Original Article

# Comparative Study Levels of Salivary Epidermal Growth Factor Between Normal Patients and Patients with Gingivitis & Periodontitis



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## ABSTRACT

**Objective:** An important polypeptide molecule known as epidermal growth factor (EGF) has significant importance in wound healing and aids in epithelial growth. Its mechanism of action is such that it binds with receptors present on the surface of cell. The main objective of present research is to access, evaluate and make comparison of levels of salivary EGF in individuals having medical conditions like oral gingivitis and advanced periodontitis along with patients with healthy oral cavity conditions.

**Study Design:** Comparative Study

**Place and Duration of Study:** This study was conducted at the Watim Medical and Dental College Rawalpindi September 2019 to January 2020.

**Materials and Methods:** The samples of saliva that were unstipulated gathered from mouth of patients having oral conditions like advanced periodontitis, gingivitis and from individuals with healthy oral conditions at Watim medical and dental college Rawalpindi at September 2019 to January 2020. The clinical parameters that were measured during this case study were bleeding on probing (BOP), plaque index (PI), clinical attachment level (CAL) and probing pocket depth (PPD) with the help of a Williams probe. EGF levels were recorded by using enzyme-linked immune sorbent assay (ELISA). For data analysis and evaluation, One- way ANOVA was used.

**Results:** In Individuals with healthy oral conditions, EGF levels were noteworthy higher (99.00) as compared to patients suffering from gingivitis (62.49). EGF value was still higher in patients with gingivitis as compared to patients with advanced stage periodontitis (37.12) ($P<0.001$).

**Conclusion:** The decline in mean levels of EGF in individuals suffering from periodontal disease may be due to periodontal diseases.

**Key Words:** Gingivitis; Epidermal Growth Factor; Salivary Proteins and Pep-tides;Periodontitis and Salivary Glands

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## INTRODUCTION

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In the whole world, periodontal disease is considered to be the most prevailing oral problem[1,2.] Presence of deep periodontal pockets are indication of Advanced periodontal disease (≥5 mm), that influence 10–15% of adolescents globally[3.] Some researchers say that this periodontal disease has some risk factors involved, like individual poor oral hygiene, stress, usage of tobacco and alcohol and diabetes.

Chronic periodontal disease on the other hand is linked with interaction between the host's inflammatory responses and gram-negative bacteria that eventually lead to tissue destruction and finally end with tooth loss[4-6.] Microorganisms play a vital role in periodontitis pathology. Clinically, extend and severity of disease depends on host immune responses to gram negative bacteria There is release of pro-inflammatory mediators by immune cells of periodontium to fight against periodontal pathogens[7.] Cytokines plays a key role among all the immunologic and inflammatory

mediators in the saliva as well as in gingival crevicular fluid (GCF). Epidermal growth factor is an important multifactorial cytokine along with biological effects. A polypeptide molecule EGF, has its unique property of aiding in wound healing and epithelial growth. Its mechanism of action is that it attaches itself on cell surface receptors. In the gingival epithelium, EGF receptors are present in great quantity on basal cell surface[8].

Periodontal disease can be defined as mixture of acute and chronic inflammatory reactions of body as a result of bacterial invasion. The detection of disease is done on basis of extracellular matrix destruction as well as bone resorption. Due to increased activity of proteinases, there is brutal destruction of periodontal tissues. Proteinases are derived from gelatinase and collagenase since as EGF is a potent activator of gelatinase as well as collagenase, its presence in saliva and tissues of gingiva showed confirmation [9,10]. Moreover, during inflammatory process, expression of gingival receptors of EGF was found. Therefore, EGF proved to be the main mediator in periodontal pathogenesis [11]

In periodontal disease, the important source that could serve for evaluation of inflammatory mediators includes saliva, GCF and urine. According to the research work done by Laurina et al., in case of periodontal disease there is quantitative and qualitative relationships of expression of cytokines, growth factors, apoptosis and defensing[12]. The results of his case research depicted that large number of epithelial cells cause expression of IL-10 in individuals suffering from periodontitis.

According to the Oxford et al. research, study there was decline in mean EGF serum levels in individuals suffering from diabetes [13]. Their study proved that it was due to decline levels of EGF that was responsible for systemic and oral health destruction especially in diabetic patients.

This case study also analyzes the salivary EGF levels in individuals suffering from gingivitis and severe periodontitis and also compared these individuals with healthy controls. The main hypothesis that was under test was that decrease in EGF expression might cause bone loss of high degree.

# MATERIALS AND METHODS

**Study Population**: This cross-sectional study was performed on samples obtained from patient's saliva who were healthy as well as one having oral conditions like gingivitis and advanced periodontitis at Watim Medical and Dental College Rawalpindi.

