Ministry of Higher Education & scientific research

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Association between the levels of interleukin-8 in gingival crevicular fluid and generalized periodontitis

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الاهداء

الى من خلقني فسواني .....الى من

بلطفه وكرمه احياني .....الى من

ارشدني وهداني .....الى من

بذكره تطيب النفوس ..... وتطمئن القلوب

ربي

اليك ياسندي في هذه الحياة.....اليك يا تاج الزمان وكل الحنان..... اليك يامن زرعت

في طموحاً ..... صار يدفعني نحو المستقبل

اليك يا اعذب كلمه نطق بها لساني .....

_ ابي الحبيب _

الى ملاكي الجميل وحبي الذي لا يزول .... رمز الحنان والعطاء ..... الى ربيع حياتي وملكه قلبي .....كل الحب والوفاء الى شمسي المشرقه وبدري المنير ..... اجمل عطايا الرحمن

_ امي الحبيبه_

الى اجمل كواكب في سمائي ..... الى اعز مافي حياتي.

_ اخوتي واصدقائي _

الى كل من علمني حرفاً وانار امامي طريق المستقبل ..... الى من بهم جننت واليهم انتميت .... الى اساتذتنا الاعزاء اهدي لكم ثمرة ما صنعت ايديكم ..

_اساتذتنا الأعزاء _

Abstract

In periodontal diseases, inflammatory mediators, including interleukin IL-8 may promote the degeneration of inflamed periodontal tissues. In previous studies, levels of IL-8 were demonstrated to be elevated in inflammatory gingival tissues and gingival crevicular fluid. The aim of the present study was to quantify IL-8 levels in the GCF of patients with generalized periodontitis and compare it with healthy subjects. In this study, GCF samples were collected from 40 patients with generalized periodontitis and 10 healthy subjects. Enzyme-linked immunosorbent assay (ELISA) was used in the measurement of IL-8 levels. The mean levels of IL-8 were significantly higher ($p<0.001$) in periodontitis patients than in healthy subjects. These results suggest that the levels of IL-8 in GCF may be relevant in the pathophysiology of periodontitis, and the measurement of this cytokine may be beneficial in the identification of patients with periodontitis.

Introduction:

Interleukin 8 (IL-8 or chemokine (C-X-C motif) ligand 8, CXCL8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Endothelial cells store IL-8 in their storage vesicles, the Weibel-Palade bodies. In humans, the interleukin-8 protein is encoded by the

CXCL8 gene. IL-8 is initially produced as a precursor peptide of 99 amino acids which then undergoes cleavage to create several active IL-8 isoforms. In culture, a 72 amino acid peptide is the major form secreted by macrophages.

IL-8 is an important protein related to inflammation, where it plays a key role in the recruitment of ne be utrophils and other immune cells to the site of infection.

Interleukin-8 (IL-8) is an important chemokine of interest in periodontal diseases. IL-8 is a potent chemoattractant cytokine and activator of neutrophils in inflammatory regions, which is released from endothelial cells, gingival fibroblasts, neutrophils, monocytes, and phagocytes in the gingival crevice. The unique coordinated expression of IL-8 facilitates the transit of neutrophils from the highly vascularized gingival tissue to the gingival crevice.

Gingival crevicular fluid (GCF

A mixture of different substances derived from host inflammatory cells, serum, microbes and structural cells of the periodontium is collectively known as gingival crevicular fluid (GCF). GCF is derived from adjacent vessels of gingival plexus and travels through the junctional epithelium and the external basement membrane to appear in the gingival sulcus. It can be extracted in trace amounts from healthy gingival sulcus.

GCF isolated from healthy gingival sulcus results as a consequence of shift in osmotic gradient (Alfano, 1974). GCF contains substances released due to the inflammatory response. Information regarding these substances can be beneficial in assessing disease status and/or therapeutic outcomes (Toker et al., 2006.

Biomarkers in GCF can be identified to diagnose and monitor periodontal disease (Loos & Tjoa, 2005). The easy and non-invasive collection of GCF and the diverse array of biochemical and cellular molecules isolated from GCF explain its diagnostic potential. In healthy gingival sulcus, GCF exists as transudate, whereas in case of periodontal disease, it exists as an inflammatory exudate, which contains substances derived from the serum, periodontal tissues and colonizing periodontopathogens (Delima et al., 2003). GCF collected from specific sites can be used to assess the microbial relationships with the host mediators (Shimada et al., 2013.

