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ORIGINAL ARTICLE

Gingival levels of monocyte chemoattractant protein-1 (MCP-1) in diabetes mellitus and periodontitis: an experimental study in rats

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Abstract The objectives of this study were to investigate and compare the monocyte chemoattractant protein-1 (MCP-1) levels of gingival tissues in diabetes mellitus (DM) and periodontitis and to reveal the effects of MCP-1 on periodontal inflammation and destruction in these diseases. DM was created in 15 rats (group 1) by streptozotocin injection, and periodontitis was obtained by ligature induction in 15 rats (group 2). Fifteen systemically and periodontally healthy rats were used as control (group 3). Gingival MCP-1 levels were measured by enzyme-linked immunosorbent assay (ELISA). Periodontal inflammation was quantified by the inflammatory cell infiltration in the gingival samples, whereas periodontal destruction was assessed by the alveolar bone loss in the experimental regions. MCP-1 concentrations were higher in groups 1 and 2 than in group 3 ($p<0.001$). Increased gingival inflammatory cell infiltration and alveolar bone loss were observed in groups 1 and 2 compared to group 3 ($p<0.001$). There were positive correlations among the MCP-1 level, gingival inflammatory cell infiltration, and alveolar bone loss in groups 1 and 2 ($p<0.001$). Our results suggest that (1) DM may lead to enhanced MCP-1 production in periodontal tissues likewise for periodontitis and (2) there may be a positive correlation between the MCP-1 concentration and diseased nature of periodontium in both diseases.

Keywords MCP-1 · Diabetes mellitus · Periodontitis · Gingival inflammation · Alveolar bone loss

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Introduction

Monocyte chemoattractant protein-1 (MCP-1) is a C–C group chemokine that has chemotactic activity for lymphocytes and monocytes, and it is considered as the major signal for the chemotaxis of mononuclear leukocytes [2], particularly in a group of diseases such as atherosclerosis, rheumatoid arthritis, and delayed-type hypersensitivity reactions which are characterized with chronic inflammation [23]. MCP-1 can be synthesized by various cells such as leukocytes, fibroblasts, kerotinocytes, and endothelial cells due to different endogenous and exogenous stimuli [22]. Monocyte functions have also been reported to be important for periodontal breakdown in periodontal disease [8]. Enhanced MCP-1 expression in the periodontal tissues of patients with periodontitis was demonstrated in previous studies [4, 6, 7, 9, 13, 15, 17, 30, 34, 35], and endothelial cells, together with mononuclear phagocytes, were reported to be the main source of MCP-1 expression in chronic periodontitis [34, 35]. The relationship between MCP-1 and host response was also suggested for aggressive periodontitis [4, 7]. All these investigations may suggest that MCP-1 concentration may be an important factor that influences the severity of the periodontal disease. However, a detailed and direct effect MCP-1 on tissue inflammation and destruction has not been demonstrated for periodontitis.

Diabetes mellitus (DM) is a metabolic disorder in which chronic inflammatory reactions affect the structure and function of different tissues. There are several studies that investigate the characteristics of host response in DM in various tissues and organs. These studies have mostly revealed that enhanced levels of C–C chemokines were important in the inflammatory aspects of systemical diabetic complications [12, 22]. Increased MCP-1 levels in blood and inflamed tissue were also reported in diabetic patients compared to systemically healthy patients, and an association with the severity of tissue inflammation and destruction was revealed for MCP-1 in DM [22, 28]. Revealing a similar correlation between DM and periodontal tissues (healthy and/or diseased) may facilitate to explain the molecular mechanisms which modify the periodontal disease status in DM. However, although a well-known and strong relationship has been established between DM and periodontitis [6–8, 23, 25, 32, 34], there were no data about the effects of DM on MCP-1 production in periodontal tissues.

In the present study, we aimed to investigate MCP-1 levels in DM and periodontitis and to correlate the MCP-1 production with the diseased nature of periodontal tissues (periodontal inflammation and destruction) in both diseases.

