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Correlation of MCP-4 and high-sensitivity C-reactive protein as a marker of inflammation in obesity and chronic periodontitis

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ABSTRACT

Objectives: Obesity is increasing in prevalence worldwide and has emerged as a strong risk factor for periodontal disease. Conversely, the remote effects of periodontal disease on various systemic diseases have been proposed. The aim of this study is to determine the presence of MCP-4 and high sensitivity C reactive protein (hsCRP) levels in gingival crevicular fluid (GCF) and serum in obese and non-obese subjects with chronic periodontitis and to find a correlation between MCP-4 and hsCRP in GCF and serum.

Materials and methods: Forty subjects (20 males and 20 females) were selected and divided into four groups (10 subjects in each group), based on clinical parameters: group NOH (non-obese healthy), group OH (obese healthy), Group NOCP (non-obese with chronic periodontitis) and group OCP (obese with chronic periodontitis). The levels of serum and GCF MCP-4 were determined by ELISA and hsCRP levels were determined by immunoturbidimetry method.

Results: The mean GCF and serum concentration of MCP-4 was highest for group OCP followed by group NOCP, group OH (in GCF); group OH, group NOCP(in serum) and least in group NOH. The mean hsCRP concentration was highest for group OCP followed by group OH, group NOCP and group NOH. A significant positive correlation was found between serum and GCF MCP-4 and hsCRP levels.

Conclusion: GCF MCP-4 concentrations increased in periodontal disease compared to health and correlated positively with the severity of disease indicating it as a novel marker of periodontal disease. The serum concentration of MCP-4 was found to be more in obese group as compared to nonobese group indicating it as a marker of obesity. Furthermore, based on the positive correlation of MCP-4 and hsCRP found in this study, it can be proposed that MCP-4 and hsCRP may be the markers linking chronic inflammation in obesity and periodontal disease.

1. Introduction

Obesity is increasing worldwide and is becoming the most common health problem not only in developed countries, but also in many developing countries [1]. Obesity is associated with several life threatening diseases including hypertension, cardiovascular disease, and type 2 diabetes [2]. Recently, obesity has emerged as one of the risk factor for periodontal disease and conversely, the remote effects of periodontal disease on various systemic diseases have been proposed [3]. It has been suggested that obesity is second only to smoking as the strongest risk factor for inflammatory periodontal tissue [4].

Periodontal diseases are multifactorial infections elicited by a complex of bacterial species that interact with host tissues and cells causing the release of a broad array of inflammatory cytokines, chemokines, and mediators, some of which lead to destruction of the periodontal structures [5]. The possible causal relationship between obesity and periodontitis and the molecular mechanisms by which obesity may affect the periodontium are not well understood. However, adipose tissue derived cytokines and hormones that are involved in inflammatory processes, point towards similar pathways involved in the pathophysiology of obesity and periodontitis [6].

Chemokines are a large family of chemotactic cytokines and the monocyte chemoattractant proteins-1, -2, -3, and -4 constitute a small subfamily within the CC chemokine group [7–9]. Monocyte chemoattractant protein-4 (MCP-4) is a CC chemokine which is a potent chemoattractant for monocytes and eosinophils, and stimulates histamine release from basophils [10]. It binds to cell surface G-protein linked chemokine receptors such as CC chemokine receptor 3 (CCR3) and induces inflammation and contributes to eosinophil migration [11].

MCP-4 has been implicated in conditions like rheumatoid arthritis, symptomatic carotid atherosclerosis, asthma and renal inflammation [12–15]. Recently a study showed that MCP-4 is a critical molecule that links obesity and chronic inflammation and serum levels of MCP-4 correlated with Body Mass Index (BMI)

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and other obesity related parameters. Furthermore, it was found that hsCRP independently correlated with MCP-4 levels [11]. Expression of MCP-1 and MCP-3 in gingival tissue samples with chronic periodontal disease and chemotactic activity of monocytes in gingival crevicular fluid (GCF) have been investigated in previous studies [9,16]. However till date no study has determined the levels of MCP-4 in GCF and also GCF levels of MCP-4 in obesity.

Previous research suggests that mediators of low grade chronic inflammation, such as cytokines and acute phase reactants, contribute to the development of these co-morbid conditions like obesity, type 2 diabetes and also periodontitis [17,18]. C-reactive protein (CRP) is an acute-phase reactant synthesized by the liver and adipocytes and has been recognized as an important biomarker of a wide spectrum of conditions such as systemic inflammation, osteomyelitis, rheumatoid arthritis and vasculites [19,20]. Recently, studies demonstrated a correlation between periodontitis and elevated serum CRP levels [21,22]. Several lines of evidence suggest that obesity is a low-grade systemic inflammatory condition accompanied by increased levels of circulating CRP and other inflammatory cytokines such as TNFα and IL-6 [23]. Adipocytokines such as TNF-α or IL-6 are generally elevated with increasing adiposity and are closely correlated with CRP [24]. Aside from being used as a marker of inflammation, CRP is now believed to itself have potent proinflammatory properties [25].

Till date, no study has reported levels of MCP-4 and high-sensitivity C-reactive protein (hsCRP) in GCF and serum in subjects with chronic periodontitis among obese and non-obese individuals. Therefore this clinico-biochemical study was undertaken to evaluate the GCF and serum levels of MCP-4 and hsCRP in obese and non-obese subjects with clinically healthy periodontium and chronic periodontitis and to find the correlation between MCP-4 and hsCRP.

