Correlation of Serum and Salivary Cytokines Level With Clinical Parameters in Metabolic Syndrome With Periodontitis

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Background: Metabolic Syndrome (MS) and chronic oral condition (periodontitis [PD]) are state of inflammation. The study was conducted to determine alterations in serum and salivary cytokines level in MS and/or chronic PD in the North Indian population. **Materials and Methods:** This cross-sectional study carried out in northern part of India. The study subjects of similar ethnicity were recruited according to International Diabetes Federation (IDF) criteria for MS, while chronic PD was diagnosed on the basis of packet depth and clinical attachment level. ELISA method was employed to assess cytokine level. All subjects were divided in four groups Gr A (MS + PD), B (MS), C (PD), and a control Gr D. **Results:**

The serum and salivary tumor necrosis factor alpha (TNF- α) level in Gr A, B, and C was significantly higher than Gr D ($P < 0.05$). Serum interleukin-10 (IL-10) level in Gr A, B, and C was lower than Gr D ($P < 0.05$), but this difference was not significant between Gr C and Gr D. Serum IL-10 level in Gr A was significantly lower than Gr C ($P < 0.05$). Salivary IL-10 level was not significantly altered in any group. **Conclusions:** Proinflammatory marker TNF- α has correlation with clinical parameters in patients of MS having PD. The study suggests level of salivary TNF- α may be utilized as a surrogate marker of MS and PD. J. Clin. Lab. Anal. 30:649–655, 2016. © 2016 Wiley Periodicals, Inc.

Key words: TNF- α ; IL-10; salivary cytokine level; metabolic syndrome; periodontitis

INTRODUCTION

Metabolic Syndrome (MS) is a global problem and has an alarming prevalence among populations. It prompts the individuals to a high risk for the development of type 2 diabetes mellitus (T2DM) and cardiovascular events (1). It is well established that MS is coupled with a proinflammatory state. The evidence lies in the presence of elevated concentrations of various inflammatory molecules and cytokines, along with a concomitant decrease in anti-inflammatory molecules (2). Periodontal diseases include a group of inflammatory diseases that affect the periodontal supporting tissues of teeth and encompass destructive and nondestructive diseases (3). Periodontal diseases are multifactorial in nature. Because of this multiplicity of factors, periodontal disease presents different degrees of progression, from the appearance of initial alterations to the periodontal tissues (such as gingivitis) to various severity levels of periodontitis (PD). Periodontal pathogens

affect the immune system and promote local and systemic inflammatory responses (4).

Both MS and PD are associated with inflammation, one manifest systemically and other locally. Both the diseases are a risk factor for atherogenic cardiovascular disease because of associated inflammation (5). Metabolic disorders directly related to the obesity and periodontal diseases, studies reported that MS is associated with PD in Korean population (6). However, the mechanism of association of MS with PD is not clear. The present cross-sectional

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study was undertaken to comparatively evaluate saliva and serum levels of tumor necrosis factor alpha (TNF- α) and interleukin-10 (IL-10) in patients with MS and/or chronic PD in north Indian population. We make an attempt to find relation between two diseases by using pro- and anti-inflammatory markers as tool in north Indian population.

MATERIAL AND METHODS

Study Subjects

For this cross-sectional study, participants were divided into four groups (Gr):

Group A (Gr A), MS with chronic PD ($n = 25$).

Group B (Gr B), MS ($n = 38$).

Group C (Gr C), chronic PD ($n = 34$).

Group D (Gr D), control ($n = 25$).

Participants in the study were enrolled from the outpatient department (OPD) of internal medicine and OPD of periodontics of King George's Medical University. A total 122 participants were enrolled in study during period of August 2012 to June 2013. All the participants in the study were unrelated individuals of similar ethnicity from Lucknow and other adjoining areas of Northern India. Information on demographic features was obtained through personal interview using a standard clinical pro forma. The study was approved by Institutional Ethics Committee (IEC) and an informed consent was obtained from each enrolled participant.

The history regarding the habit of smoking, tobacco chewing, drug taken (currently and past 3 months), demographic characteristics was recorded. BG Prasad scale of socioeconomic status was used (7). Patients diagnosed as MS cases on the basis of diagnostic criteria given by International Diabetes Federation for asian population (8). Healthy controls were recruited from staffs of university and people residing in the vicinity of university campus. All patients attending OPD of internal medicine were examined for waist circumference (WC) and blood pressure (BP). Blood and saliva samples were taken for further laboratory investigations before patients underwent dental examination. After dental examination patients were allotted to respective groups according to diagnosis.