Materials and methods

Our study was carried out at the Ondokuz Mayıs University Medical and Surgical Research Center and approved by the Official Experimentational Committee of this Center. Forty-five male and systemically healthy (aged 9 weeks) Sprague–Dawley rats were used in this study. Fifteen rats were utilized for the DM group (group 1), 15 rats were utilized for the periodontitis group (group 2), and 15 rats were served as the negative control group (group 3). All animals were housed in separated cages (1 animal per cage) at standard temperature and humidity with a 12-h light/dark cycle, and their body weights were recorded before the test procedures. The animals were anesthetized with intraperitoneal ketamine-HCL (60 mg/100 kg) injection for all surgical interventions. Their periodontal health was standardized with daily irrigation of 0.2% chlorhexidine digluconat, and this chlorhexidine digluconat regimen was left before the experimental procedures. Mandibular first molar teeth were included in the study, and commissurotomy was performed to reach to the areas. Only one side of the mandible was used for the experimental procedures to facilitate the alimentation of animals during the study period.

Experimental DM creation

An irreversible DM creation method was utilized as reported in several studies [14]. Blood glucose levels were measured with a glycometer before the procedure and the rats received a single intraperitoneal injection of 70 mg/kg streptozotocin (Sigma-Aldrich Chemie., Taufkirchen, Germany) in 0.2 ml citrate buffer solution (0.1 M, pH 4.5). Differences in the blood glucose levels were compared 3 days after the injections, and the animals were sacrificed 21 days after the DM creation procedure. DM creation was confirmed by the increase in the blood glucose level and the body weight loss as described in the referred studies.

Experimental periodontitis creation

Experimental periodontitis was induced by ligature placement [16]. After flap elevation, a 5.0 silk suture was adapted around the mandibular first molars at the cemento–enamel junction (CE) adjacent to the gingival margin, and then the flap was replaced to its original position. The ligatures were left in their places for a 40-day of induction period. For enhancing the plaque accumulation, the animals were also fed with soft diet during the periodontitis creation. Periodontitis production was assessed by the inflammatory cell infiltration in the gingival biopsies and alveolar bone loss quantification in the experimental sites.

Gingival biopsy collection

The gingival specimens were taken approximately in 3×3 mm^2 dimensions (including whole gingiva) from the vestibular site of the teeth before animal killing at the required days. Each specimen was divided into two samples which were approximately in similar sizes. One sample was stored in 0.25 M sucrose solution at −70°C for MCP-1 detection, whereas the other sample was stored in 10% formalin solution for histopathological assessment.

MCP-1 analysis

The gingival samples were transferred in a phosphate buffer solution (+4°C, pH 7.0). The gingival biopsies were firstly processed to obtain supernatants via homogenization (at 27.845 g, four times, for 30 s, at 10-s intervals), freeze–thawing procedures, ultrasonification (at 5 μm, four times, for 30 s, at 10-s intervals), and centrifugation (at 20.375 g, for 16 min). All these supernatant preparation processes were carried out on ice medium approximately at 0–4°C. The supernatants were then brought to room temperature for enzyme-linked immunosorbent assay (ELISA). Gingival tissue MCP-1 concentrations were analyzed in each 50 μl sample by standard ELISA procedures at 450–550 nm using a commercially available MCP-1 Kit (Endogen Rat MCP-1 ELISA Kit, Endogen, Woburn, MA, USA).

Assessment of inflammatory cell infiltration

Monocyte and lymphocyte infiltrates were calculated as the number of cells in the histological preparations of the gingival biopsies obtained from the study groups. The cell counts were performed in a 45×70 μm^2 area separately, including connective tissue and epithelium by a light microscope transferred to a monitor with a camera apparatus (Panasonic F10 CCD Camera, Objective ×3.3) at ×4,165 magnification.