This case study included about 11 individuals having periodontitis of advance stage, 17 individuals suffering from gingivitis and 20 individuals having optimum oral conditions, free from disease. The sampling was done non-randomly. The selection of patients was made on basis of following criteria:

**Inclusion Criteria**: In case of advanced stage periodontitis, the inclusion criteria include attachment loss ≥5 mm, bone loss visible on radiograph and systemic health. In case of gingivitis, the inclusion criteria include age range between 16–55 years, no radiographic bone loss, depth of periodontal pocket ≥5 mm and systemic health.

**Exclusion Criteria**: This include patient history suffering from systemic disease that has its effects on periodontal tissues, antibiotic intake history in the past month, history of positive periodontal treatment in past, any prophylactic procedures performed, stubborn patient, compliance and pregnancy.

**Registry of Clinical Findings:** With the help of Williams probe, the clinical parameters like BOP, PI, CAL and PPD were recorded (Hu-Friedy, Chicago, IL, USA). By spitting, salivary samples (unstimulated )were taken from the patients between 9 to 11 a.m. The test samples were shifted to Eppendorf tubes, immediately that consist of Tris-HCl buffer solution because of fear of disruption of the test results as samples contain protease enzymes (Figure 1).we prepared the buffering solution by dissolving 1.18 g of Tris (hydroxymethyl) amino- methane in water 80 mL in a flask of 100-mL flask. By the use of 0.5 mmol/L hydrochloric acid, the PH of solution was maintained at 7.8. Finally, with the use of distilled water, the **vo**lume was adjusted at 100 mL.The preparation of solution this way aids in its stability and also increases its shelf life for 6 months at - 4°C. By transferring 300 μL of the solution to each Eppendorf tube, we can achieve same ideal conditions for all the rest of samples. Observation of these samples was done for about half an hour before they were submitted the laboratory. All samples were at 4°C in refrigerator -The samples were further preserved in laboratory at -20°C until sufficient samples were taken with the aid of ELISA kit. Then, evaluation of each sample was carried out.

**Procedure Steps:** The steps followed during the procedure were as follows:

1. In the buffering solution the antigen that was dissolved was transferred to the respective wells with the help of sampler
2. The antigens in smaller quantities were absorbed by plastic surfaces.
3. Antigens that were free were separated by rinsing. Neutral protein was used to avoid binding of other proteins.
4. All the unbounded proteins were washed away.
5. Addition of another binding agent was done at this stage that has ability of antibodies identification. There was also covalent bond found between binding agent and enzyme like peroxidase. The binding molecule has ability to attach to the antibody under test.
6. The antibodies not attaching the antibody were all rinsed away.

7. Addition of coloring agent was done. This agent has ability to change itself into a colorful material as a result of enzymatic reactions so that we could identify the complex.
8. The antigen concentration was recorded was by scanning colored product optical density.

**Statistical Analysis:** For data analysis SPSS 24.0 was used. The mean salivary EGF level and mean age of patients were analyzed and documented. one-way ANOVA was applied for observing differences in EGF levels between patients suffering from periodontitis advance stage, patients suffering from gingivitis and individuals with in healthy oral conditions. Statistical significance was defined at $P \leq 0.05$.

# RESULTS

The mean levels of salivary EGF in this case study were observed and documented in patients having pathologies like gingivitis, periodontitis at advance stage and individual with healthy oral cavity. As recorded from our tests, the mean age of patients having periodontitis of advance stage, gingivitis and individual with healthy oral conditions was 47.18 (SD=6.5), 31.13 (SD=1.4) and 31.24 (SD=4.4) years, respectively.

**Table No.1: Pair wise comparison of groups**

| Group 1 | Group 2 | Mean | P |
|---|---|---|---|
| Healthy controls | Gingivitis | 6.97 | 0.001 |
| Healthy controls | Periodontitis | 24.10 | 0.001 |
| Gingivitis | Periodontitis | 16.00 | 0.001 |

**Table No.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 | 20 | 99.00 | 28.12 | 6.72 | 84.58 | 113.88 |
| Gingivitis | 17 | 62.49 | 19.62 | 5.18 | 51.02 | 76.27 |
| Periodontitis | 20 | 37.12 | 9.18 | 2.85 | 30.00 | 43.07 |

**Table No.3: Pairwise comparison of groups using the post hoc Tukey's test**

| Group1 | Group2 | Mean | P |
|---|---|---|---|
| Healthy | Gingivitis | 38.93 | 0.001 |
| Healthy | Periodontitis | 63.12 | 0.001 |
| Gingivitis | Periodontitis | 25.40 | 0.14 |

Significant difference was recorded among three groups when pair wise comparisons done with post hoc Tukey's test. (P<0.001) (Tables 1). The mean salivary level of EGF in patients suffering from gingivitis. Periodontitis of advance stage and individual with healthy oral conditions was 62.49±19.62, 36.14±9.17 and 99.00±28.12 ng/mL, respectively (Table 3).

