ARTICLE IN PRESS
+ MODEL
Journal of the Formosan Medical Association (2017) xx, 1–7

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Levels of high-mobility group box-1 in gingival crevicular fluid in nonsmokers and smokers with chronic periodontitis

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Received 3 November 2016; received in revised form 4 January 2017; accepted 12 January 2017

KEYWORDS
gingival crevicular fluid;
high-mobility group box-1;
periodontitis;
smoking

Background/purpose: High-mobility group box-1 (HMGB1), a proinflammatory cytokine, plays a role in inflammatory disorders. Smoking is a well-established risk factor for periodontal disease. The aim of this study was to compare the levels of HMGB1 in the gingival crevicular fluid from periodontally healthy nonsmokers, chronic periodontitis nonsmokers, and chronic periodontitis smokers. Furthermore, the relationship between levels of HMGB1 and periodontal parameters was examined.

Methods: Periodontal parameters of 17 nonsmokers with chronic periodontitis, nine smokers with chronic periodontitis, and nine periodontally healthy nonsmokers were examined. Gingival crevicular fluid samples were collected, and the levels of HMGB1 were analyzed using the enzyme-linked immunosorbent assay.

Results: The median level of HMGB1 was statistically significantly higher in chronic periodontitis nonsmokers (37.5 ng/mL) than in chronic periodontitis smokers (9.5 ng/mL) and periodontally healthy nonsmokers (3.7 ng/mL). There was no significant difference in the levels of HMGB1 between chronic periodontitis smokers and periodontally healthy nonsmokers. Levels of HMGB1 were positively correlated with plaque index, gingival index, probing depth, and clinical attachment level of nonsmokers. However, no significant correlations were found between levels of HMGB1 and all periodontal parameters examined in chronic periodontitis smokers.

Conclusion: Chronic periodontitis nonsmokers had elevated levels of HMGB1 in gingival crevicular fluid. Moreover, the levels of HMGB1 were correlated with severity of periodontitis.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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http://dx.doi.org/10.1016/j.jfma.2017.01.006

Please cite this article in press as: Lin Y-C, et al., Levels of high-mobility group box-1 in gingival crevicular fluid in nonsmokers and smokers with chronic periodontitis, Journal of the Formosan Medical Association (2017), http://dx.doi.org/10.1016/j.jfma.2017.01.006

Chronic periodontitis smokers exhibited lower levels of HMGB1 than chronic periodontitis nonsmokers. Further research is needed for understanding the role of HMGB1 in smoking and pathogenesis of periodontitis.

Introduction

Periodontal disease is an inflammatory disease with multiple factors for pathogenesis. The interaction of microbial challenge with host immunoinflammatory response results in periodontal tissue breakdown. The process of periodontal disease is also modified by environmental factors such as smoking.[1] Smoking may affect prevalence, extent, and severity of periodontal disease.[2–4] Moreover, smoking has negative effects on the treatment outcome of nonsurgical[5] and surgical therapies,[6–8] as well as maintenance therapies.[9,10] In addition, smoking has been suggested to have an influence on cytokine levels in the gingival crevicular fluid (GCF) of patients with chronic periodontitis.[11–13]

High-mobility group box-1 (HMGB1) is correlated with many autoimmune diseases and inflammatory diseases such as rheumatoid arthritis,[14] systemic lupus erythematosus,[15] sepsis,[16] and chronic rhinosinusitis.[17] HMGB1, named for its rapid migration properties on electrophoretic gels, is a nuclear nonhistone protein and organizes chromatin structure, DNA replication, and transcription.[18] HMGB1 can be actively secreted into the extracellular space by monocytes and macrophages,[19] or passively released from the nuclei of necrotic or damaged cells.[20] HMGB1 can bind with receptors including Toll-like receptor 2,[21] Toll-like receptor 4,[21] and the receptor for advanced glycation end products,[22] which are known to be involved in inflammatory processes. Interestingly, extracellular HMGB1 can activate several other cells involved in the immune responses or inflammatory reactions, and act as a multifunctional cytokine.[18] HMGB1 can induce release of inflammatory cytokines.[23,24] Macrophages from synovial fluid express the receptor for advanced glycation end products and release tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 upon stimulation with HMGB1 *in vitro*.[24] Stimulation of synovial fibroblasts with HMGB1 in complex with lipopolysaccharide (LPS) or IL-1β enhances production of TNF, IL-6, and IL-8.[23] In addition, HMGB1 also regulates receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis *in vitro* and *in vivo*.[25]

HMGB1 can be found in gingival tissues and GCF. In chronic periodontitis, higher expression of HMGB1 has been presented in gingival tissues.[26,27] The expression of HMGB1 is also higher in the GCF of chronic periodontitis patients than in healthy individuals.[26,28] However, the relationship between the levels of HMGB1 and periodontal parameters is not examined. TNF-α and IL-1β are key proinflammatory cytokines in periodontal diseases.[29] The increased production of HMGB1 in gingival epithelial cells following stimulation with TNF-α[26] and IL-1β[30] has been shown *in vitro*.

GCF can be collected easily by a minimally invasive method. Multiple biomarkers in GCF might contribute to the pathogenesis of periodontal diseases. In the systemic review by Buduneli and Kinane,[31] the authors have concluded that highly specific and sensitive biomarkers for the diagnosis and monitoring of periodontal diseases are still needed for early and better detection of periodontal tissue destruction. The potential role and mechanisms of HMGB1 involved in periodontitis are still unclear. Smoking is a well-established risk factor for periodontal disease. The possible correlation of HMGB1 and periodontal disease in smokers is also unknown.

The purpose of the present study was to assess the levels of HMGB1 in the GCF from periodontally healthy nonsmokers, chronic periodontitis nonsmokers, and chronic periodontitis smokers. Furthermore, the relationship between levels of HMGB1 and periodontal parameters was examined.

Materials and methods

Participants

The study population, consisting of 17 nonsmokers with chronic periodontitis (10 males and 7 females, aged 36—63 years; median age, 53.0 years; interquartile range, 16 years), nine smokers with chronic periodontitis (7 males and 2 females, aged 36—63 years; median age, 46.0 years; interquartile range, 11 years), and nine periodontally healthy nonsmokers (3 males and 6 females, aged 31—57 years; median age, 39.0 years; interquartile range, 14 years), was recruited between 2012 and 2014. Written informed consent was obtained from each patient and healthy volunteer in the Division of Endodontics and Periodontology, Department of Stomatology, Taipei Veterans General Hospital, Taiwan. This study was approved by the Institutional Review Board (IRB) of Taipei Veterans General Hospital (VGH IRB No: 2012-08-012BC). In this study, patients with chronic periodontitis had sites with probing depth (PD) $\geq$5 mm and clinical attachment level (CAL) $\geq$4 mm. Periodontally healthy volunteers had PD $\leq$ 3 mm and CAL $\leq$ 3 mm. Individuals were excluded if they had any systemic condition that would influence the course of periodontal disease or medical conditions that would require antibiotic prophylaxis for routine dental procedures. Individuals who had taken antibiotics in the previous 6 months or who were pregnant were also excluded. Clinical examinations and collection of GCF were performed before patients received periodontal treatments.

Clinical examinations

Clinical parameters were evaluated in all teeth, excluding third molars. Six sites, including mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual sites, were examined for each tooth. Clinical measurements included

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