All the patients of chronic PD in OPD of Periodontics were examined for WC and BP and those fulfilling above criteria were called for fasting blood sugar level on the next day. Blood and saliva samples were collected and patients were enrolled in the respective groups.

Diagnosis of Chronic PD

Subjects with two or more tooth sites with pocket depth ≥ 4 mm and clinical attachment level ≥ 4 mm that bleed on probing were categorized as chronic PD (9). Subjects with pocket size < 4 mm and clinical attachment loss (CAL) < 3 mm with no bleeding on probing (BOP) were considered normal control. Similarly controls were examined for WC, BP, Fasting Blood Sugar (FBS), and full mouth dental examination and not having any of these.

Periodontal Clinical Measurements

Clinical periodontal recordings, including plaque index (PI), calculus index (CI), BOP, gingival index (GI), pocket depth in millimeter, CAL in millimeter were performed at all sites on each tooth present, except for the third molars, using a University of North Carolina-15 (UNC 15) periodontal probe (10). CAL was assessed from the cement enamel junction to the base of the probable pocket. BOP (deemed positive if it occurred within 15 sec after periodontal probing) was recorded dichotomously by visual examination.

Biochemical Investigations

In biochemical investigations, we estimated fasting plasma glucose (FPG) in a blood sample without preliminary treatment by using a Cobas C-111 analyzer (Roche, USA) and related kit. Serum lipid profile was also investigated with estimation of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) by using a Cobas C-111 analyzer (Roche) and a related kit.

Quantification of Cytokines

Both TNF- α and IL-10 were measured in serum and saliva samples by using sandwich ELISA method. Cytokines were measured using the human sensitive cytokine antibody kit (Diaclone, France) for serum and saliva (Invitrogen, USA).

Statistical Analysis

Data were summarized as mean $\pm$ SD. The sociodemographic characteristics of two groups were compared by chi-square (χ^2) test. Descriptive statistics such as mean, standard deviation (SD) for continuous variables, and frequency percentage for categorical variables were determined. Fisher's test, Student's t -test for independent sample, and one-way ANOVA with Games-Howell post hoc test were used to show the relation between independent and dependent variables. The level of significance was

TABLE 1. Frequency Distribution of Sociodemographic Characteristic

Characteristic		MS + PD (Gr A)	MS (Gr B)	PD (Gr C)	Control (Gr D)	Total	P-value
Age (years)	25–35	(4.0)	15.8	17.0	36.0	18.0	>0.05
	36–45	31.6	31.6	58.8	32.0	39.3	
	46–55	40	34.2	23.5	20.0	29.5	
	56–65	24.0	18.4	0	12.0	13.1	
Gender	Male	60	55.3	50	56	54.91	0.895
	Female	40	44.7	50	44	45.09	
Education	Up to 8th	52	52.6	52.9	28	47.54	<0.05
	9th to 12th	28	34.2	38.2	16	30.33	
	Graduation and above	20	13.2	8.8	56	22.13	
Socioeconomic class	I	0	0	5.9	44.0	10.7	<0.05
	II	40	42.1	8.8	28	18.0	
	III	28	10.5	38.2	24.0	29.6	
	IV	24	34.2	5.9	4.0	24.5	
	V	8	13.2	41.2	0	17.2	
Brushing per day	Once	100	81.6	91.2	84	88.52	0.12
	Twice	0	18.4	8.8	16	11.48	

All values represented in percentage (%).

MS, metabolic syndrome; PD, periodontitis.

set at <0.05. Data were analyzed using the SPSS, version 17.0.

RESULTS

Sociodemographic Characteristics

There were more male (54.91%) participants than female (45.09%). Highest frequency of subjects was belonging to 36–45 years age group ($P < 0.05$). Most of the subjects were belonging to lower education (up to eighth standard) group ($P < 0.05$) and socioeconomic class III and IV (BG Prasad scale) that is middle and lower middle class ($P < 0.001$; Table 1).

Profiling of MS Risk Factors

Serum HDL was low in Gr A, Gr B, Gr C as compared to Gr D. While mean FPG, TG, WC, BP were increased in Gr A, Gr B, Gr C as compared to Gr D. There was

significant difference in mean FPG and TG of Gr A, Gr B, Gr C with Gr D ($P < 0.05$), also difference was significant among three groups ($P < 0.05$). Difference in mean HDL level among Gr A, Gr B, and Gr C was significant ($P < 0.05$). No significant difference was seen in mean WC, systolic blood pressure (SBP), and diastolic blood pressure (DBP) among Gr A and Gr B, and Gr C and Gr D (Table 2).

