

Comparison of Salivary Epidermal Growth Factor Levels in Patients with Gingivitis and Advanced Periodontitis and Healthy Subjects

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Abstract

Objective: Epidermal growth factor (EGF) is a polypeptide molecule, with important functions in epithelial growth and wound repair. It exerts its effects on cells by binding to receptors on the cell surface. The aim of this study was to evaluate and compare salivary EGF levels in patients with gingivitis and advanced periodontitis as well as in healthy controls.

Material and Methods: Unstimulated salivary samples were collected from patients with gingivitis and advanced periodontitis and healthy individuals. The clinical parameters of plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) were measured and recorded using a Williams probe. The enzyme-linked immunosorbent assay (ELISA) was used to determine salivary levels of EGF. One-way ANOVA was used for data analysis.

Results: The mean salivary level of EGF in healthy individuals (99.27) was significantly higher than that in patients with gingivitis (61.53). This value in patients with gingivitis (61.53) was also significantly higher than that in subjects with periodontitis (36.14) ($P < 0.001$).

Conclusion: The reduction in salivary level of EGF in patients with periodontal disease may be related to the pathogenesis of periodontitis.

Keywords: Epidermal Growth Factor; Gingivitis; Periodontitis; Salivary Proteins and Peptides; Salivary Glands

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INTRODUCTION

Periodontal disease is a major oral health problem, with a high prevalence worldwide [1,2]. Advanced periodontal disease is associated with deep periodontal pockets (≥ 5 mm), affecting 10–15% of adolescents all over the world [3]. Evidence available now associates the periodontal disease with some important risk factors, including poor oral hygiene, to-

bacco use, stress, diabetes and excessive use of alcohol.

On the other hand, chronic periodontal disease results from the interaction between gram-negative bacteria and the host's inflammatory responses, leading to eventual tissue destruction and tooth loss [4-6]. In fact, microorganisms are the etiologic agents for periodontitis; however, the clinical manifestations of the

disease (severity and extent) depend on the quality of host response to bacterial invasion. In response to periodontal pathogens and their endotoxins, the immune cells of the periodontium secrete some pro-inflammatory mediators [7]. Of a large array of known immunologic and inflammatory mediators in the saliva and gingival crevicular fluid (GCF), cytokines have attracted a lot of attention.

Epidermal growth factor is a multifactorial cytokine with various biological effects, including cell proliferation by binding to EGF-specific receptors. EGF is a polypeptide molecule, with important functions in epithelial growth and wound repair processes. It exerts its effects on cells by binding to receptors on the cell surface. RGF receptors are expressed in large numbers on the surface of basal cell layers in the gingival epithelium [8].

Periodontal disease is in fact a series of acute and chronic inflammatory reactions in response to certain bacterial products or compounds; the condition is diagnosed by the breakdown of extracellular matrix, including bone resorption. Severe destruction of periodontal tissues is probably associated with the increased activity of proteinases derived from collagenase and gelatinase and since EGF is an important stimulator of collagenase and gelatinase, its presence in the gingival tissue and saliva has been confirmed [9,10]. In addition, expression of gingival receptors of EGF has been observed during the inflammatory process. Therefore, it appears that EGF is an important mediator in the pathogenesis of periodontal disease [11].

Some of the sources for sampling and evaluation of inflammatory mediators in periodontal disease include saliva, serum, urine and GCF.

Laurina et al. evaluated the qualitative and quantitative relationships and expression of growth factors, cytokines, defensins and also apoptosis in the pathogenesis of periodontal disease [12]. The results showed that large number of epithelial cells resulted in the expression of IL-10 in patients with periodontitis

and a large number of positive cells were found in the controls. In addition, variable IL-10 expression rates were noted in the connective tissues of patients and healthy controls. The results of their study showed a lower expression rate of growth factors and their receptors in the sulcular epithelium compared to the gingival epithelium, which might be specific for periodontitis.

