Evaluation of Thrombomodulin Level in Periodontal Pockets

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Abstract: This study aims to investigate thrombomodulin level in periodontal pockets with respect to its depth. This study included 27 patients, 13 women and 14 men. Probing depth with 5 mm or more were recorded as deep pockets and probing depth < 5 mm recorded as shallow pockets. Gingival crevicular fluid was collected with paper strips from 3 deep and 3 shallow pockets of canine to canine tooth in the same patients. The fluid volume in the periopaper strip was calculated with Periotron 8000. Thrombomodulin levels were analyzed by enzyme-linked immunosorbent assay.

There was a significant difference between shallow and deep pockets with respect to the thrombomodulin levels. Increasing of thrombomodulin levels were correlated with shallow pockets, deep pockets and gingival index scores. Thrombomodulin level increases in bleeding sites and inflammation sites of gingival crevicular fluid.

Keywords: Bleeding, gingival crevicular fluid, inflammation, thrombomodulin

1. BACKGROUND

Thrombomodulin (TM) is an important cofactor for thrombin in circulation to activate protein C and inhibit coagulation. Thrombomodulin is a cofactor presented on the surfaces of endothelial cells. When thrombin binds to TM, protein C activation increase 1000 fold [1, 2]. This effect contribute a direct anti-coagulant activity. It means where the thrombomodulin levels increase, bleeding also increased [3]. Previous studies regarding with wound healing reported decrease in TM anticoagulant activity level in epidermis does not appear to alter the reepithelization, but influences collagen production with fibroblasts in the wound matrix [4, 5]. It was reported that diseased sites of periodontium usually found in the regions where the clot clotting chamber is activated [6]. This activation may be monitored among the molecules found in gingival crevicular fluid (GCF). TM was released from the gingival epithelial cells via neutrophil enzymes in periodontitis patients. The increased level of TM in GCF points out the injury of cell-wall membrane and it may speculate its potential role to ascertain the disease entity [7]. Therefore, relation with TM in deep and shallow periodontal pockets in the same subject was studied in this study.

2. MATERIALS

2.1 Study Design

The study was carried out in Department of Periodontology, Ondokuz Mayıs University Dental Faculty. This study was approved by the ethics committee of Ondokuz Mayıs University (2008/9) and written informed consent was obtained from the patients. This study included 27 patients, 13 women and 14 men. All of them were non-smokers. Subjects had periodontal disease by multiple sites with a probing depth (PD) of 5 mm or more and bone loss evaluated by radiographs. Their ages ranged from 31 to 57 years. They were untreated periodontal patients and they had no medical history or any systemic disease. The exclusion criteria were taking antibiotics within the previous 6 months, taking any medications within the previous 3 months, pregnancy or taking oral contraceptive, having less than 20 natural teeth and receiving periodontal therapy within the previous 6 months. Probing depth with 5 mm or more were recorded as deep pockets and up to 5 mm recorded as shallow pockets. The periodontitis
subjects included in this study had shallow and deep pockets in the same mouth and maxillary anterior teeth sites. The remaining all teeth had varying degrees of untreated periodontal disease.

All the clinical data were collected by same examiner. The following periodontal variables were recorded; PD and gingival index (GI) [8]. PD was recorded at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual). PD was obtained from sample sites with the Florida Probe (FP32/7.2.2 version, Florida Probe Corporation, Gainesville, FL, USA). The tip diameter of the probe was 0.45mm and the probing force applied was 20gr. The probe was used parallel to the long axis of the teeth.

2.2 Collection of Samples

Supragingival plaque was removed and sampling sites were isolated with cotton rolls and sampling sites were air dried to eliminate the contamination of plaque and saliva. A suction pipe was used to prevent salivary contamination of the samples. Each GCF sample was collected with paper strips (Periopaper Strips®, Amityville, NY, USA) from selected 3 deep and 3 shallow pockets of maxillary anterior teeth of same patients. The strips were inserted 1mm into the crevice for 30 seconds. Paper strips with GCF were immediately placed in a eppendorf tube containing 200µlPBS. These samples were stored at -80°C until being analyzed.

2.5 Thrombomodulin ELISA Assay

Paper strips with GCF were incubated in phosphate buffered solution for 60 seconds at 4°C included 1% bovine serum albumin and protease inhibitors and extracted at pH 7.4. 200 µL dilution of thrombomodulin standards, control sera and GCF samples were analyzed with imubindelisa kit (American Diagnostica Stamford, CT, USA) by ELX50 ELISA device with sandwich method at 450 nm. Thrombomodulin values, estimated as nanogram, and periotron volume values were calculated together and concentration values (ng/ml) were obtained.