Periodontal Clinical Parameters

Mean PI and CI of Gr A, Gr B, Gr C were significantly increased than Gr D ($P < 0.05$) and the difference of mean PI among Gr A, Gr B, and Gr C was also significant ($P < 0.05$). Though mean CI of GrA was higher than Gr C, but the difference was not significant. Mean BOP and GI in Gr A and Gr C were significantly increased than Gr D ($P < 0.05$), while there was no significant difference between Gr A and Gr C in GI cases. Pocket

TABLE 2. Comparison of MS Diagnostic Criteria's Between GROUPS

MS diagnostic criteria	Groups			
	MS + PD (Gr A)	MS (Gr B)	PD (Gr C)	Control (Gr D)
FPG (mg/dl)	127.83 $\pm$ 9.00 ^{1,2}	113.78 $\pm$ 7.54 ^{1,3}	96.39 $\pm$ 5.96 ^{2,3}	92.26 $\pm$ 5.23
TG (mg/dl)	153.63 $\pm$ 15.76 ^{1,2}	143.99 $\pm$ 16.62 ^{1,3}	118.97 $\pm$ 7.85 ^{2,3}	110.56 $\pm$ 11.06
HDL (mg/dl)	43.18 $\pm$ 6.62 ^{1,2}	47.54 $\pm$ 6.77 ^{1,3}	49.03 $\pm$ 4.91 ^{2,3}	49.32 $\pm$ 4.91
WC (cm)	94.84 $\pm$ 8.42	93.61 $\pm$ 5.85	82.91 $\pm$ 6.46	81.72 $\pm$ 6.61
SBP (mmHg)	133 $\pm$ 5.61	134.68 $\pm$ 4.86	126 $\pm$ 8.41	125.36 $\pm$ 3.90
DBP (mmHg)	87.80 $\pm$ 3.82	87.52 $\pm$ 3.09	84.00 $\pm$ 8.70	81.36 $\pm$ 2.87

Superscript 1, 2, and 3 denote their significant difference among individual group.

All values are represented as mean $\pm$ SD, P -value < 0.05 considered significant.

WC, waist circumference; FPG, fasting plasma glucose; TC, total cholesterol.

TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

TABLE 3. Comparison of Periodontal Clinical Parameters Between Groups

Periodontal indices	MS + PD (Gr A)	MS (Gr B)	PD (Gr C)	Control (Gr D)
PI	2.49 ± 0.58 ^{1,3}	1.12 ± 0.20 ^{1,2}	2.17 ± 0.41 ^{2,3}	0.93 ± 0.12
CI	2.31 ± 0.59 ¹	0.99 ± 0.17 ^{1,2}	1.98 ± 0.33 ²	0.84 ± 0.95
BOP	81.26 ± 10.32 ^{1,2}	22.04 ± 5.86 ^{1,2}	74.09 ± 13.61 ^{1,2}	21.08 ± 5.20
GI	2.56 ± 0.38 ¹	1.29 ± 0.14 ^{1,2}	2.47 ± 0.40 ²	1.18 ± 0.08
Pocket depth (mm)	4.60 ± 0.88 ^{1,3}	2.43 ± 0.45 ^{1,2}	4.15 ± 0.52 ^{2,3}	2.40 ± 0.27
CAL (mm)	5.23 ± 0.92 ^{1,3}	2.97 ± 2.74 ^{1,2}	4.76 ± 0.59 ^{2,3}	2.49 ± 0.27

Superscript 1, 2, and 3 denote their significant difference among individual group.

All values represented as mean ± SD, *P*-value < 0.05 considered significant.

PI, plaque index; CI, calculus index; BOP, bleeding on probing; GI, gingival index; CAL, clinical attachment loss.

depth and CAL were significantly increased in Gr A and Gr C than Gr D and Gr B (*P* < 0.05). Both parameters were significantly increased in Gr A than Gr B (*P* < 0.05; Table 3).

Serum and Salivary Cytokine Level

We compared the serum and salivary levels of TNF-α and IL-10 among designated groups. Mean serum and salivary TNF-α level in Gr A, Gr B, and Gr C were significantly increased than Gr D (*P* < 0.05), and difference was also significant among Gr A, Gr B, and Gr C (*P* < 0.05). Mean serum IL-10 level in Gr A, Gr B, and Gr C was lower than Gr D (*P* < 0.05), but the difference was not significant between Gr C and Gr D, while mean serum IL-10 level in Gr A was significantly lower than Gr C (*P* < 0.05). Mean salivary IL-10 level in Gr A, Gr B, and Gr C was lower than Gr D, but difference was not significant (Table 4).

