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Association of thalassemia major and gingival inflammation: A pilot study

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ABSTRACT

Objectives: This cross-sectional study aimed to investigate the relationship between thalassemia major (TM) and gingival inflammation through the salivary, serum, and gingival crevicular fluid (GCF) levels of matrix metalloproteinase (MMP)-8, MMP-9 and tissue inhibitor of MMP (TIMP)-1.
Methods: Biofluid samples and full-mouth clinical periodontal recordings were obtained from 29 otherwise healthy patients with TM and 25 systemically healthy (SH) individuals. Biofluid samples were evaluated by immunofluorometric assay (IFMA) and enzyme-linked immunoassays (ELISAs). Data were tested statistically by Kolmogorov Simirnov, Mann–Whitney *U* tests, Spearman correlation analysis.
Results: Age, smoking status, bleeding on probing, plaque index were similar in the study groups, but probing depth, gender data exhibited significant differences ($p = 0.037$ for both). Salivary MMP-8, MMP-9, TIMP-1 concentrations were significantly higher in the TM than SH group ($p = 0.014$; $p < 0.001$; $p = 0.042$, respectively). Serum TIMP-1 concentrations were significantly higher; MMP-8/TIMP-1, MMP-9/TIMP-1 molar ratios were significantly lower in the TM than SH group ($p < 0.001$; $p = 0.005$; $p = 0.022$, respectively). Very few GCF samples revealed biochemical data above the detection limits. Numerous correlations were found between clinical periodontal parameters and biochemical data.
Conclusions: It may be suggested that TM may exacerbate the local inflammatory response as manifested in salivary MMP-8, MMP-9, TIMP-1 levels.

1. Introduction

Thalassemia is considered as the most common genetic disorder worldwide, presenting major public health and social problems particularly in the high incidence areas. Based on genetic heterogeneity, clinical and haematological variability, thalassemia can be homozygous, heterozygous, or compound heterozygous. The heterozygous form of the disease (beta-thalassemia) is the most common form with minimal clinical expression (Hattab, 2012). Thalassemia major (TM), which is the homozygous type of beta-thalassemia, is an autosomal recessive hereditary anaemia (Skordis & Toumba, 2011). Systemically-released inflammatory markers due to the iron overload play a key role in multi-organ complications seen in patients with TM (Aggeli et al., 2005) Moreover, low levels of sexual hormones together with iron overload lead to decreased inhibition of osteoclast activity and bone metabolism. Therefore, skeletal complications like osteopenia/osteoporosis, pain, and fractures are quite common in TM (Aydinok, 2012).

Gram-negative, anaerobic and microaerophilic bacteria that colonize in microbial dental plaque are considered as the main etiological factor of inflammatory periodontal diseases. These bacteria are capable of stimulating host immune cells to increase their matrix metalloproteinase (MMP) release, thereby leading to the irreversible tissue destruction seen in periodontitis (Sorsa et al., 1992; Sorsa et al., 2006). MMPs can collectively degrade almost all components of extracellular matrix and basement membrane and their excess activity play a major role in periodontal tissue destruction. MMPs can also process bioactive non-matrix substrates such as cytokines, chemokines, growth factors and immune modulators, thus they mediate anti-inflammatory and pro-inflammatory processes (Kuula et al., 2009; Sorsa et al., 2006). Upon bacterial insult triggered leukocytes migrate to

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the site of inflammation and release MMP-8 and MMP-9, which are activated locally [Tissue inhibitors of MMPs (TIMPs) regulate the activities of these enzymes and TIMP-1 is more effective on interstitial collagenases (Howard, Bullen, & Banda, 1991). An imbalance between MMPs and TIMPs is a key element in the pathological tissue destruction observed in periodontitis (Aiba, Akeno, Kawane, Okamoto, & Horiuchi, 1996; Bıyıkoğlu et al., 2009; Özçaka et al., 2011).

The possible relationship between TM and gingival inflammation is unclear. Iron release from tissue stores and reactive oxygen species production are considered as adequate stimuli for the production of MMPs (Zamboni et al., 2005). It is hypothesized that the iron overload in TM patients may affect levels of MMPs in saliva, serum and/or gingival crevicular fluid (GCF) and thereby deteriorate clinical periodontal status. Therefore, the aim of the present study was to evaluate salivary, serum, and GCF levels of MMP-8, MMP-9, TIMP-1 together with clinical periodontal status in TM patients and systemically healthy counterparts.

