Hussein SH. Ridha et al /J. Pharm. Sci. & Res. Vol. 10(11), 2018, 2998-3000

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Assessment of some immunological biomarkers in saliva and serum of Iraqi patients with chronic periodontitis disease

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Abstract

Chronic periodontal diseases (CPDs) are serious chronic infections that involve destruction of the tooth-supporting apparatus and can result in tooth loss if left untreated, and chemokines and growth factors have been suggested to have a role in its pathogenesis. A total of 64 patients with CPD (30 mild, 26 moderate and 8 severe) and 24 healthy control were enrolled in the study. Their age mean was 40.53±2.22, 39.30±2.05, 40.50±2.87 and 40.58±2.30 years, respectively. Saliva and venous blood samples were collected and ELISA method was employed to assess levels of monocyte chemoattractant protein-1(MCP-1/CCL2), macrophage inflammatory protein-1 alpha (MIP-1α/CCL3) and Connective tissue growth factor (CTGF/CCN2). As compared control, the levels of the three biomarkers were significantly increased in saliva and serum of patients, and the highest increase was observed in the severe group of patients. Moreover, the levels in saliva were significantly higher than those of serum. In conclusion, MCP-1, MIP-1α and CTGF are suggested to be important saliva biomarkers of CPD, especially severe cases.

Key words: Saliva, serum, MCP-1, MIP-1α, CTGF, Periodontitis.

INTRODUCTION

Chronic periodontal diseases (CPDs) are serious chronic bacterial infections that involve destruction of the tooth-supporting apparatus and can result in tooth loss if left untreated. It is caused by hereditary factors, in addition to pathogenic bacterial factors (1). As well as there are many factors related to the appearance and evolution of periodontitis such as age, living standards, frequency of dental monitoring, hormone change in females, and systemic diseases, which all can decrease host immune functions and result in increased susceptibility to disease (2,3). Therefore, CPD is a multi-factorial complex disease (4).

The identification of periodontitis is based on clinical findings including the presence and extent of periodontal pockets, loss of clinical attachment, pattern and extent of alveolar bone loss, or a combination of these findings (5). The most specific organisms that are associated with CPDs include *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Treponema denticola* (6,7,8). The bacterial challenge induces the production of cytokines, chemokines and growth factors by the gingival epithelium, resulting in the expression of adhesion molecules, increased permeability of gingival capillaries and chemotaxis of polymorphonuclear neutrophils through the junctional epithelium and into the gingival sulcus (9). If this process continues, the inflammation extends deep into the tissues and causes loss of supporting connective tissue and alveolar bone (10), in which cytokines, chemokines and growth factors play important role (11).

They contribute to inflammation-induced bone resorption because they can stimulate one or more steps of bone resorption, including the recruitment, differentiation, or fusion of precursor cells to form osteoclasts or enhance osteoclast survival. They also affect periodontal bone loss by their role in recruiting cells, such as neutrophils, which protect against bacterial invasion (12). Therefore, any change in the profile of cytokines, chemokines and growth factors signaling can have detrimental effects leading to pathogenic involvement in inflammatory diseases as well as cancer (13).

Accordingly, the present study was planned to examine the role of monocyte chemoattractant protein-1(MCP-1/CCL2), macrophage inflammatory protein-1 alpha (MIP-1α/CCL3) and Connective tissue growth factor (CTGF/CCN2) in pathogenesis of CPD in a sample of Iraqi patients. Their potential as biomarkers of disease in saliva and serum was also evaluated.

SUBJECTS, MATERIALS AND METHODS

Subjects: A total of 64 Iraqi patients of both gender (32 males and 32 females) with CPD and 24 healthy persons (control) were enrolled in the study. The patients attended the Specialized Health Center for Dental Medicine / Al-Kadhimiya (Baghdad) during the period October 2017 - February 2018 for diagnosis and treatment. They were clinically examined and evaluated by the consultant medical staff at the Health Center, and accordingly, they were distributed into three clinical groups (30 mild, 26 moderate and 8 severe cases); probing pocket depth (PPD), clinical attachment level (CAL) and plaque index (PLI) (14). Smokers, pregnant women, patients with chronic diseases or under any type of therapy were excluded.

Serum and saliva samples: Venous blood was collected from participants and left for 15 minutes to clot at room temperature, and then it was centrifuged (3000 rpm for 15 minutes). Saliva samples were also collected under unstimulated conditions by spitting into a sterile universal tube and the samples were placed immediately on ice and before prior to freezing. The separated sera and collected saliva were frozen at -80°C until assessment (15).

***Methods*:** MCP-1, MIP-1α and CTGF were determined in serum and saliva by means of ELISA using commercially available kits (Human MCP-1, MIP-1alpha; Komabiotech and Human CTGF; MyBioSource), and the instructions of manufacturer were followed.

Statistical Analysis: The results were statistically analyzed using SPSS (Statistical Package for Social Sciences) version 13. Their data were given as mean ± standard error (S.E.), and differences between means were assessed by ANOVA (Analysis of Variance), followed by LSD (Least Significant Difference) or Duncan test.

RESULTS AND DISCUSSION

Through studies that showed role of numerous factors like aging, smoking, hormonal changes, life standards and systemic diseases, in the initiation and progression of CPD; patients with these factors were excluded, and only patients that have the inflammation due to bacterial challenge were included. The focus was on the role of MCP-1, MIP-1α and CTGF in progression of periodontitis and if there is a relationship between their levels in saliva and serum.

