Biomarkers in Metabolic Syndrome Patients with Chronic Periodontitis

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Objectives: *To investigate whether the levels of serum C-reactive protein (CRP), salivary interleukin (IL)-6 and IL-lβ in metabolic syndrome (MS) patients can be potential monitors for inflammation in MS patients with severe periodontitis.*

Methods: *A total of 114 MS patients and 49 systemically healthy subjects were enrolled. CRP in serum and IL-1β and IL-6 in non-stimulated whole saliva were collected from these patients and subjects and analysed by enzyme-linked immunosorbent assay (ELISA). Dental examinations were performed and the participants completed a questionnaire.*

Results: *The serum CRP level of MS patients was higher than that of systemically healthy subjects, and increased as the number of components increased ($P < 0.05$). No difference was observed in the salivary level of IL-6 and IL-1β between MS patients and controls or between MS patients with different components. The level of salivary IL-6 in MS patients with moderate/severe periodontitis was significantly higher than in MS patients with good periodontal health/mild periodontitis ($P < 0.05$). After adjustment for age, sex and smoking habits, multivariate analysis showed that the corresponding odds ratio (OR) for MS combined with moderate/severe periodontitis was 1.21 (95% confidence interval [CI] 1.04–1.39, $P = 0.012$) for subjects with high serum CRP and salivary IL-6 and IL-1β were not risk indicators for MS combined with moderate/severe periodontitis.*

Conclusion: *MS patients might be burdened by high levels of serum CRP. Serum CRP could be a potentially valuable biomarker to detect inflammation in MS patients with severe periodontal disease.*

Key words: *cytokines, inflammation, metabolic syndrome, periodontitis*
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Metabolic syndrome (MS) has emerged as one of an important cluster of risk factors for atherosclerosis. Five components of MS are central obesity, high triglyceride (TG) levels, reduced high-density lipoprotein (HDL) cholesterol levels, elevated blood pressure (BP) and elevated fasting plasma glucose (FPG) or type 2 diabetes mellitus. The presence of MS is a predictor of future cardiovascular events[1]. The prevalence of MS in China was 14.39%, and the age-adjusted prevalence was 7.78% in men and 6.76% in women in Chinese adults aged 18 to 96 years, and it has become a critical public health problem in China[2]. Many people with MS have low-grade systemic inflammation, which may increase their risk of future adverse events[3]. Periodontitis is a well-known chronic and long-lasting low-grade inflammatory disease. Some studies have shown that periodontal conditions were poorer in MS patients than

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those without MS[4,5]. It is therefore important to pay greater attention to patients with MS combined with severe periodontitis.

Serum C-reactive protein (CRP) is a marker of systemic inflammation and patients with MS have been found to have higher CRP levels than those without this syndrome[3]. In systemically healthy individuals, patients with severe periodontitis also have higher serum CRP levels than those without periodontitis[6,7]. However, little is known about the serum CRP levels in Chinese MS patients with chronic periodontitis.

Salivary testing is a non-invasive way to diagnose some diseases. The rich mixture of substances makes saliva a source for identifying unique biomarkers that reflect both oral and systemic health changes[8]. Some studies have shown that salivary biomarkers such as interleukin (IL)-6 and IL-1β can reflect the inflammation that occurs in periodontal conditions[9,10]. However, little is known about whether levels of salivary IL-6 and IL-1β can reflect periodontal conditions in MS patients, and whether these two salivary biomarkers can be used as atraumatic monitors for MS patients with severe periodontal inflammation.

The aims of this study were as follows:

- to quantify the levels of CRP in serum and IL-1β and IL-6 in whole saliva from subjects with MS and compare them with those from systemically healthy individuals;
- to investigate whether these cytokines change with the number of metabolic components;
- to investigate whether serum CRP and salivary IL-6 and IL-lβ can be used as potential monitors for MS patients with severe periodontal inflammation.

Materials and methods

In this cross-sectional study, 114 MS patients and 49 systemically healthy subjects were enrolled.

According to the definition released by the International Diabetes Federation (IDF) in 2005[11], Chinese patients with MS must have central obesity (defined as waist circumference ≥ 90 cm for men and ≥ 80 cm for women) plus any two of the following four factors: high TG levels, ≥ 150 mg/dl (1.7 mmol/l) or specific treatment for this lipid abnormality; reduced HDL cholesterol levels, < 40 mg/dl (1.03 mmol/l) in men and < 50 mg/dl (1.29 mmol/l) in women, or specific treatment for this lipid abnormality; elevated BP, systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment of previously diagnosed hypertension; and elevated FPG ≥ 100 mg/dl (5.6 mmol/l) or previously diagnosed type 2 diabetes.