Moreover, when comparison was made with the post hoc Tukey's test also showed a noticeable differences between healthy individuals and individuals gingivitis (P<0.001), healthy individuals and individuals with periodontitis (P<0.001) and individuals with gingivitis and those having periodontitis (P=0.14); i.e. the mean levels of EGF in case of healthy individuals in was notably higher as compared to individuals with gingivitis; and it was observed that EGF value in patients suffering from gingivitis was noteworthy greater when comparison was made with individuals suffering from pe-riodontitis (Table 3).

# DISCUSSION

With the help of the ELISA technique, EFG levels in saliva were detected in normal individuals as well as in individuals suffering from periodontitis and gingivitis. The benefit of using The ELISA test as compared to other tests was that it is cost- friendly, scientifically very accurate and implementation is way too easy as it does not require any complicated and expensive tool. It has similarity with other radioimmunoassay tests but the only difference between ELISA and other radioimmunoassay test is that it involves the use of color change reaction because of the action enzyme over substrate rather than radioisotope work as indicator. In our current studies, ELISA was used to obtain data which helps in making our study reliable from future point of view.

From the results of our study, the mean salivary levels of EGF observed and came out to be 62.49, 37.12 and 99.00 ng/mL for gingivitis, periodontitis and healthy individuals, respectively. From results it was clearly noted that EGF salivary levels were lowest in patients with periodontitis of advance stage as compared to individuals with healthy oral conditions and the one gingivitis. This trend showed that that as the disease progressed from gingivitis to periodontitis, there was decreasing value of salivary EGF was observed.

The studies by some researchers showed that in animals, EGF present in the saliva can be taken up systematically by the intestines and mucosa of oral cavity.[15]

Moreover, high salivary levels of EGF aids in enhancing the heeling mechanism in injured parts by attaching itself to receptors of EGF and by activation of tyrosine kinase pathway. In addition, by more and more attachment of EGF to its receptors there was activation of different biological effects such as angiogenesis, epithelial proliferation, and gastric juice release inhibition. So, it was seen that with the temporary increased in levels of salivary EGF, there was increase in oral mucosal injuries.

The levels of salivary EGF were recorded by Oxford et al. pre and post oral and juxta-oral operations The unstimulated salivary flow was obtained approximately at different intervals. The samples of saliva were collected from patients who were candidates for periodontal surgery before and after 6-, 12-, 18-, 24-, 30-, 36- and 42 hours and 2 weeks after the surgery. Then afterwards, the mean levels of salivary EGF were analyzed by the Quantikine Human EGF Immunoassay. The values from all these tests clearly showed that local cells have ability to produce as well as to secrete growth factor at the operated sites. So increased in secretion and production of salivary EGF was observed at operated site that ultimately aided in wound healing and repair. Growth factors play a vital biological role in regulation and proliferation of connective tissue cells and generation of protein synthesis as well as other extracellular matrix constituents. The target cells reaction to growth factors relay upon their particular receptors expression; they are considered as membrane antigens that help in production of intercellular signals, the time they attach themselves to growth factors and results in chemo taxis stimulation as well as, cellular growth, cellular differentiation and synthesis of the extracellular matrix[5.] This is due to this reason, growth factor receptors play a vital and important role in the starting and progression of periodontal disease and as well as in regeneration process[2.] Also, from some researchers EGF proved to play a key role in wound healing[13,14.] The researches thought that this wound healing property of EGF might be because of the biological factor that controls the pathogenesis involved in periodontal disease. It was also shown by various researches that decline levels of EGF specially in patients suffering from diabetes mellitus is the main cause of destruction of peridontium[9,13.] This finding supports our current study research.

Gelatinase, prostaglandin E2, Collagenase, activators, TNF, IL-1 and plasminogen has a key involvement in destroying peridontium. It was also proven that EGF was considered to be important regulator in periodontal pathogenesis. Thus, the researchers believe that in future a strong focus should be paid on gingival receptors specifically regarding EGF or other cytokines expression involved in various periodontal pathogenesis.

# CONCLUSION

In a nutshell, it was concluded that main differences were seen when comparison was made between three groups of patients with respect to salivary level of EGF, and proved that with the progression of periodontal pathologies, there was decreasing trend in salivary EGF was observed and noted So decrease in the mean levels of salivary EGF is an important mechanism linked with periodontal destruction.

**Author's Contribution:**

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|---|---|
| Concept & Design of Study: | Saqib Ghafoor Kayani |
| Drafting: | Syed Ali Asad Raza Naqvi, Muhammad Farooq |
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| Revisiting Critically: | Saqib Ghafoor Kayani, Syed Ali Asad Raza Naqvi |
| Final Approval of version: | Saqib Ghafoor Kayani |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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