Oxford et al. evaluated the decrease in EGF levels in diabetic patients [13]. Given the role of EGF in maintaining oral health and in wound healing, they compared EGF levels in diabetic patients and healthy controls. Based on the results, EGF salivary levels expressed relative to one milliliter volume of saliva in type I and type II diabetic patients were significantly less than those in healthy controls (873.43 ± 106.5 vs. 1101.09 ± 116.8 pg/mL in diabetics and healthy controls, respectively). Their study showed that decreased salivary levels of EGF in diabetic patients might have a role in the deterioration of oral and systemic complications in diabetic patients.

On the other hand, Hormia et al. [14] demonstrated increasing salivary levels of EGF in juvenile periodontitis patients utilizing radioimmunoassay technique and proposed that this elevation may be associated with the pathogenesis mechanism of this kind of periodontitis. This study sought to assess the salivary EGF levels in patients with gingivitis and severe periodontitis in comparison to healthy controls to evaluate the amount of this growth factor in health and disease conditions. The main hypothesis tested was that lower expression of EGF could result in greater bone loss.

MATERIALS AND METHODS

A. Study Population

This cross-sectional, analytical study was carried out on salivary samples of patients with gingivitis and advanced periodontitis as well as the healthy individuals, referring to the Faculty of Dentistry, Tehran University of Medical Sciences.



Fig1. An Eppendorf tube

The study population consisted of 11 patients with advanced periodontitis, 13 patients with gingivitis and 16 healthy controls. A non-random sampling procedure was used based on the subjects available. The study subjects were selected based on the following inclusion and exclusion criteria:

Inclusion Criteria

The inclusion criteria for patients with advanced periodontitis consisted of attachment loss ≥ 5 mm, radiographic signs of bone loss and systemic health.

The inclusion criteria for patients with gingivitis consisted of an age range of 20–48 years, absence of radiographic bone loss, periodontal pocket depth ≥ 5 mm and systemic health.

Exclusion Criteria

The exclusion criteria consisted of a history of any systemic disease affecting periodontal tissues, positive history of antibiotic therapy within the past month before the study, positive history of any periodontal treatment during the previous year, pregnancy, positive history of prophylactic procedures, and lack of patient cooperation and compliance.

B. Data Registry

The patients were briefed on the study design and written consent forms were obtained. The demographic data of the subjects were recorded, which included name, age, gender, occupation, educational status, underlying systemic conditions, antibiotic therapy and dosing interval, pregnancy, and history of periodontal treatment.

C. Registry of Clinical Findings

The clinical parameters including PI, BOP, PPD and CAL were measured and recorded using a Williams probe (Hu-Friedy, Chicago, IL, USA). Unstimulated salivary samples were collected from the subjects at 9–11 a.m. by spitting. Due to the presence of proteolytic enzymes in the saliva and the possibility of disruption of the test results, the samples were immediately transferred to Eppendorf tubes containing Tris-HCl buffer solution (Figure 1). The buffering solution was prepared as follows: 1.18 g of Tris (hydroxymethyl) amino-methane was dissolved in 80 mL of distilled water in a 100-mL flask; the pH of the solution was adjusted at 7.8 by incorporating hydrochloric acid (0.5 mmol/L). Finally, the volume was adjusted at 100 mL by adding distilled water. The solution produced this way is stable and can be preserved for 6 months at -4°C . In order to achieve an identical test condition for all the samples, 300 μL of the solution was transferred to each Eppendorf tube. A maximum time of 30 minutes was observed before submitting the samples to the laboratory. During this period, all the samples were kept in a refrigerator at -4°C . The samples were kept at -20°C in the laboratory until sufficient samples were collected for using an ELISA kit. Then, the samples were simultaneously evaluated.

D. ELISA Test

The optical density and the intensity of the color changes depend on the concentration of antigen determined by using an ELISA reader. Finally, a standard curve was used to determine the concentration of the samples in ng/mL.