Patients and controls were older than 35 years of age and were residents of GuCheng, Beijing who had undergone a comprehensive general examination, including height, weight, waist circumference, BP, FPG, total cholesterol (TC), TG, HDL, low-density lipoprotein cholesterol (LDL), parameters reflecting the liver and kidney metabolism, routine urine test and electrocardiogram. The diagnosis of MS was determined based on these results. The control population was judged to be generally healthy based on BP, serum biochemical examination (e.g. FPG, TC, TG, HDL and LDL), parameters reflecting lipid, liver and kidney metabolism, routine urine test and electrocardiogram. The exclusion criteria for participants were as follows:

- unwilling to cooperate;
- edentulous;
- use of antibiotics for > 1 week in the last 6 months before the dental examination;
- oral mucosal lesion or medical history of salivary gland disorders;
- any known condition for which a preventive antibiotic treatment is required before dental examination.

All subjects gave written informed consent to participate in the study. The study protocol was approved by the Ethics Committee at Peking University Health Science Centre.

Interview and dental examination

All individuals were interviewed by a trained interviewer using a standardised questionnaire that focused on medical history and cardiovascular risk factors. Dental examinations were performed by a specially trained dental practitioner. The examiner was blinded to the subjects' group information before the examination.

For assessment of periodontitis, the attachment loss (AL) and probing depth (PD) were measured at two sites of each tooth (mesiobuccal and distolingual). AL was measured as the distance from the cementoenamel junction to the bottom of the pocket. Attachment levels were analysed as a continuous variable, and the percentage of sites with AL of 3 mm or greater in all examined sites of each subject were categorised as 0%, > 0% to 33%, > 33% to 67% and > 67%[12], defined respectively as no periodontitis, mild, moderate and severe periodontitis. Bleeding Index (BI)[13] and Plaque Index (PI)[14] were also recorded. Mean values were calculated on a subject basis. Smoking was divided into current smoker (more than 1 cigarette/day for a period of over 1 month) and current non-smoker.

Serum and saliva collection and biomarker analysis

Vein blood and unstimulated whole saliva from each subject were collected before dental examination. The subjects refrained from eating, drinking and chewing gum, etc., for at least 2 hours prior to sample collection. Blood samples were stored on ice immediately and transported to the laboratory. Serum CRP levels were tested by enzyme-linked immunosorbent assay (ELISA). To obtain salivary samples, subjects rinsed their month with tap water and tilted their head forward to pool saliva in the mouth for 5 minutes without swallowing. During the 5 minutes, the overflowing saliva was collected in a sterile cup held under the mouth, and finally the remaining saliva was spat into the same cup. Saliva samples were stored on ice immediately, transported to the laboratory and centrifugated at 4ºC, 13,000 rmp/min for 15 minutes. Supernatants were stored at −70ºC for assay. IL-1β and IL-6 were tested by ELISA according to the manufacturer's protocol. The data were read at 450 nm with wavelength correction to 570 nm (Bio-Rad Model 450, Bio-Rad, Hercules, CA, USA).

Statistical analysis

All data were entered in a database and were double-checked to avoid any errors. Continuous variables were presented as mean and standard deviation (SD) for normally distributed data or median and range for abnormally distributed data. A Mann-Whitney U test was used to compare periodontal parameters and serum parameters. Log base 10 transformation was used to transform levels of IL-1β, IL-6 and CRP, because these three indices were not normally distributed. One-way analysis of variance (ANOVA) was used to compare the difference in IL-1β, IL-6 and CRP among the different groups and the least significant difference (LSD) method was used for post hoc tests. Logistic regression analysis was used to analyse the association of the investigated parameters with MS combined with moderate/severe periodontitis. Variables were entered into the multivariate model if they were significant in univariate analysis ($P < 0.05$) or if they were variables of interest. The software package SPSS (SPSS 17.0, standard version, SPSS, Chicago, IL, USA) was used for the analyses.

Results

Demographic data, risk factors, MS and periodontal parameters

There was no significant difference in age or education level between the MS and control groups. The percentage of men and smokers in the control group was significantly higher than in the MS group. BMI and serum TG and FPG levels in the MS group were all significantly higher, while HDL levels were lower than in the control group (Table 1).