GCF has been previously used as a rich source of biomolecules which represent periodontal disease status (Bakri et al., 2013; Luo et al., 2011). More than 65 oral biomarkers representing possible disease progression have been identified from GCF samples. These biomarkers include host-response modifiers, inflammatory mediators, tissue breakdown products, host-derived enzymes and their inhibitors (Khongkhunthian et al., 2014.

The proximity of GCF to the diseased periodontal tissues increases its ability to provide more relevant information as compared to saliva. As saliva originates from salivary glands it is suspected that it could better serve as a predictor of salivary gland diseases rather than periodontal disease (Ozmeric, 2004).

Methods of GCF collection:

Intracrevicular washing technique

One of the sampling techniques used to collect GCF employ two injection needles, in which one needle is placed inside another needle. During sampling the 18 gauge “collection needle” is placed at the gingival margin whereas a 27 gauge “ejection needle" is positioned at the deepest point in the gingival sulcus. An incessant suction is used to collect the expelled solution from the crevice (Salonen & paunio, 1991).

Microcapillary technique

Different types of microcapillary pipettes are used to collect the GCF. These include non-calibrated pipette of known volume and calibrated volumetric pipette (Figure 1). To collect the sample without causing an irritation to gingival tissues, supragingival plaque is carefully removed by cotton pellets or suitable instruments. Then cotton rolls or swabs are used to achieve isolation and

then the oral cavity is gently dried with air. Following the extracrevicular approach, volumetric micro-capillary pipette is used to collect one, two, or three µl of GCF as required. GCF samples that had an inadequate volume and/or were suspected to be contaminated are excluded.

Figure 1: Microcapillary pipettes (Drummond 2µL)

Absorption technique

Paper strips (Figure 2) or paper points (Figure 3) have been mainly used to perform this technique. Briefly, cotton rolls or swabs are used to achieve isolation and then the oral cavity is gently air dried. Then, paper points or strips are positioned in the deepest point in the sulcus or pocket until minimum resistance for 30 seconds. Contaminated paper points or strips are ideally discarded.

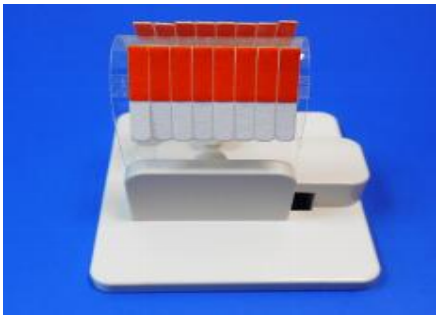

Figure 2: Periopaper, Oraflow

Figure 3: Absorbent paper points

Materials and methods

Periodontal examination

Periodontal examination was performed to enable categorization of the selected subjects into two groups (healthy and generalized periodontitis). Six sites per tooth were scored using a Williams's periodontal probe (Hu-Friedy, Chicago, IL), which were: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual for the entire dentition; and the third molar was excluded. This was performed by measuring the following periodontal parameters:

1_ Plaque Index (PI) The method of Silness and Loe (1964) was followed in recording the plaque index.

2 -Bleeding On Probing (BOP)BOP was measured using Ainamo & Bay's (1975) index. Williams's periodontal probe was inserted in the bottom of the gingival pocket or sulcus. If there was no bleeding, a score of zero was given; whereas if bleeding ensue within 10-15 seconds, a score of one was given.

3 -Probing Depth (PD)This index was performed by measuring the linear distance between the crest of the gingival margin and the point where the probe first meets resistance. PD was recorded in millimetres .

4 -Clinical Attachment Loss (CAL (CAL was measured by determining the linear distance from the cementoenamel junction to the base of the periodontal pocket.

Gingival crevicular fluid collection

Following site selection, the sites were isolated with cotton rolls and the tooth surface were air dried to avoid contamination with saliva. The GCF samples were collected using absorbent paper points size 30, which were placed into the sulcus / pocket

until mild resistance was felt and then held in place for 30 seconds. Paper points that contaminated with saliva or blood were excluded. Four paper points were obtained from each subject.

All the paper points with GCF were immediately placed into a sterile polypropylene tube and kept at −20°C until further analysis. Then, 100 µl of phosphate buffer saline was added to each tube and the tubes were shaken gently for 1 hour at room temperature.

Enzyme-linked immunosorbent assay (ELISA) procedures

The manufacturer protocol followed to calculate the level of IL-8.

Results

Socio-demographic data of study sample

The demographic data of the study groups shown in table 1. As its clearly shown the age range for the patients with periodontitis is more than those of health subjects (42.861± 12.259, 22.500±0.7559), respectively. Moreover, the gender of the participants show more female involvement than male in both healthy and periodontitis groups (75%, 61%), respectively.

