. The AUC is 0.80, indicating the model's satisfactory predictive ability.

4 | DISCUSSION

To the best of our knowledge, this is the first study to contrast glucose and lipid metabolism indexes and blood

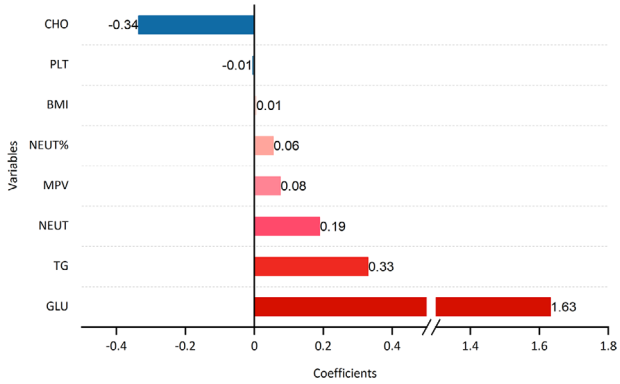

FIGURE 3 The coefficients for LASSO regression between blood indexes and severe periodontitis outcomes among younger subjects when the value of λ was taken as 0.025. Red colors signify positive and blue signifies negative correlations, with the coefficient of each variable labeled at the top of the corresponding bar. BMI, body mass index; CHO, total cholesterol; GLU, fasting glucose; MPV, mean platelet volume; NEUT, neutrophil count; NEUT%, neutrophil percentage; PLT, platelet count; TG, triglycerides

inflammatory biomarkers of patients suffering from severe periodontitis with moderate periodontitis and periodontally healthy individuals who are all in good systemic health. Interestingly, patients with moderate periodontitis showed no significant difference in glucose and lipid metabolism indexes and blood inflammatory biomarkers compared with healthy controls; however, there were significant differences in various glucose and lipid metabolism indexes (including CHO, TG, HDL, and GLU) and blood inflammatory biomarkers (including WBC, MPV, NEUT, and NEUT%) between severe periodontitis patients and healthy controls. Also, the YP group presented a greater degree of statistical difference in glucose and lipid metabolism indexes and blood inflammatory biomarkers compared to the YC group, while differences between the elder subgroups are more minor. Additionally, we firstly applied the logistic LASSO regression model, which minimizes multicollinearity between dietary variables, to assess multiple glucose and lipid metabolism indexes and blood inflammatory biomarkers with the generalized Stage III/IV periodontitis outcome in subjects aged under 35 years.

A few prior studies have noted the associations of glucose and lipid metabolism indexes with periodontitis in comparisons between systemically healthy periodontitis patients and periodontally healthy controls.[17–19] A large cross-sectional study has revealed that greater serum TG was associated with higher localized Stage II/III periodontitis risk.[20] However, there were no associations for CHO and HDL.[20] These findings were consistent with our findings of TG but not CHO. A possible explanation for this might be that the periodontal status of patients from the previous study was better than ours, which may lead to a minor but statically insignificant difference in CHO. Also, elevated GLU has been identified as an indicator of higher periodontitis risk by a large cohort study in the non-diabetic population.[21] Our results further supported the idea of a strong relationship between GLU and periodontal conditions in non-diabetic subjects.[22,23] Besides the glucose and lipid metabolism indexes taken singly, the combination of these indexes[24] and the level of serum lipoprotein antibodies[25,26] may serve as markers of severe periodontitis.

Several reports have shown that peripheral blood indexes could serve as periodontal inflammatory biomarkers.[27–29] Previous studies demonstrated that greater peripheral WBC and NEUT numbers were related to generalized aggressive periodontitis,[28,30] which is in agreement with our findings. However, how platelet characteristics reflect the periodontal condition was still ambiguous.[9,31] In accordance with our results about MPV, some previous studies have demonstrated that plateletcrit, MPV, and platelet distribution width were elevated in periodontitis patients.[31] Moreover, individuals with severe periodontitis have higher PLT levels than moderate and healthy controls.[32] However, this differed from Zhan et al.'s finding that lower MPV is related to more severe periodontal inflammation.[33,34] This inconsistency may be due to patients in these studies suffering different levels of severity of periodontitis.

Since fair and definite correlations have been found between some glucose and lipid metabolism indexes and blood inflammatory biomarkers, it suggests that severe periodontal inflammation may influence glucose and lipid metabolism indexes via raising systemic inflammatory responses.[35,36] It is generally accepted that periodontitis as an oral infection leads to the entry of bacteria (or their products) into the bloodstream, therefore promoting systemic low-grade inflammation (SLGI).[37,38] SLGI is considered a major link between periodontal disease and systemic disease.[39] Our findings showed that the main mediators were platelets and neutrophils. The mediation of neutrophils may be explained by periodontitis-induced trained immunity.[36] Subgingival plaque unleashes sustained, persistent low-level lipopolysaccharide from the periodontal pocket into the peripheral blood. Subsequently, the neutrophils become hyper-responsive.[40] Cytotoxic neutrophil proteases and histones[41] and the formation of neutrophil extracellular traps[42] may be responsible for ulcers on the pocket epithelium, which foster endotoxemia and consequently SLGI. Since platelets express several molecules involved in antigen recognition and can directly react with microbes, an increase in their number and activity has been subsequently observed during periodontitis-induced bacteremia.[43] Characterized by