Correlation Analysis

We performed a correlation analysis in Gr A and Gr C to investigate whether any possible association existed between the serum and salivary cytokine levels with periodontal parameters. In both the groups, serum and salivary IL-10 levels have negative correlation with periodon-

tal parameters, while TNF-α has positive correlation. In Gr A, serum TNF-α with PI while serum and salivary IL-10 level with pocket depth was not significantly associated (Table 5).

We have analyzed Gr A and Gr B to find out if any association occurs between MS diagnostic criteria and cytokine levels. In Gr A, serum and salivary IL-10 levels have negative correlation with all criteria except HDL, which is positive. TNF-α has positive correlation with all the criteria except HDL, which is negative. While in Gr B, TNF-α was associated with FPG, TG, and HDL, while IL-10 was associated with TG (Table 6).

DISCUSSION

There was male preponderance in each group except for PD group. Out of five metabolic criteria, the mean level of FPG, TG, and HDL were significantly increased in patients with Gr A as compared to patients of Gr B and Gr C alone. Similar results were found in other studies where patients having PD with MS were compared with those who had PD only (11). Early reports suggested that subjects with PD exhibited lower HDL cholesterol, higher low-density lipoprotein (LDL) cholesterol, and increased nonfasting serum glucose levels compared to controls. Similarly, other studies also found an association of PD with diabetes (12) and obesity (13, 14). There was no

TABLE 4. Comparison of Level of Inflammatory Cytokines Between GROUPS

Inflammatory markers (pg/ml)	Groups			
	MS + PD (Gr A)	MS (Gr B)	PD (Gr C)	Control (Gr D)
Proinflammatory				
Serum TNF-α	26.92 ± 8.1 ^{1,3}	20.58 ± 5.73 ^{2,3}	6.39 ± 2.54 ^{1,2}	5.58 ± 1.53
Salivary TNF-α	3.25 ± 0.85 ^{1,3}	1.98 ± 0.61 ^{1,2}	2.67 ± 0.85 ^{2,3}	1.66 ± 0.38
Anti-inflammatory				
Serum IL-10	5.68 ± 1.77 ¹	7.01 ± 3.42	8.03 ± 3.45 ¹	11.49 ± 7.78
Salivary IL-10	1.49 ± 0.77	3.02 ± 2.59	1.99 ± 1.39	3.10 ± 3.63

Superscript 1, 2, and 3 denote their significant difference among individual group.

All values represented as mean ± SD, *P*-value < 0.05 considered significant.

TNF-α, tumor necrosis factor alpha; IL10, interleukin 10.

TABLE 5. Correlation of Inflammatory Markers With Clinical Periodontal Parameters

Periodontal indices	MS + PD (Gr A)				PD (Gr C)			
	TNF- α		IL-10		TNF- α		IL-10	
	Serum	Saliva	Serum	Saliva	Serum	Saliva	Serum	Saliva
PI	0.250	0.814**	-0.601*	-0.513*	0.898**	0.951**	-0.785**	-0.842**
CI	0.455*	0.702**	-0.945**	-0.347	0.840**	0.909**	-0.827**	-0.909**
BOP	0.500*	0.596**	-0.261	-0.630**	0.748**	0.846**	-0.898**	-0.981**
GI	0.569**	0.588**	-0.781**	-0.557**	0.946**	0.972**	-0.811**	-0.842**
Pocket depth	0.483*	0.618**	-0.305	-0.321	0.913**	0.966**	-0.851**	-0.894**
CAL	0.646**	0.516**	-0.566**	-0.166	0.893**	0.922**	-0.834**	-0.864**

*Correlation is significant at 0.05 level.

**Correlation is significant at 0.01 level.

PI, plaque index; CI, calculus index; BOP, bleeding on probing; GI, gingival index; CAL, clinical attachment loss.

significant difference in BP of Gr A and Gr B, which was quite same as some studies in which three of five components of MS, except for abdominal obesity and high BP, showed higher odds ratios (ORs) for PD (15). So, BP and WC were found not to be associated in our study as well as others. But this is not in agreement with the INVEST study where they evaluated periodontal infection for cardiovascular risk and found gingival bleeding (a marker of periodontal inflammation) was significantly associated with high systolic BP and increase the probability of hypertension (16). Despite WC is central criteria for diagnosis, this component of MS is not significantly associated with PD in cohort studies (17). As discussed earlier, dyslipidemia caused by increased abdominal fat is responsible for inflammation in MS, this significant difference in lipid profile in Gr A and Gr B indicates that associated PD may cause poor control of dyslipidemia. This fact is supported by previous studies evaluating the effect of periodontal therapy in patients with MS, showing significant modulation of serum triglyceride and HDL-C (18).

