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Original

Hepatocyte growth factor in saliva is a potential marker of symptomatic periodontal disease

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Abstract: Evidence has been emerging that hepatocyte growth factor (HGF) - a pluripotential regenerative cytokine - is a key factor in the pathogenesis and progression of periodontal disease, mostly through its over-stimulation of gingival epithelial cell growth and impairment of the regeneration of collagenous structures. We measured the levels of immunoreactive HGF in unstimulated whole mixed saliva from 26 patients referred for treatment of periodontal disease, and from 20 healthy subjects. HGF was detected in all saliva samples from the patients, the concentration ranging from 0.06 to 5.38 ng/ml, with a mean concentration of 1.87 ± 1.32 ng/ml. In healthy individuals, the median salivary HGF level was 0.68 ng/ml (range: 0 - 7.33 ng/ml), being almost 3-fold lower ($P < 0.0001$) than that in the patients. Periodontal parameters in the patients were: gingival index (GI) 2.0 (0 - 2.8), papillary bleeding index (PBI) 2.2 (0 - 3.2), plaque index (PI) 2.0 (0 - 3.0), probing depth (PD) 3.0 (1.8 - 5.9) mm, and loss of clinical attachment level (CAL) 4.7 (1.1 - 10.6) mm. We found that the salivary HGF level was positively correlated with GI ($P = 0.004$), PBI ($P = 0.046$) and PI ($P = 0.001$), but not with PD ($P = 0.351$), CAL loss ($P = 0.172$), number of teeth ($P = 0.279$) or patient age ($P = 0.362$). Our findings suggest that salivary HGF concentration may be a novel marker of symptomatic periodontal disease, and that it warrants further validation. (J. Oral Sci. 48, 47-50, 2006)

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Introduction

Hepatocyte growth factor (HGF) is a multifunctional cytokine involved in embryonic development and the repair and regeneration of various tissues/organs and their protection from injury (1,2). It exhibits mitogenic and antiapoptotic activities, and enhances the motility of different cell types, including not only hepatocytes but also epithelial cells and vascular endothelial cells. Following tissue damage, HGF is expressed in mesenchymal cells (i.e. fibroblasts, mononuclear cells, megakaryocytes), while its high-affinity receptor c-Met is expressed by almost all epithelial cells, endothelial and erythroid progenitor cells (1,2).

Remarkably, HGF is also involved in the development of periodontal disease (3-10). Recently, it was shown that HGF levels in unstimulated whole mixed saliva are directly correlated with probing depth and the percentage of sites positive for bleeding on probing in the general population (8). This finding provided evidence to corroborate previous *in vitro* and *in vivo* investigations indicating a novel link between HGF and periodontal disease (3-7).

In the present study, we investigated the relationship between HGF concentration in unstimulated whole mixed saliva and several indices of the severity of periodontal disease. Unlike a previous study that involved random subjects visiting a health insurance company in Japan (8), our cohort comprised patients with periodontal disease who had been referred for specialized treatment in Poland. We also compared salivary HGF levels between these patients

and healthy subjects.

Materials and Methods

Twenty-six patients (10 women, 16 men; mean age 49 ± 8 years) were enrolled in the study. We included only subjects with two or more teeth present (which were not indicated for extraction) in a minimum one tooth-sextant, according to the WHO recommendations (11). The patients were free of systemic diseases, were not receiving any medication on a regular basis, and had not been treated with antibiotics within the previous 6 months.

Clinical measures of the severity of periodontal disease, such as gingival index [GI], papillary bleeding index [PBI], plaque index [PI], probing depth [PD] and loss of clinical attachment level [CAL], were determined according to the original and/or WHO recommendations (11-14). We used a standardized periodontal probe (11).

Twenty healthy subjects (no periodontitis, systemic diseases or medications) matched for age and gender (8 women, 12 men; mean age 47 ± 10 years) with the patients served as controls for salivary HGF quantification.