2. Materials and methods

The study was conducted from April 2011 to July 2011. The study group consisted of 40, age and gender balanced subjects (25–45 years; gender: 20 males and 20 females) attending the outpatient section, Department of Periodontics, Government Dental College and Research Institute, Bangalore. Written informed consent was obtained from those who agreed to participate voluntarily. Subjects with aggressive periodontitis, diabetes, hypertension, a smoking habit, alcoholics, pregnant and lactating females, gross oral pathology, heart diseases, rheumatoid arthritis, tumors, or any other systemic disease that can alter the course of periodontal disease, or those who had any course of medication affecting periodontal status such as phenytoin, cyclosporins, or calcium channel blockers; or had taken antibiotics, anti-inflammatory drugs (NSAIDS) or had received periodontal therapy in the preceding 6 months were excluded from the study. The ethical clearance was approved by Institutional Ethical Committee and Review Board, Government Dental College and Research Institute, Bangalore.

Each subject underwent a full-mouth periodontal probing and charting. BMI charting was done as per WHO guidelines [26] and periapical radiographs were taken using the long cone technique. Subjects were having BMI in the range of 18.5–22.9 kg/m^2 (in non-obese groups) and BMI $\geqslant$ 25 kg/m^2 with waist circumference (WC) $\geqslant$ 90 cm in men and $\geqslant$80 cm in women (in obese groups). Subjects with minimum of 20 natural teeth were selected in this study. Radiographic bone loss was recorded dichotomously (presence or absence) to differentiate patients with chronic periodontitis from other groups.

Subjects were categorized into four groups based on gingival index (GI), probing depth (PD), clinical attachment level (CAL), with radiographic evidence of bone loss, bleeding on probing, BMI and WC. The four groups were: (1) Group NOH (non-obese healthy) consisted of 10 subjects with clinically healthy periodontium with no evidence of disease, (GI = 0, PD $\leqslant$ 3 mm, CAL = 0 and BMI = 18.5–22.9 kg/m^2) without any crestal bone loss as determined from radiograph; (2) Group OH (obese healthy) consisted of 10 subjects with clinically healthy periodontium with no evidence of disease (GI = 0, PD $\leqslant$ 3 mm, CAL = 0 and BMI $\geqslant$ 25 kg/m^2) with no crestal bone loss as determined from radiograph; (3) Group NOCP (non-obese with chronic periodontitis) consisted of 10 subjects, who showed clinical signs of gingival inflammation (GI > 1, PD $\geqslant$ 5 mm, CAL $\geqslant$ 3 mm and BMI = 18.5–22.9 kg/m^2) with radiographic evidence of bone loss and (4) Group OCP (obese with chronic periodontitis) consisted of 10 subjects, who showed clinical signs of gingival inflammation (GI > 1, PD $\geqslant$ 5 mm, CAL $\geqslant$ 3 mm and BMI $\geqslant$ 25 kg/m^2) with radiographic evidence of bone loss.

2.1. Site selection and GCF fluid collection

All clinical examinations, radiographs, group allocations, and sampling site selections were performed by one examiner (ARP), and the samples were collected on the subsequent day by a second examiner (MK) who was blinded to the groups allotted. This was to prevent contamination of GCF with blood associated during probing of inflamed sites. Examiners were calibrated and performed all the clinical assessments using a University of North Carolina (UNC)-15 periodontal probe[1] to ensure adequate intra-examiner reproducibility. In subjects with chronic periodontitis, the site showing the greatest CAL and signs of inflammation, along with radiographic confirmation of bone loss, was selected for sampling.

After making the subjects sit comfortably in an upright position on the dental chair, the selected test site was air dried and isolated with cotton rolls. Without touching the marginal gingiva, supragingival plaque was removed to avoid contamination of the paper strips[2] using the intracrevicular 'superficial' method developed by Loe and Holm-Pederson [27]. The absorbed GCF volume of each strip was determined by electronic impedance. Two Periopaper strips that absorbed GCF for each subject were pooled and the Periopaper strips were placed in a sterile eppendorff vial containing 400 μl of phosphate buffer saline and were kept at −70 °C until analyzed. Periopaper strips contaminated with blood and saliva were excluded or discarded. Periodontal treatment (scaling and root planning) was performed for periodontitis subjects at the same appointment after GCF collection.

2.2. Blood collection

Two milliliters of blood was collected from the antecubital fossa by venipuncture using a 20-gauge needle with 2-ml syringe and immediately transferred to the laboratory. The blood sample was allowed to clot at room temperature and, after 1 h serum was separated from blood by centrifuging at 3000*g* for 5 min. The serum was immediately transferred to a plastic vial and stored at −70 °C until the time of assay.

2.3. MCP -4 analyses

The GCF and serum samples were then assayed for MCP-4 using enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's instructions[3] by a technician who was blinded to the groups allotted and who was not involved in sample collection.

[1] Hu Friedy, Chicago, IL, USA.
[2] Periopaper, Ora Flow Inc., Amityville, NY, USA.
[3] Raybiotech, Inc., USA.