Periodontal clinical parameters in patients of Gr A were found to be significantly increased than patients of Gr B and Gr C alone. PI, BOP, pocket depth, and CAL were the parameters significantly increased. This shows that associated coexistence of MS increases the severity of PD. It has been shown in earlier studies that patients with MS have abnormal clinical periodontal parameters as compared to those who do not have MS (19, 20). On the other hand, studies also show that chronic generalized PD in patients with MS was characterized by an active and aggressive course (11).

Serum and salivary concentrations of TNF- α were significantly higher in Gr A as compared to Gr B and Gr C, while lower serum concentration of IL-10 in Gr A was in agreement with previous studies (21, 22). It has been reported in many studies that serum TNF- α levels were increased in PD and in MS (23–25). MS and T2DM result from sustained hyperglycemia. The expression of the proinflammatory mediators implicated in hyperglycemia (IL-6, TNF- α , and CRP) was reported to be associated with oral infection and increased risk for cardiovascular

TABLE 6. Correlation of Inflammatory Markers With MS Diagnostic Criteria

MS diagnostic criteria's	MS + PD (Gr A)				MS (Gr B)			
	TNF- α		IL-10		TNF- α		IL-10	
	Serum	Saliva	Serum	Saliva	Serum	Saliva	Serum	Saliva
WC	0.407	0.469*	-0.201	-0.062	0.001	0.046	0.182	0.238
FPG	0.192	0.467*	-0.048	0.027	0.477*	0.433*	-0.236	-0.120
TG	0.155	0.588**	-0.505*	-0.317	0.606**	0.494**	-0.433*	-0.331*
HDL	-0.301	-0.513**	0.414*	0.011	-0.533**	-0.490**	0.277	0.136
SBP	0.422*	0.453*	-0.525**	-0.198	-0.093	-0.013	0.090	0.057
DBP	0.476*	0.552**	-0.511**	-0.332	0.083	0.095	0.004	0.126

*Correlation is significant at 0.05 level.

**Correlation is significant at 0.01 level.

WC, waist circumference; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

disease among persons who had periodontal disease (26). This increased level in serum and saliva shows that inflammation in systemic and oral compartments is correlated. This assumption is supported by some studies that concluded that in chronic PD level of TNF- α are sufficiently high in GCF (gingival crevicular fluid) to be dumped systemically falling within detectable range of biological serum assays (27, 28). Alternately, serum level of TNF- α were found to be decreased in chronic PD patients after periodontal therapy (29). On the other side, IL-10 levels decreased in patients of Gr A as compared to patients of Gr B and Gr C alone. The decrease in the IL-10 level was not significant in serum as well as in saliva and similar result was reported in other study as well (30). Serum and salivary level of TNF- α increased consistently with increasing number of MS criteria and peaked at presence of all five criteria. Serum IL-10 level decreased with the number of criteria, but salivary levels were inconsistent, suggesting either reliability of salivary IL-10 is questionable or anti-inflammatory process in serum and saliva has limitation to handle increased TNF- α . This notion is supported by studies in which inhibitory effect of IL-10 was observed in the ex vivo study (31) and found TNF- α converting enzyme accumulates even in the presence of IL-10. In the present study, clinical periodontal parameters are significantly increased in patients with MS with PD as compared to patients with PD alone. There are involvement of complex inflammatory and immune responses in the progression of periodontal disease along with MS. The most common metabolic factor, obesity, may have deleterious consequences on the host response, leading to alterations in T cells and monocytes/macrophages and increased cytokine production (32), all of which would contribute to PD. The inflammatory cytokines, abnormally altered in PD having MS, mostly originate from the activated macrophages infiltrating the adipose tissue or related to glucose abnormality (33).

In conclusion, this is the first study performed in population from north India. In the present study, most of the PD patients belong to low socioeconomic class and more than half of them had poor education. The study explores obesity (WC) and high FPG were most common screening criteria for metabolic abnormality. The study reinforces increased metabolic abnormalities as well as periodontal symptoms to be poorly controlled in patients of MS with PD as compared to MS and PD alone. Level of serum and salivary TNF- α increased with increasing number of metabolic abnormality in patients of MS as well as in patient of MS and/or PD. Salivary cytokines (pro- and anti-inflammatory) reflected similar changes (increased or decreased) as were in participants' serum, but was not statistically significant in case of salivary IL-10. Future investigations including larger cohorts of subjects along with the inclusion of subjects from other regions, as well

as studies in other populations, are needed to substantiate these findings.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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