Assessment of alveolar bone loss

The amount of destruction in periodontium due to experimental DM and ligature-induced periodontitis was measured by the quantification of alveolar bone loss in the experimental regions (mandibular first molars). The mandibles were removed, boiled for 10 min, and all soft tissues were cleaned leaving in 0.2 N NaOH solution at room temperature for 5 min to expose all bone surfaces. CE was utilized as the reference point of measurements, and the jaws were stained with 1% methylene blue solution for adequate identification of CE. Measurements were performed between CE and the upper level of alveolar bone (the vertical distance from the CE to the alveolar bone crest) at 22 certain and different sites in mandibular molar teeth with a stereomicroscope at ×40 magnification.

Statistical assessment

The differences between the study groups were assessed with *Kruskal–Wallis* test for statistical significance. The comparisons between two groups were done with *Mann–Whitney U* test according to the *Bonferonni* adjustment of "*p*" value ($p<0.05/3=0.017$). The correlations among the MCP-1 level, inflammatory cell infiltration, and alveolar bone loss were analyzed by *Spearman's Correlation* test. These calculations were carried out using a statistical Software Package Program (SPSS version12.0, Software Package Programme, Chicago, Illinois, USA).

Results

The blood glucose levels of group 1 increased 3 days after streptozotocin injection (135.8±9.9 mg/dl pre-injection; 290.7±93.9 mg/dl postinjection), and there were body weight losses in the animals of group 1 after 21 days (248.4±93.77 g pre-injection; 236.3±93.54 g postinjection).

MCP-1 increased in groups 1 and 2 after the experimental procedures compared to group 3 (Table 1). Comparison of the differences among the groups revealed statistical significance between groups 1–3 and groups 2–3, but not between groups 1–2 (Table 1). The amount of inflammatory cell infiltration was higher in groups 1 and 2 than in group 3 (Table 1). The differences were found to be significant between groups 1–3, groups 2–3, and groups 1–2 (Table 1). Enhanced alveolar bone loss was observed in groups 1 and 2 unlike group 3 (Table 1). There were statistically significant differences between the alveolar bone losses of groups 1–3 and groups 2–3, but not between groups 1–2.

A strong and positive relationship was noted between the MCP-1 concentration of gingiva and inflammatory cell infiltration in groups 1 ($r=0.930$, $p<0.001$) and 2 ($r=0.956$, $p<0.001$) (Fig. 1a and b), likewise for the MCP-1 concentration of gingiva and alveolar bone loss in groups 1 ($r=0.977$, $p<0.001$) and 2 ($r=0.757$, $p<0.001$) (Fig. 2a and b).