Table 1: The demographic data for the periodontitis patients and control subjects:

Criteria	Patients with	Control subjects

	Generalized periodontitis	
Age	42.861± 12.259	22.500±0.7559
Gender	39% male 61% female	25% male 75% Female

Table 2: clinical periodontal parameters (plaque index, BOP, PPD and CAL) for the periodontitis patients and control subjects:

Clinical parameters	Patients with Generalized periodontitis	Control subjects	t- test	p- value
Plaque index	1.2527±.36436	0.6535±.21544	4.44509.	0.000032.
Bop	0.3306±0.34975	0.00±0.00	2.64855.	0.005712.
Ppd	3.2472±0.62393	1.2163± 0.73868	8.06921.	< .00001.
Cal	2.6840±1.08523	0.00±0.00	6.93066.	< .00001.

Comparison of IL-8 levels between healthy and periodontitis groups

Mann-Whitney U-test was used to show the statistical differences in the IL-8 levels between the study groups. There was high statistical difference between the groups ($p<0.000$) as shown in figure 4.

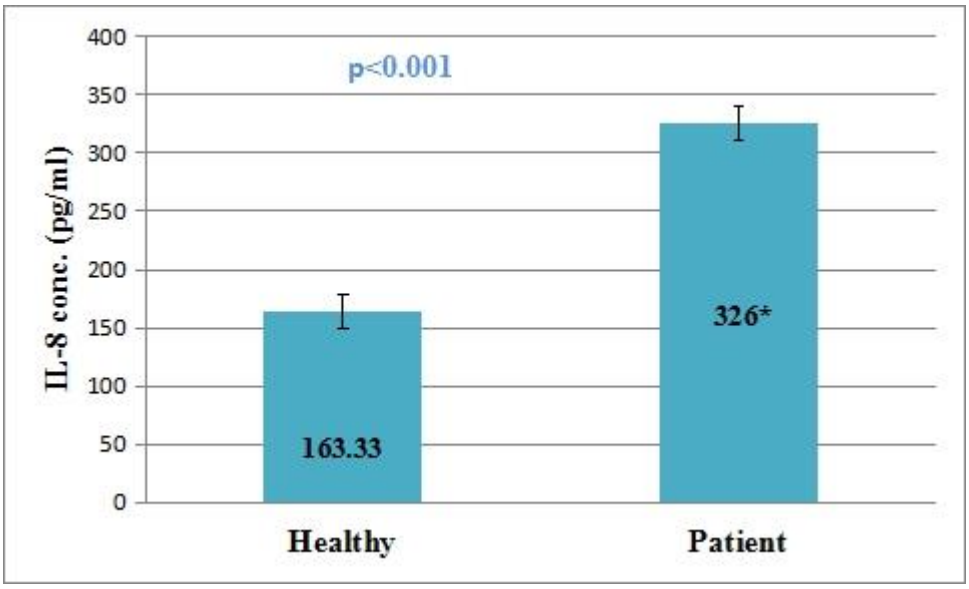

Figure 4: IL-8 levels in GCF of healthy and patients with generalized periodontitis.

Discussion

Periodontal diseases may lead to chronic inflammatory conditions and the destruction of the supporting structures of the

dentition. Periodontal diseases are caused by gram-negative bacterial infections, which produce lipopolysaccharides (LPS) (Van Dyke TE and Serhan CN, 2003). The bacterial infection and inhabitation is the etiological factor of periodontitis, due to the fact that the host response and immune reaction to such infectious organisms mediate the development of the periodontitis, rather than infection itself. Previous studies have revealed the roles of IL-8 (Seymour GJ and Gemmell E, 2001) as proinflammatory cytokines. Consistent with other inflammatory diseases, proinflammatory cytokines, including IL-8, appear to be major mediators in periodontitis (Bastos et al., 2009). Therefore, in the current study, the levels of IL-8 expressed in the human GCF of patients with periodontitis were investigated.

IL-8 is an established chemotactic protein and is released in inflamed human gingival tissues (Okada H and Murakami S,2009). Gingival fibroblasts affected by infectious organisms express higher levels of IL-8 mRNA in comparison with the baseline expression (Botero et al., 2008). Our data showed that IL-8 was highly expressed in the gingival tissues of all the patients with periodontitis. The present results were consistent with those from previous studies.

Conclusion

It was concluded based on the results of this study, that the increased levels of IL-8 in periodontitis may have diagnostic and prognostic potentials for the monitoring of the disease and the therapeutic decisions.

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