A minimum of 5 ml of unstimulated whole saliva was collected by the spit-out method into a sterile vessel after a 10-min oral rinse with distilled water. The saliva samples were kept on melting ice for no longer than 1 hour; afterwards they were centrifuged at 3800 rpm for 10 min. The supernatant (middle 1/3) was collected and stored at -70˚C until analysis.

The HGF levels in saliva were determined using an immunoenzymatic (ELISA) kit (Quantikine® human HGF immunoassay by R&D Systems, Minneapolis, MN, USA; cat. No: DHG00; minimum detection limit: 0.04 ng/ml) according to the manufacturer's instructions. All the measurements were performed in duplicate using a 400 SFC photometer (SLT-Labinstruments, Gröding/Salzburg, Austria, EC), and calibrated using the recombinant human HGF reference samples and standards that were provided.

Inter-group comparison of salivary HGF levels (patients *vs* healthy subjects) was performed by the non-parametric Mann-Whitney *U* test. Relationships between HGF level and periodontal parameters, number of teeth and patient age were analyzed by Spearman's regression method. The tests were two-sided, and differences at $P < 0.05$ were considered significant. For all calculations, a computer and a curve-fitting program were used. The software used was Statistica (version 6.0 PL, StatSoft, Tulsa, OK, USA).

Results

HGF was detected in all saliva samples obtained from the patients and in 18 of the 20 saliva specimens (90%) from the healthy controls. In the patients, HGF concentration ranged from 0.06 to 5.38 ng/ml with a mean value of 1.87 ± 1.32 ng/ml. In the healthy subjects, the median salivary HGF level was 0.68 ng/ml (range: 0 - 7.33), and was markedly lower ($P < 0.0001$) than in the patients.

Periodontal index values in the patients were: GI 2.0 (0 - 2.8), PBI 2.2 (0 - 3.2), PI 2.0 (0 - 3.0), PD 3.0 (1.8 - 5.9) mm, and loss of CAL 4.7 (1.1 - 10.6) mm.

We found positive correlations between the salivary HGF level and GI (r = 0.550, $P = 0.004$, Fig. 1A), PBI (r = 0.394, $P = 0.046$, Fig. 1B), and PI (r = 0.590, $P = 0.001$, Fig. 1C). There were no significant associations between HGF and PD (r = 0.165, $P = 0.351$) or CAL loss (r = 0.276, $P = 0.172$; scatter plots not shown). There were also no