As shown in Table 2, PI was statistically significantly higher in the MS group than in the control group. BI and PD, AL, missing teeth and the percentage of sites with AL ≥ 3 mm and PD ≥ 5 mm were higher in MS than in the control group, but not statistically significant.

Table 1 Demographic variables and risk factors in all subjects.

Variable		MS (n = 114)	Control (n = 49)	P value
Age (y), mean ± SD (range)		60.36 ± 8.65 (37–78)	60.37 ± 10.43 (42–78)	0.952
Sex	Female	81	22	0.002
	Male	33	27	
Education (y), n (%)	≤ 9	66 (57.9)	28 (57.1)	0.629
	10–12	31 (27.2)	11 (22.5)	
	≥ 13	17 (15)	10 (20.4)	
Current smoker, n (%)	Yes	13 (11.4)	12 (24.5)	0.034
	No	101 (88.6)	37 (75.5)	
BMI (kg/m^2; mean ± SD)		26.64 ± 2.96	22.50 ± 2.22	< 0.001
Triglycerides (mmol/l, mean ± SD)		3.31 ± 2.26	1.06 ± 0.29	< 0.001
HDL (mmol/l, mean ± SD)		1.28 ± 0.28	1.55 ± 0.28	< 0.001
FPG (mmol/l, mean ± SD)		8.49 ± 3.45	5.09 ± 0.36	< 0.001

Serum CRP levels and salivary IL-1β and IL-6 levels in different general conditions

Serum CRP levels were significantly higher in the MS group than in the control group ($P < 0.001$). Salivary IL-1β and IL-6 levels were higher in the MS group than in the control group, but not statistically significant (Table 3).

Biomarkers in MS with different components

According to the definition of MS, the five components are central obesity, high TG levels, reduced HDL cholesterol levels, elevated BP and elevated FPG or type 2 diabetes. In MS patients, serum CRP levels increased as the number of components increased and were significantly lowest in the 0-component group (control), and highest in the 5-component group ($P < 0.001$). The levels of salivary IL-1β and IL-6 did not change significantly as the number of MS components increased (Table 4).

Biomarkers in different general and periodontal conditions

Subjects were classified into four groups according to general and periodontal condition:

1. Individuals who were generally healthy with no or mild periodontitis (n = 18);
2. Patients with moderate or severe periodontitis but who were generally healthy (n = 31);
3. Patients with no or mild periodontitis but MS (n = 28);
4. Patients with moderate or severe periodontitis and MS (n = 86).

Table 2 Periodontal variables in different groups (mean ± SD).

Variables		MS (n = 114)	Control (n = 49)	P value
PI		1.92 ± 0.48	1.56 ± 0.49	< 0.001
BI		2.14 ± 0.61	2.01 ± 0.50	0.534
PD (mm)		2.87 ± 0.86	2.66 ± 0.68	0.420
Sites with PD ≥ 5 mm		16.21 ± 14.85	14.20 ± 12.99	0.638
AL (mm)		3.03 ± 1.94	2.58 ± 1.86	0.143
Missing teeth (n)		4.91 ± 6.08	4.14 ± 5.71	0.283
Residual root/crown (n)		0.80 ± 1.76	1.04 ± 2.19	0.194
Sites with AL ≥ 3 mm, n (%)	0 (no periodontitis)	2 (1.8)	4 (8.1)	0.143
	> 0 to 33% (mild periodontitis)	26 (22.8)	14 (28.6)	
	> 33% to 67% (moderate periodontitis)	41 (36)	17 (30.7)	
	> 67% to 100% (severe periodontitis)	45 (39.5)	14 (28.6)	

Table 3 Levels of cytokines between MS and systemic healthy subjects.

Variables	MS (n = 114), median (range), mean ± SD	Control (n = 49), median (range), mean ± SD	P value
sCRP (mg/l)	2.56 (0.44–16.15), 3.45 ± 3.03	0.81 (0.04–3.77), 0.92 ± 0.80	< 0.001
IL-1β (pg/ml)	631.77 (21.07–1557.59), 656.58 ± 351.31	488.06 (34.09–1533.59), 567.89 ± 315.01	0.316
IL-6 (pg/ml)	6.41 (0.28–290.83), 16.82 ± 32.58	5.41 (0.45–89.46), 10.84 ± 14.94	0.398

sCRP, C-reactive protein in serum

Table 4 Cytokine variables by metabolic components.