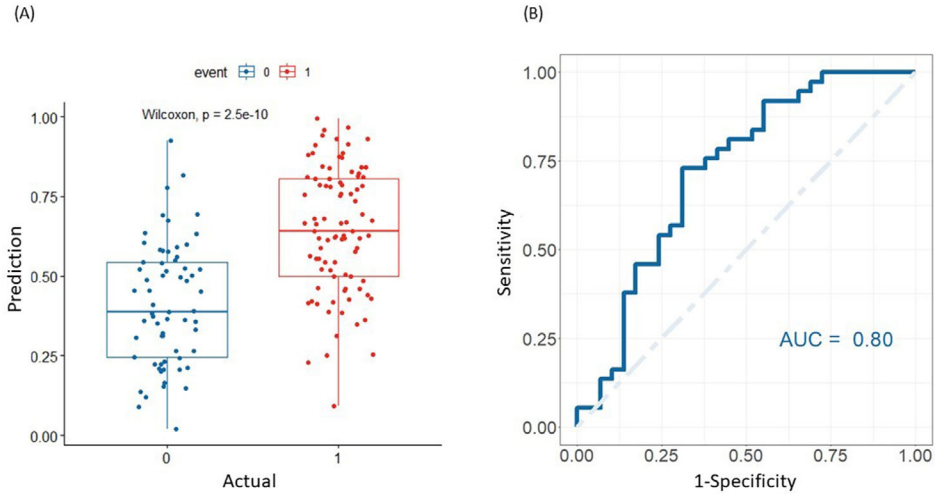

FIGURE 4 Assessment of the predictive performance of the LASSO regression model. (**A**) Box plots for predicted results in the test in the validation set. The X-coordinate of every dot represents the actual periodontal status (0 for control, and 1 for severe periodontitis), and the Y-coordinate shows the value calculated from the LASSO regression model. In the test dataset, when a value exceeding 0.5 of the outcome variable was generated from the fitted model, it was interpreted as severe periodontitis; otherwise, it was interpreted as control. The predicted results of subjects whose actual periodontal status is control are shown in blue, and those with severe periodontitis are shown in red. (**B**) Receiver operating characteristic (ROC) analysis results for the test in the validation set. The blue curve represents the prediction ability of severe periodontitis risk. AUC (area under the curve) = 0.80 indicates satisfactory predictive ability

more granules, aggregating rapidly with collagen, having a higher thromboxane A2 level, and expressing more glycoprotein Ib and IIb/IIIa receptors,[32] activated platelets also contribute to increased systemic inflammatory burden[44] and an elevated risk for adverse cardiovascular events.[45]

According to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions,[1] patients in our case group were all classified as Stage III or IV, and Grade C. However, a significantly higher mean BL/age of the YP group was found than in the EP group, which indicated more rapid progress of periodontal tissue destruction. It can therefore be assumed that there is still a difference in the periodontal inflammation level among patients diagnosed with Stage III/IV, Grade C periodontitis. As an undoubtedly major effector of acute inflammation,[46] significantly higher NEUT and NEUT% in the YP group than in the EP group may suggest a higher systemic inflammation level in the YP patients. According to the SGLI theory mentioned above, this finding may also partly explain the greater differences in glucose and lipid metabolism indexes between the YP and the YC than between the EP and the EC groups. Thus, the present study raises the possibility that the systemic inflammatory level may distinguish younger severe periodontitis patients from the elder ones, who could be characterized by significantly different levels of glucose and lipid metabolism indexes and blood inflammatory biomarkers compared to healthy controls.

This is the first study that depicts the characteristics of glucose and lipid metabolism of severe periodontitis in systemically healthy patients. Although changes in glucose and lipid metabolic indexes in patients with severe periodontitis in our study were still in the medical reference range, a constellation of metabolic imbalances could lead to a greater potential for future occurrence of these diseases (including type 2 diabetes, lipid disorders, cardiovascular disease, hepatic steatosis, and other circulatory disorders, etc.).[47,48] In contrast to former studies which mainly focus on the relationship between periodontitis and diagnosed systemic diseases,[49] our study based on systemic healthy subjects implied that severe periodontal inflammation may not only be serving as an aggravating factor for already established pathologies but also act as a risk factor for the development of potential systemic diseases, especially in YPs. The systemic prognosis of these individuals needs to be confirmed by cohort studies. Despite these promising results, limitations remain. The present cross-sectional study design could not examine the causal relationships between blood indexes and severe periodontitis. Therefore, prospective studies are needed to further explore the relationships between them. Additionally, the data from only one hospital may not be representative, so a population-based study will be needed to extrapolate the findings cautiously.

5 | CONCLUSION

The present study revealed that various glucose and lipid metabolism indexes (including CHO, TG, HDL, and GLU)

and blood inflammatory biomarkers (including WBC, MPV, NEUT, and NEUT%) significantly differ between severe periodontitis patients and healthy controls. Furthermore, the younger severe periodontal patients presented a greater degree of statistical difference compared to controls in glucose and lipid metabolism indexes and blood inflammatory biomarkers, while differences between the elder subgroups are more minor. We also showed that a LASSO regression model containing multiple blood indexes could be a viable option to assess the Stage III/IV periodontitis risk for individuals aged 35 years and younger.

AUTHOR CONTRIBUTIONS

Xiaoyuan Guan, Xiane Wang, and Huanxin Meng conceived and presented this study. Yi Li, Jingling Xu, Lu He, Xinran Xu, and Xu Li were responsible for collecting clinical data. Guan Xiaoyuan carried out data analysis and drafted the manuscript. All authors contributed to and approved the final version of the manuscript.

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CONFLICT OF INTEREST

All the authors report no conflicts of interest related to this study.

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