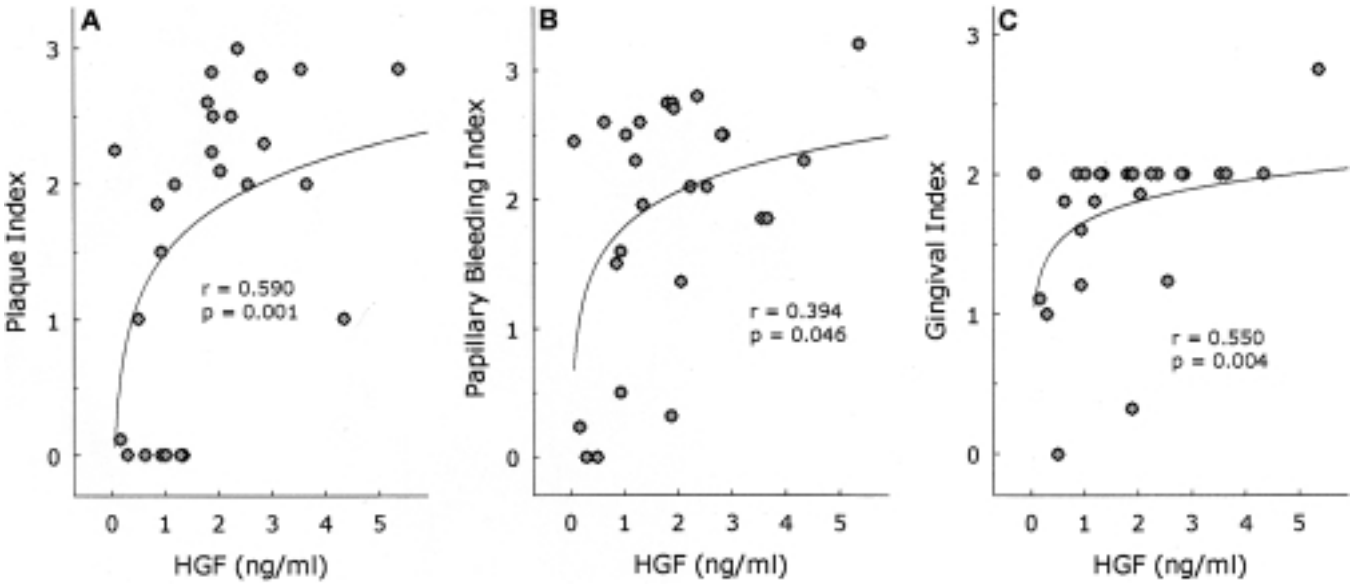

Fig. 1 Correlations between salivary HGF levels and periodontal parameters.

significant correlations between salivary HGF, number of teeth (median 22.5; range: 7 - 28) and patient age (r = 0.220, $P = 0.279$ and r = -0.186, $P = 0.362$, respectively).

Discussion

Extensive studies of periodontal pocket formation over more than half a century have revealed that it is a complex process involving loss of connective tissue attachment followed by epithelial cell downgrowth (15,16).

Ohshima et al. reported for the first time that HGF had demonstrable chemotactic activity for gingival epithelial cells, which was not observed for other cytokines such as transforming growth factor, epidermal-like growth factor, keratinocyte growth factor and interleukin-8 (3). Furthermore, based on a series of *in vitro* studies, Ohshima et al. formulated a hypothesis that HGF stimulates excessive proliferation and invasion of gingival epithelial cells, which in turn impair the proper regeneration of deep collagenous structures in the periodontium (4-6). In other words, it was postulated that HGF may be closely involved in the pathogenesis and progression of periodontal disease. In a subsequent clinical study, it was found that the HGF content of gingival crevicular fluid from patients with periodontitis was 15 times higher than that in the general population, and almost 300 times higher than that in normal human serum (7). In 2002, Japanese authors found significant correlations between the salivary level of HGF and periodontal parameters such as PD and GI, and postulated that salivary HGF might be useful as a screening marker for early detection of periodontal disease (8). At the same time, some of the above *ex vivo* and clinical data (4-8) were confirmed by Daikuhara and coworkers (9,10). Notably, a recent study has shown that HGF over-production by inflamed human gingival fibroblasts is mediated by protease-activated receptors (17), thus corroborating the HGF/periodontal disease relationship on a molecular basis.

Our present clinical study provides for the first time evidence to corroborate the findings of Ohshima et al. (8). Using a complementary but somewhat distinct approach, we examined the relationship between HGF levels in whole mixed saliva samples and indices of periodontal disease such as PI and PBI, as well as PD, GI, and loss of CAL. We employed the same methodology for saliva preparation and ELISA that had been used previously, although our patients (who were confirmed to have periodontal disease) differed from the apparently symptom-free individuals who were enrolled in the Japanese investigation (8). The diverse characteristics of the patients in these two corresponding studies may explain some of the dissimilarities in the results, especially those pertaining to the depth of periodontal pockets. However, we also found statistically significant, direct, non-linear and logarithmic associations between salivary HGF levels and the other parameters of periodontal disease studied. To further strengthen our findings, we showed that the salivary HGF concentration in patients with periodontal disease who required specialized treatment was almost 3 times higher than that in periodontitis-free healthy subjects.

In conclusion, salivary HGF concentration appears to be a novel and promising marker of not only early, but also symptomatic periodontal disease, and warrants further study.

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