2. Materials and methods

2.1. Study population

A total number of 54 individuals were recruited for the present study between September 2012 and March 2013. Twenty-nine TM patients (aged 18–58 years) followed for at least three years by the outpatient clinic of Haematology Department, Aydın State Hospital, Aydın, Turkey were included. None of the TM patients had had splenectomy. Independent factors likely to be associated with low bone mass such as history of gonadal or pubertal dysfunction, history of iron chelating therapy, history of treatment with calcium and vitamin D, pre-transfusion haemoglobin level, serum levels of calcium, phosphorus, alkaline phosphatase, and thyroid function indices (T3, T4 and TSH) were determined and patients with any of these factors were excluded. Inclusion criteria for TM patients were age $\geq$18 years, absence of hepatitis B, C or HIV infection, and treatment with chelation therapy using deferasirox and regular erythrocyte transfusion, vitamin B and C. Patients with any other known systemic disease that can affect periodontal status and those, who received antibiotic therapy or periodontal treatment within the last three months, patients having less than 10 teeth were also excluded from the study. Smoking history was recorded, but smokers were not excluded. Twenty-five systemically healthy individuals (aged 18–40 years) seeking dental treatment in the School of Dentistry, Ege University volunteered for the control group. The study was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki. The study was approved by the Ethics Committee of the Ege University with the protocol number 13-11/72. The study protocol was explained and written informed consent was received from each individual before clinical periodontal examination and biofluid sampling. Medical and dental histories were also obtained.

2.2. Saliva sampling

Whole saliva samples were obtained simply by expectorating into polypropylene tubes prior to clinical periodontal measurements or any periodontal intervention and in the morning following an overnight fast during which individuals were requested not to drink (except water) or chew gum.

2.3. Serum sampling

Five millilitres of venous blood were taken from antecubital vein by a standard venipuncture method and the plasma was separated from blood by centrifugation at $1500 \times g$ for 10 min.

2.4. GCF sampling

From each individual participating in the study, GCF samples were obtained from the buccal aspects of two interproximal sites in single-rooted teeth. Sites with obvious plaque accumulation and visible signs of inflammation, such as hyperaemia were selected for GCF sampling. Before GCF sampling, supragingival plaque was removed carefully by sterile curettes and the surfaces were air-dried and isolated by cotton rolls. Filter paper strips (PerioPaper, ProFlow, Amityville, NY, USA) were placed in the orifices of gingival sulcus/pocket for 30 s. Care was taken to avoid mechanical trauma, and strips visually contaminated with blood were discarded. The absorbed GCF volume was estimated by a precalibrated instrument (Periotron 8000, Oraflow, Plainview, NY, USA). The strips from each patient were placed into separate polypropylene tubes before freezing at $-40\,^{\circ}C$. The actual volumes of the GCF samples were calculated in μL by reference to the standard curve.

All biofluid samples were immediately frozen and stored at $-40\,^{\circ}C$ until the sample collection period was completed and thawed immediately before assays.

2.5. Clinical measurements

Subsequent to biofluid samplings, clinical periodontal recordings, plaque index (PI) (Löe, 1967), probing depth (PD), clinical attachment level (CAL), and presence of bleeding on probing (BOP; as present or absent within 15 s after periodontal probing) were performed at 6 sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations) on each tooth present, except the third molars, using a Williams periodontal probe (Hu Friedy, Chicago, IL, USA). CAL was assessed from the CEJ to the base of the probable pocket. BOP (deemed positive if it occurred within 15 s after periodontal probing) (Bıyıkoğlu et al., 2009; Nizam et al., 2014). All measurements were performed by a single calibrated examiner (ÖÖ).

2.6. Measurement of MMP-8, MMP-9 and TIMP-1 in saliva and serum samples

2.6.1. MMP-8 analysis by immunofluorometric assay (IFMA)

MMP-8 levels in the salivary and serum samples were determined by a time-resolved immunofluorescence assay (IFMA) as described previously by (Gürsoy et al., 2010). The monoclonal MMP-8 specific antibodies 8708 and 8706 (Medix Biochemica Oy Ab, Kauniainen, Finland) were used as a catching and tracer antibody respectively. The tracer antibody was labelled using europium-chelate. The assay buffer contained 20 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 5 mM $CaCl_2$, 50 μM $ZnCl_2$, 0.5% bovine serum albumin, 0.05% sodium azide, and 20 mg/l dietyhlenetriaminepentaacetic acid (DTPA). Samples were diluted in assay buffer and incubated for 1 h, followed by incubation for 1 h with the tracer antibody. Enhancement solution was added, and after 5 min fluorescence was measured using a fluorometer (1234 Delfia Research Fluorometer, Wallac, Turku, Finland). The specificity of the monoclonal antibodies (Gürsoy et al., 2010) against MMP-8 was the same as that of polyclonal MMP-8 antibodies (Sorsa et al., 1999). The salivary and serum concentrations of MMP-8 were expressed as ng/ml and the detection limit for MMP-8 was 0.8 ng/ml.