Variables	0 components (n = 49), median (range), mean ± SD	3 components (n = 41), median (range), mean ± SD	4 components (n = 43), median (range), mean ± SD	5 components (n = 30), median (range), mean ± SD	P value
sCRP (mg/l)	0.81* (0.04–3.77), 0.92 ± 0.80	1.83 (0.44–14.16), 3.07 ± 2.89	2.25 (0.48–9.96), 3.00 ± 2.31	3.05† (0.65–16.15), 4.62 ± 3.81	< 0.001
IL-1β (g/ml)	490.68 (34.09–1533.59), 567.89 ± 315.01	532.47 (21.07–1493.24), 587.62 ± 348.41	635.28 (38.11–1557.59), 658.85 ± 350.42	758.18 (104.69–1323.20), 747.56 ± 346.89	0.21
IL-6 (pg/ml)	5.63 (0.45–89.46), 10.84 ± 14.94	6.90 (0.28–290.83), 20.73 ± 46.90	7.48 (0.54–106.60), 15.26 ± 23.16	5.29 (0.54–57.61), 13.71 ± 17.06	0.81

*, significant difference between 0 components and other 3 groups, P < 0.05
†, significant difference between 5 components and other 3 groups, P < 0.05

Salivary IL-6 and serum CRP levels were significantly different among the four groups. However, no difference was observed in salivary IL-1β concentration among the four groups. Salivary IL-6 levels were highest in MS patients with moderate or severe periodontitis. Serum CRP levels in the generally healthy groups were significantly lower than those in the MS groups, irrespective of the periodontal conditions. As shown in Table 5, whether in the general healthy group or in the MS group, serum CRP levels were not significantly different between patients with different periodontal conditions.

Multivariate analysis

In the 163 patients, after adjustment for age, sex, smoking habits, PI, BI and PD, the relationship between biomarkers and MS combined with moderate/severe periodontitis was observed by logistic regression (backward). The adjusted odds ratio (OR) of serum CRP for MS combined with moderate/severe periodontitis was 1.21 (95% confidence interval [CI] 1.04–1.39), and salivary IL-6 and IL-1β were not risk indicators for MS combined with moderate/severe periodontitis (Table 6). Meanwhile, in 77 subjects in the control group with no or mild periodontitis or moderate or severe periodontitis and in the MS group with no or mild periodontitis combined with no or mild periodontitis (Table 5), the adjusted OR of serum CRP levels for MS combined with no/mild periodontitis compared to general healthy subjects was 5.00 (95% CI 2.27–11.03, $P < 0.0001$) and salivary IL-6 and IL-1β were still not risk indicators.

Discussion

In the present study, serum CRP levels were higher in the MS group (Table 3) and highest in MS with five metabolic components (Table 4); these results were similar to a previous study[3] in which the age-adjusted prevalence of an elevated CRP concentration was 29.0% for participants with MS and 12.1% for participants without MS compared with participants with normal values in any of the five components. The age-adjusted ORs were 1.91, 3.00, 5.01, 5.97 and 6.79 for participants with 1, 2, 3, 4 and 5 metabolic abnormalities, respectively. This supported the fact that the MS population suffer from low-grade inflammation and that CRP is closely connected to MS components. In the present study, no difference was found in the serum CRP levels in patients with different periodontal conditions in the generally healthy group (Table 5). Even in the multivariate analysis, serum CRP levels were not a risk indicator for periodontitis (data not shown), which was not coincident with some previous studies[6,7]. Yamazaki et al[15] and Ide et al[16] also showed that serum CRP levels did not change significantly in patients with periodontitis who had received periodontal treatment. The possible reason for this diversity was investigated by Singer et al[17], who found that oxidative stress and the serum immunoglobulin G (IgG) response appear to function in opposing directions to modify serum CRP levels and the association with periodontitis, while individuals with increased serum IgG antibodies to plaque bacteria exhibit lower serum CRP levels. Thanakun et al[18] also supported this negative correlation between serum IgG antibodies and serum CRP levels in a Thai population with MS and periodontitis. Feng et al[19] showed that in Chinese chronic periodontitis patients, serum IgG antibody levels against Aggregatibacter actinomycetemcomitans were significantly higher than in a control group. Increased IgG antibody levels may be due to lower CRP levels, so no difference was observed between periodontitis groups and groups with good periodontal health, whether in MS patients or controls. A multivariate analysis showed, after adjustment for sex, age, smoking habits and PI, that the OR of serum CRP levels for MS combined with moderate/severe periodontitis was 1.21, which suggested that serum CRP levels were a risk indicator for MS patients combined with periodontal inflammation.