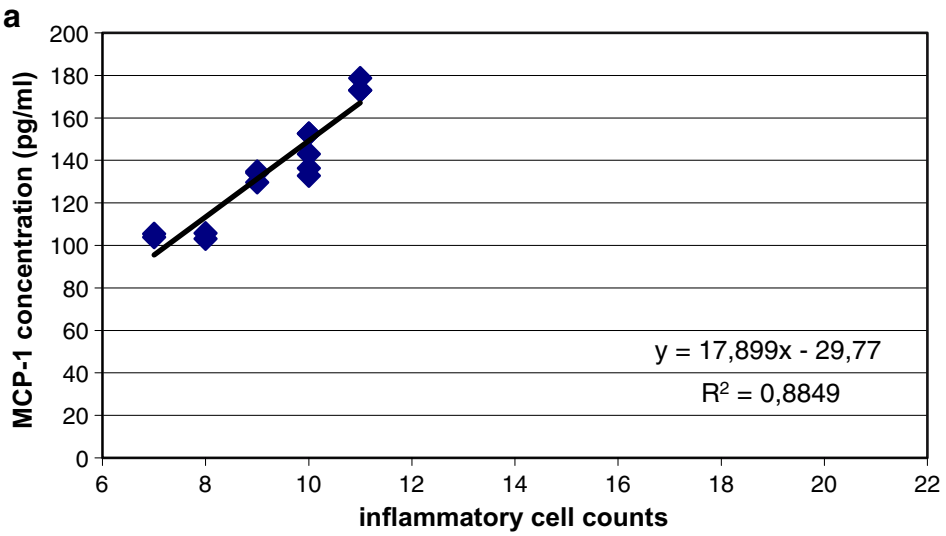
Discussion

There is evidence that in the monocyte/macrophage and lymphocyte recruitment during inflammation, MCP-1 plays an important role to consequently produce the pathogenic state of disease, and DM may trigger this process [22]. A similar role of MCP-1 and its regulatory effects have also been proposed in the inflammatory reactions of periodontal disease [6, 7, 13, 15, 30]. Nevertheless, there are not any studies which investigate the MCP-1 concentration of periodontium in DM alone and compare the MCP-1 levels in DM and periodontitis. Moreover, a possible correlation between the MCP-1 concentration and diseased nature of periodontium

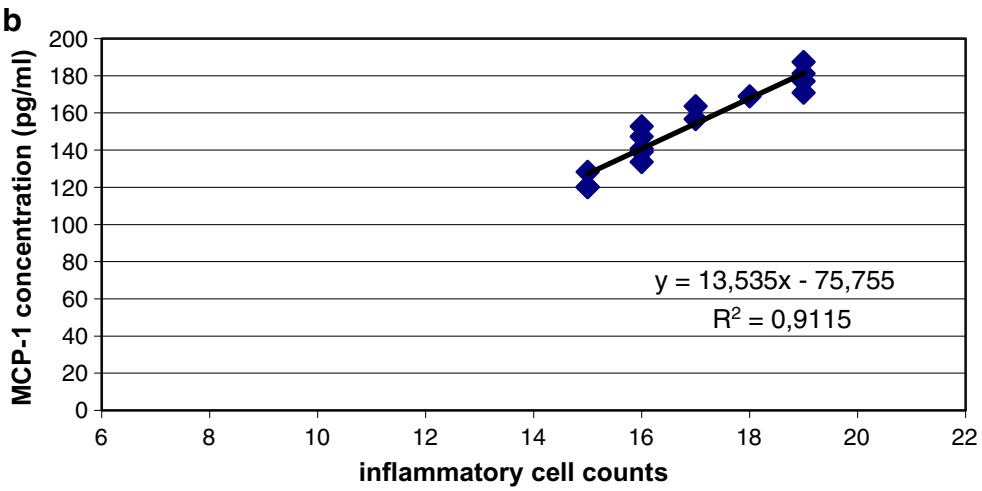
Table 1 Distribution and comparison of the study parameters among the groups

Groups	MCP-1 levels (pg/ml) median (min.–max.)	Inflammatory cell infiltration (cell counts) median (min.–max.)	Alveolar bone loss (mm) median (min.–max.)
Group 1 (DM)	134.8 (103.2–178.8)*	10.0 (7.0–11.0)*·***	0.69 (0.61–1.15)*
Group 2 (periodontitis)	152.8 (120.1–187.4)**	16.0 (15.0–19.0)**·***	0.71 (0.67–0.79)**
Group 3 (healthy)	79.4 (60.9–120.1)*·**	4.0 (2.0–5.0)*·**	0.25 (0.22–0.29)*·**

*Significant difference between groups 1 and 3 ($p=0.000$).
**Significiant difference between groups 2 and 3 ($p=0.000$).
***Significiant differences between groups 1 and 2 ($p=0.000$).



95% confidence interval;
MCP-1 level upper bound:6.682, lower bound:-66.223
Inflammatory cellcounts upper bound:21.768, lower bound:14.031