Procedural Steps

The following steps were followed:

1. The antigen dissolved in the buffering solution was transferred to the respective wells using a sampler.
2. Small amounts of antigens were adsorbed to plastic surfaces.

3. All the free antigens were removed by rinsing. A neutral protein was used to block the surface in order to prevent unspecific binding of other proteins during the forthcoming stages of the test.
4. All the unbound proteins were rinsed away.
5. At this stage another binding agent was used, which consisted of a molecule capable of identifying antibodies. The molecule was also covalently bound to an enzyme such as peroxidase. The binding molecule was bound to the antibody being tested.
6. The antibodies not binding the antibody under question were rinsed away.
7. A coloring agent was added; the agent was able to change into a colored material due to the action of the enzyme so that the complex could be identified.
8. The concentration of the antigen was determined by scanning the optical density of the colored final product and reported by the use

Statistical Analysis

SPSS 15.0 was used for data analysis. The mean age and the mean salivary EGF level were calculated and reported. Due to the normal distribution of data (based on Kolmogorov-Smirnov test and establishment of the equality of variances based on Leven's test), one-way ANOVA was used to evaluate differences in EGF levels among patients with periodontitis and gingivitis and healthy controls. Statistical significance was defined at $P \leq 0.05$.

RESULTS

In this study, the salivary levels of EGF were evaluated in patients with gingivitis and advanced periodontitis and healthy controls. Based on the results, the mean age of patients with gingivitis and advanced periodontitis and healthy controls was 31.13 (SD=1.4), 47.18 (SD=6.5) and 31.24 (SD=4.4) years, respectively.

Table 1. Pairwise comparison of groups using the post hoc Tukey's test

Group 1	Group 2	Mean	P
Healthy controls	Gingivitis	7.18	0.001
Healthy controls	Periodontitis	23.06	0.001
Gingivitis	Periodontitis	15.87	0.001

Table 2. The mean salivary EGF level in patients with gingivitis and advanced periodontitis and healthy controls

Group	No.	Mean	SD	Std. Error	95% CI	
					Minimum	Maximum
Healthy	16	98.27	27.35	6.83	83.69	112.84
Gingivitis	13	61.53	18.51	5.13	50.34	72.72
Periodontitis	11	36.14	9.17	2.76	29.98	42.30

Table 3. Pairwise comparison of groups using the post hoc Tukey's test

Group1	Group2	Mean	P
Healthy	Gingivitis	39.73	0.001
Healthy	Periodontitis	62.12	0.001
Gingivitis	Periodontitis	25.39	0.14

Pairwise comparisons with the post hoc Tukey's test revealed significant differences among the three groups ($P < 0.001$) (Tables 1). The mean salivary level of EGF in patients with gingivitis and advanced periodontitis and healthy controls was 61.53 ± 18.51 , 36.14 ± 9.17 and 98.27 ± 27.35 ng/mL, respectively (Table 3).

In addition, pairwise comparisons with the post hoc Tukey's test revealed significant differences between healthy controls and patients with gingivitis ($P < 0.001$), healthy controls and patients with periodontitis ($P < 0.001$) and patients with gingivitis and those with periodontitis ($P = 0.14$); i.e. the mean salivary level of EGF in healthy individuals was significantly higher than that in patients with gingivitis; and this value in patients with gingivitis was significantly higher than that in patients with periodontitis (Table 3).

DISCUSSION

In this study, the salivary level of EGF was determined in patients with periodontitis and gingivitis as well as in healthy controls using the ELISA technique. The ELISA is based on very precise scientific principles and, is cost-effective and easy to implement because it does not require expensive or complicated equipment compared to other tests. It is similar to other radioimmunoassay tests with the exception that it uses a color change reaction due to the effect of an enzyme on a substrate as an indicator instead of a radioisotope. Since our data were obtained from ELISA, the authors believe that they are reliable for future studies.