Table 5 Cytokine variables by general and periodontal condition.

Variables	Control, median (range), mean ± SD		MS, median (range), mean ± SD		P value
	No or mild periodontitis (n = 18)	Moderate or severe periodontitis (n = 31)	No or mild periodontitis (n = 28)	Moderate or severe periodontitis (n = 86)	
IL-1β (pg/ml)	518.57 (34.09–1533.59), 574.14 ± 365.56	490.68 (196.02–1386.11), 564.26 ± 288.12	672.25 (141.45–1493.24), 697.06 ± 331.80	632.67 (21.07–1557.59), 643.39 ± 358.31	0.47
IL-6 (pg/ml)	4.68* (0.45–22.78), 5.96 ± 5.54	7.48 (1.30–89.46), 13.67 ± 17.80	3.68* (0.54–50.63), 9.81 ± 12.94	7.52 (0.28–290.83), 19.10 ± 36.56	0.05
sCRP (mg/l)	0.98*# (0.04–3.77), 0.98 ± 0.82	0.66*# (0.08–3.62), 0.88 ± 0.80	2.68 (0.44–15.72), 3.54 ± 3.09	2.50 (0.48–16.15), 3.42 ± 3.02	< 0.001

* compared to moderate or severe periodontitis MS group, P < 0.05
\# compared to no or mild periodontitis MS group, P < 0.05

Some advantages of observing salivary biomarkers when studying periodontal medicine include the fact that whole saliva represents a pooled sample from all periodontal sites and oral mucosa, offering a way of assessing subject-level dentition status; that the salivary level of biomarkers may reflect current disease activity as well as severity; and that collection of whole saliva is easy, noninvasive and rapid and requires no special equipment or expertise. Some previous studies showed that salivary IL-6 can reflect the inflammation of both the blood and oral cavity[9,20]. In MS, hyperglycaemia induces nonenzymatic glycation of proteins and the resultant advanced glycation end products are known to stimulate macrophages to express cytokine IL-6. IL-6 induces the secretion of acute phase reactants from the liver, which are implicated in the inflammatory process related to the pathogenesis of cardiovascular disease. IL-6 is a multifunctional cytokine that also contributes to the terminal differentiation of B-lymphocytes to plasma cells and stimulates the secretion of IgA and IgG[21]. Particularly significantly, IL-6 can induce bone resorption, both by itself and in conjunction with other bone-resorbing agents[22]. In the present study, MS patients with moderate/severe periodontitis showed higher levels of salivary IL-6 than MS patients with no/mild periodontitis (Table 5), while in multivariate analysis, after adjusting for confounding factors, salivary IL-6 levels were not a risk indicator (Table 6). Torumtay et al[23] found that salivary IL-6 levels were always significantly higher in patients with MS and periodontitis than in patients who were systemically healthy but had periodontitis at baseline and 3 or 6 months after nonsurgical periodontal treatment. Salivary IL-6 levels have also been shown to increase significantly in patients with calculus-associated chronic periodontitis compared to healthy controls[9].

As some studies had shown that ulcers[24] and lichen planus[25] also contributed to higher salivary cytokine levels, patients with diagnosed mucosal diseases and salivary gland disease were not included in this study. The high concentration of salivary cytokines may be due to cytokines with high serum levels and basement membrane abnormalities in salivary glands in MS patients[26], or perhaps due to the low salivary flow rate in MS patients[27].

Table 6 Association of biomarkers and MS combined with moderate/severe periodontitis in a logistic model (n =163).

Variables	OR	95% CI	P value
sCRP	1.21	1.04–1.39	0.012
PI	14.69	5.56–38.84	< 0.001
Sex (female/male)	2.95	1.30–6.68	0.01

Conclusion

In conclusion, MS patients were burdened with high levels of serum CRP. Serum CRP might be a potential candidate as a valuable biomarker to detect inflammation in MS patients with severe periodontal disease

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Peng LI collected the data and wrote the manuscript; Dr Zhi Bin CHEN directed the experiments in the lab; Drs Lu HE and Qing Xian LUAN revised the manuscript.

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