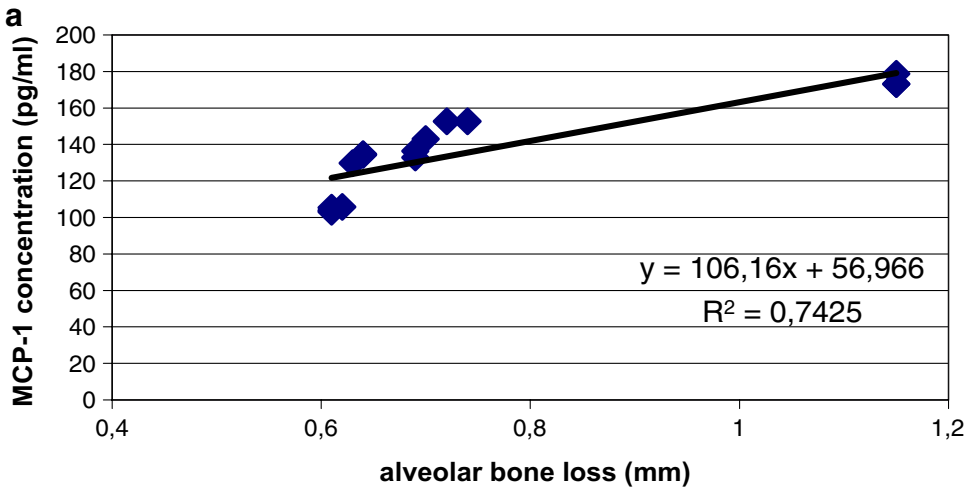
95% confidence interval;
MCP-1 level upper bound:-32.967, lower bound:-118.543
Inflammatory cellcounts upper bound:16.061, lower bound:11.008

Fig. 1 Relationship between the MCP-1 level and inflammatory cell infiltration in **a** group 1 (diabetic group) and **b** group 2 (periodontitis group)

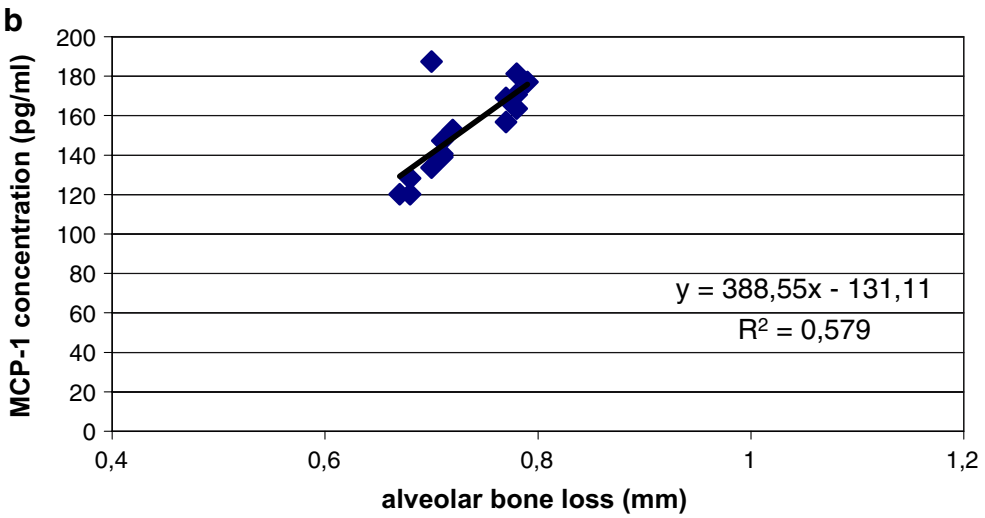
has not yet been revealed in both diseases. This is the first study which attempts to reveal some data about these points.

We utilized periodontally healthy diabetic rats as one of the test groups to compare the study parameters with a systemically healthy periodontitis group and negative control group. The main reasons of such a study design were to investigate the direct influence of diabetic conditions on periodontally healthy tissues and to compare the findings with the peridontitis group almost independently.