According to the results of the present study the mean salivary levels of EGF in patients with gingivitis and advanced periodontitis and healthy controls were 61.53, 36.14 and 98.27 ng/mL, respectively. Therefore, EGF salivary levels in patients with periodontitis were significantly lower than those in patients with gingivitis, which were in turn significantly lower than those in healthy controls, indicating

that with the progression of the condition from gingivitis to periodontitis, the salivary levels of EGF decreased.

In the same context, animal studies have shown that EGF in the saliva can be systematically absorbed by the oral mucosa and the intestines [15-17].

In addition, increased levels of salivary EGF can enhance the regenerative processes in traumatized areas by binding to EGF receptors and stimulating the tyrosine kinase pathway; the tyrosine kinase phosphorylates molecules. In addition, increased binding of EGF to its receptors might result in various biological effects, including epithelial proliferation, angiogenesis and inhibition of the release of gastric juices [18-20]. Therefore, it appears that a transient increase in the salivary levels of EGF is associated with the oral mucosal injuries.

Hormia et al. reported a minor increase in EGF levels in patients with juvenile periodontitis [14]. In addition, this increase was statistically significant in samples collected by stimulating the salivary flow.

However, the results of our study were different and salivary levels of EGF decreased significantly with the progression of periodontal disease. Such difference may be attributed to the technique used to evaluate the results. In this study, the authors declared that they used the whole saliva and stimulated the parotid gland (that is the main source for EGF in humans) for analyzing the EGF levels.

In addition, Oxford et al. reported decreased levels of EGF in diabetic patients and stated that decreased salivary levels of EGF might have a role in deterioration of the oral mucosal diseases and systemic complications of diabetes [13].

They also reported a significant decrease in salivary levels of EGF in diabetic patients, resulting in diabetes-related oral and systemic manifestations, including periodontal disease. Similar results were achieved in our study despite the fact that sampling was carried out in non-diabetic patients.

Oxford et al. evaluated the EGF levels before and after oral and juxta-oral surgeries [21]. Approximately 3 mL of the unstimulated salivary flow of patients were collected at certain intervals. The salivary samples of patients undergoing periodontal surgery were collected before surgery and at 6-, 12-, 18-, 24-, 30-, 36- and 42 hours and 2 weeks postoperatively. Subsequently, salivary EGF levels were determined by the Quantikine Human EGF Immunoassay. The results showed that since local cells are capable of producing and secreting growth factors at surgical sites, the surgical procedure results in an increase in the production and secretion of growth factors in the saliva. In addition, an increase in the salivary levels of growth factors can contribute to wound healing. Growth factors are biological mediators that regulate migration and proliferation of connective tissue cells and synthesis of proteins and other constituents of the extracellular matrix. Reaction of the target cells to growth factors depends on the expression of their specific receptors; they are membrane antigens that produce intercellular signals when they bind to growth factors and stimulate chemotaxis, cellular growth and differentiation and production of the extracellular matrix [22]. Therefore, growth factor receptors play a major role in the initiation of periodontal disease and regeneration process [23]. Also, some studies have demonstrated the effect of EGF as a wound healer [13, 21, 24]. The authors believe that this effect of EGF may be the biological factor that controls the pathogenesis of periodontal disease. It was shown that decreased EGF in diabetic patients may lead to periodontal destruction [13, 25]; this finding verifies our results as well.

Collagenase, gelatinase and plasminogen activators as well as TNF, IL-1 and prostaglandin E2 play a role in tissue destruction in periodontal disease. Moreover, it was shown that EGF may be an important regulator of the pathogenesis of periodontal disease due to its complex reactions with the factors mentioned

above. Thus, the authors believe that future studies must focus on the expression of gingival receptors for EGF or other cytokines in different types of periodontal disease to better elucidate this subject.

CONCLUSION

The results of this study demonstrated that:

– There were significant differences between the three understudy groups in the salivary level of EGF, with a significant decrease in EGF levels with the progression of periodontal disease.

– Given a significant decrease in the salivary level of EGF in patients with periodontal disease, it appears that change in EGF level is an important mechanism associated with the pathogenesis of periodontal disease.

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