Age distribution revealed that there was no significant difference between three studied clinical groups of CPD patients and controls, and there was no clear correlation between age and disease severity, and this may be due to the close range ages between groups (Table 1). However, other studies have created a relationship between aging and CPD (16,17), and their results suggested that prevalence and severity of periodontal disease tend to increase with age of patients due to degenerative changes in periodontal tissues that are assumed to be the cause of this condition (18,19).

Table (1): Age distribution in patients with periodontitis and control.

Groups		(Mean±SE; Years)
Chronic periodontal disease	Mild (No.=30)	40.53±2.22[a]
	Moderate (No.=26)	39.30±2.05 [a]
	Severe (No.=8)	40.50±2.87 [a]
Controls (No.=24)		40.58±2.30 [a]

*****Similar letters represent no significant difference (P > 0.05) between means in columns (Duncan test).

As shown in table 2, MCP-1/CCL2 level was significantly ($p \leq 0.001$) increased in saliva (locally production) and serum (systemic production) of the three clinical groups of patients groups as compared to controls especially severe cases (saliva: 891.7±19.9 *vs*. 361.9±60.4 pg/ml; serum: 189.6±34.9 *vs*. 42.8±5.7 pg/ml). This result is in agreement with Gupta *et al*.(20) and Babu *et al*. (21) studies, which revealed that MCP-1 concentration was significantly increase in serum and gingival crevicular fluid (GCF) of patients with chronic periodontitis, while its concentration was decreased after therapy. Furthermore, Zhang *et al.* (22) reported that the expression of MCP-1 on endothelial cells, as well as monocytes/macrophages in inflamed gingival tissues was correlated with the severity of inflammation.

Table (2): MCP-1 level in saliva and serum in patients with periodontitis.

Groups		MCP-1 (Mean±SE; pg/ml)		Probability ≤
		Saliva	**Serum**	
Chronic periodontal disease	Mild (No.=30)	478.3±57.5[c]	127.1±17.1[b]	0.001
	Moderate (No.=26)	672.7±50.9[b]	141.3±18.1[ab]	0.001
	Severe (No.=8)	891.7±19.9[a]	189.6±34.9[a]	0.001
Controls (No.=24)		361.9±60.4[c]	42.8±5.7[c]	0.001

*****Different letters represent significant difference ($P \leq 0.05$) between means in columns, while similar letters represent no significant difference (P > 0.05) between these means (Duncan test).

The same observation was also made for MIP-1α/CCL3 level (saliva: 285.3±24.5 *vs*. 125.4±29.6 pg/ml; serum: 146.0±30.6 *vs.* 100.0±5.4 pg/ml) (Table 3). These findings also agree with Raja *et al*. (23) who revealed that MIP-1α levels were significantly higher in severe periodontitis followed by mild to moderate as compared to controls in saliva and GCF. A further study on MIP-1α role in predicting bone loss recorded elevated levels of MIP-1α, which was considered as a biomarker for bone loss in both saliva and GCF (24).

Table (3): MIP-1α level in saliva and serum in patients with periodontitis.

Groups		MIP-1α (Mean±SE; pg/ml)		Probability ≤
		Saliva	**Serum**	
Chronic periodontal disease	Mild (No.=30)	169.3±22.6[b]	106.8±8.2[b]	0.01
	Moderate (No.=26)	184.9±23.8[b]	115.1±12.9[ab]	0.01
	Severe (No.=8)	285.3±24.5[a]	146.0±30.6[a]	0.01
Controls (No.=24)		125.4±29.6[b]	100.0±5.4[b]	N.S

*****Different letters represent significant difference ($P \leq 0.05$) between means in columns, while similar letters represent no significant difference (P > 0.05) between these means (Duncan test).

In addition to chemokines, a significant increase in the level of CTGF was also observed in CPD patients compared to controls, especially in patients with severe disease (saliva: 3488.7±142.3 *vs.* 1503.2±297.1 pg/ml; serum: 2980.2±156.1 *vs.* 1271.3±213.2) (Table 4). Accordingly, CTGF is a further important mediator of tissue remodeling that stimulates fibroblasts to produce extracellular matrix constituents, and its expression is correlated positively with the degree of gingival fibrosis (25,26). In agreement with such theme, Mize and colleagues (27) showed that both CTGF/CCN2 and TGFβ1 mRNA expression levels were significantly increased in individuals with periodontitis as compared to individuals without periodontitis.

Table (4): CTGF level in saliva and serum in patients with periodontitis.

Groups		CTGF (Mean±SE; pg/ml)		Probability ≤
		Saliva	**Serum**	
Chronic periodontal disease	Mild (No.=30)	2528.6±125.4[a]	1643.4±197.5[b]	0.001
	Moderate (No.=26)	2591.8±107.2[a]	1871.4±174.7[b]	0.001
	Severe (No.=8)	3488.7±142.3[a]	2980.2±156.1[a]	0.001
Controls (No.=24)		1503.2±297.1[b]	1271.3±213.2[b]	N.S

*****Different letters represent significant difference ($P \leq 0.05$) between means in columns, while similar letters represent no significant difference (P > 0.05) between these means (Duncan test).

The results also declared that the level of the three markers showed an evident increase in saliva compared to serum (Tables 2, 3 and 4). In conclusion MCP-1, MIP-1α and CTGF are important saliva biomarkers that are related to CPD and the increase in their levels is a good indicator of the severity of inflammation. Therefore, it is preferable to measure MCP-1, MIP-1α and CTGF levels in saliva (local produced) rather than in serum (systemic produced). Saliva, therefore, can give a better evaluation of the inflammatory condition in gums that are associated with these markers.

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