Our results demonstrated increased gingival MCP-1 concentration in the diabetic group, in agreement with numerous investigations that also reported increased MCP-1 production of different tissues in DM [12, 22–24, 28]. In general, these reports have proposed that prolonged pathological induction of the involved tissue by DM, activity of the advanced glycation end products, vascular changes, and abnormal immune response due to hyperglycemia may be the main starting points of more MCP-1 production. Furthermore, high glucose concentrations have been related with higher MCP-1 expressions in glomerulosclerosis [27] and atherosclerosis [1], as a result of macrophage recruitment that leads to nephropathy and angiopathy. These explanations suggest that the pathogenic reactions which influence the MCP-1 produc-



95% confidence interval;
MCP-1 level upper bound:86.291, lower bound:27.641
Alveolar bone loss upperbound:143.617, lower bound:68.697



95% confidence interval;
MCP-1 level upper bound:14.047, lower bound:-276.298
Alveolar bone loss upper bound:587.069, lower bound:190.023

Fig. 2 Relationship between the MCP-1 level and alveolar bone loss in **a** group 1 (diabetic group) and **b** group 2 (periodontitis group)

tion in DM may be independent from the reactions in an infective disease such as periodontitis. Thus, DM may have an additive role in MCP-1 production during periodontal disease process.

Enhanced MCP-1 levels were also observed in the periodontitis group of our study, which confirmed the previous investigations about more MCP-1 production in periodontal disease [4, 6, 7, 9, 11, 13, 15, 17, 30, 34, 35]. It has been reported that the amount, location, and duration of the stimulus in the induction of a disease may affect the MCP-1 production, likewise for other chemotaxic cytokines that induce migration of leukocytes in the earlier phases of inflammatory process [13, 15]. In a local infection such as plaque-induced periodontal disease, the stimulus (microbial dental biofilm) may be expected to be more potent than the stimulus in a systemically induced disease such as DM, at least in the models where only the local levels of MCP-1 are studied. However, similar MCP-1 concentrations were observed in our diabetic and periodontitis groups. We suggest that the experimental DM model of the study might have caused such a result due to its irreversible and prolonged diabetic effects on the tissues. Further studies are needed to also investigate the MCP-1 concentration of periodontal tissues in metabolically controlled diabetic conditions.

A possible relationship between the amount of MCP-1 and severity of inflammation has been reported for periodontal disease [6, 7, 30]. Furthermore, a similar

association has been demonstrated for different diabetic tissues and organs [3, 4, 18, 22]. In our study, we performed gingival inflammatory cell counts (monocyte + lymphocyte) to assess the degree of inflammation in DM and periodontitis. Our results demonstrated similarly increased inflammatory cell infiltrates and a positive correlation between MCP-1 and gingival inflammation in both diseases. Although the inflammatory cell infiltrate has been reported to increase in diseased gingival tissues in DM [21, 26], there are limited data for a similar finding in periodontally healthy periodontium. But, in an experimental rat study, Feher et al. [5] have similarly observed increased inflammatory cell infiltration in systemically healthy and diabetic gingival tissues 1 week after streptozotocin injection. These findings suggest that enhanced MCP-1 production might have also been important in DM-induced periodontal inflammation.

We observed enhanced alveolar bone resorption both in DM and periodontitis. Alveolar bone resorption is an inevitable result of periodontitis, and it has also been demonstrated in diabetic conditions [29, 31]. The mechanisms of alveolar bone metabolism and/or resorption in DM have been explained previously [19, 20, 33]. In two experimental studies which also assessed a periodontally healthy diabetic group, Mishima et al. [20] and Villarino et al. [33] suggested that streptozotocin-induced DM might have had short-term effects on bone metabolism and might have reduced the rate of bone turnover in the alveolar wall surrounding the root. In our study, alveolar bone loss was also utilized to assess the relationship between MCP-1 and tissue destruction in DM and periodontitis. It may be speculated that there are still limited data about the functional role of MCP-1 in bone resorption and/or metabolism. However, Graves et al. [10] and Volejnikova et al. [31] have revealed the role of MCP-1 in bone metabolism during tooth eruption in mice.

In conclusion, our results reveal that (1) DM may lead to enhanced MCP-1 production in periodontal tissues, and this may be as much as MCP-1 production in periodontitis, (2) there may be a positive correlation between MCP-1 levels and diseased nature of periodontium by means of tissue inflammation and destruction in both diseases, and (3) thus, MCP-1 may be of importance among the modifying mechanisms of DM on periodontal disease.

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