2.6 Statistical Analyses

The Wilcoxon test was used to statistical analyses. P value of less than .05 was considered statistically significant. Pearson Correlation test was used to correlate thrombomodulin levels with pockets depth, deep pockets’ GI score, shallow pockets’ GI score.

3. RESULTS

Descriptive values for PD, GI and TM are given in Table 1. There was a significant difference between shallow and deep pockets with respect to the thrombomodulin levels (Table2). Increasing of thrombomodulin levels correlated with shallow, deep pockets and GI scores (Table3).

Table1: Descriptive values for Pocket depth, Gingival Index and Thrombomodulin

|                   | N   | Minimum | Maximum | Mean  | SD   |
|-------------------|-----|---------|---------|-------|------|
| Shallow Pockets (mm) | 27  | 2.00    | 4.00    | 3.18  | 0.78 |
| Deep Pockets (mm)  | 27  | 5.00    | 8.00    | 6.70  | 0.91 |
| Shallow Pockets GI | 27  | 1.00    | 2.00    | 1.39  | 0.36 |
| Deep Pockets GI    | 27  | 1.00    | 2.00    | 1.67  | 0.34 |
| Shallow Pockets TM (ng/ml) | 27 | 40.88   | 124.98  | 76.41 | 23.45 |
| Deep Pockets TM (ng/ml) | 27 | 94.56   | 170.83  | 123.48| 19.72 |

SD = Standard deviation

Table2: Comparision of the thrombomodulin levels (ng/ml) between shallow and deep pockets.

| Shallow Pockets | Deep Pockets | P value |
|-----------------|--------------|---------|
| 76.4±23.45      | 123.4±19.72  | 0.000’  |

*p < 0.000

Table3: Correlation of shallow pockets, deep pockets, shallow pockets’ GI scores and deep pockets’ GI scores with thrombomodulin levels

| Thrombomodulin Levels | N  | r   | p   |
|-----------------------|----|-----|-----|
| Shallow Pockets GI    | 27 | 0.703’ | 0.000 |
| Deep Pockets GI       | 27 | 0.604’ | 0.001 |

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| Deplorability  | Count | p-value | Significance |
|----------------|-------|---------|--------------|
| Shallow Pockets| 27    | 0.592   | 0.001        |
| Deep Pockets   | 27    | 0.612   | 0.001        |

*Correlation is significant at the 0.01 level (2-tailed).

4. **Discussion**

We examined the GCF thrombomodulin levels in comparison with shallow and deeper periodontal disease subjects. In this study, thrombomodulin levels were found increased in deep pockets in comparison with shallow pockets. Matsuyama et al. [7] reported that increased thrombomodulin in GCF is related to its enzymatic effect. Destruction of epithelial cells by neutrophil enzymes (cathepsin G and elastase) cause increasing of TM in the gingival reticular fluid. Authors also reported that the soluble thrombomodulin increased as a result of independent cell death or destruction of epithelial cells. TM values between up to 3 mm pocket depths and deeper than 3 mm pocket depths show significant difference. However, TM values increased significantly deeper than 3 mm regardless of its deepness.

TM is the integral membrane protein located on endothelial cells through the vascular system. Thrombin connects with the thrombomodulin (ratio of 1:1) and this connection dramatically alters the substrate specificity of thrombin [9, 10]. Activated protein C with this complex causes inhibition of coagulation [2, 11]. TM increased in periodontitis patients at the bleeding and inflammation sites [3]. In the current study, our results showed that TM correlated with GI scores.

Free thrombin effectively binds the fibrinogen but activates the protein C poorly. In contrast thrombin thrombomodulin complex binds fibrinogen poorly, but activates protein C effectively [11, 12]. When there is failure of TM anticoagulant activity, collagen production and fibroblasts are affected due to lack of thrombin and tissue integrity is impaired. Thrombin also connects thrombomodulin which is cell-surface anticoagulant. Thrombomodulin is emerged on the endothelial cells and keratinocytes. It specifically induces the proliferation of endothelial cells and regulates fibrinolysis [13, 14, and 15]. For all these reasons thrombomodulin may play a key role in molecular relations in mechanism of periodontal disease. Furthermore, it is believed that thrombomodulin is resistant to cytokines. Apparently, TM is also monitored in non-endothelial tissues in early developmental period brings in mind that it’s various functions [11]. Where the TM increase it is likely that collagen deposition fails [16]. As this sentence capillary fragility is probably increased in these deeper sites due to collagen deposition failing and in consequence the epithelial cell membrane injury may be seen and this probability cause to thrombomodulin increase in GCF.

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