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RESEARCH ARTICLE

ASSESSMENTS OF IL-20 LEVELS PRESENT IN SALIVARY AND GINGIVAL CREVICULAR FLUID IN NON-SMOKER CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Background: Chronic periodontitis is inflammation of the tissues surrounding tooth structure. Several cytokines are involved in pathogenesis of periodontitis. For example, IL-20 promotes inflammation, angiogenesis, chemotaxis and regulates osteoclast differentiation. **Objectives:** the purpose is to evaluate the IL-20 levels found in salivary and gingival cervicular fluid (GCF) in healthy and chronic periodontitis patients. **Methods:** GCF and Saliva samples obtained from systematically free adult individuals. They were divided into ten patients with moderate and severe chronic periodontitis and ten periodontally healthy subjects. The GCF and saliva samples from chronic periodontitis patients were collected before and after nonsurgical periodontal therapy. All GCF and saliva samples were stored at -80°C till laboratory analysis. IL-20 levels were measured by ELISA according to manufacturer instructions. **Results:** IL-20 levels were significantly higher in patients with chronic Periodontitis compared with healthy periodontal patients (T=2.712, P≤0.010). However, the levels of IL-20 reduced after non surgical Periodontal treatment, but this difference was statistically insignificant (T=1.859, P≤0.079). **Conclusions:** We have shown that IL -20 may play a role in Chronic Periodontitis patients.

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INTRODUCTION

Chronic Periodontitis is one of the most common oral diseases. It means inflammation of the tissues around the teeth, and it is occurred by accumulation of dental plaque, which is the main etiological factor of periodontitis. It is portrayed by many signs and symptoms, which could appear together or alone. For example, redness of the gingival color ,gingival bleeding during brushing of teeth or dental flossing , even during hard food biting, gingival swelling, bad mouth smell, soft or hard deposit present on the tooth surfaces, gingival recession, deep pocket depth , attachment loss of alveolar bone that found during clinical examinations and x-ray of patients teeth at the end. (Michael G. Newman *et al*, 2015). There are many local and systemic causes of chronic Periodontitis, but the major risk factors are Presence of dental plaque, smoking, systemic diseases like diabetes mellitus and bad oral hygiene .

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(Christopher J *et al*, 2015). There are two types of cytokines that are involved in progression of periodontitis. The pro inflammatory cytokines include interleukin -1 beta (IL-1β), IL-6, tumor necrosis factor- alpha (TNF-α) and IL-17, while the anti-inflammatory cytokines involve IL-4 and IL-10. (Zhang JM *et al*, 2007; Moore K.W *et al*,2001 ; Rousset F *et al*, 1992 ; Thompson-Snipes L *et al* , 1991 ; Moore K.W *et al*,1990 and Go N.F *et al* 1990). IL-20 is considered as one of the member of the IL-10 family due to the similarity in the amino acid composition between them. (Chi-Chen Wei *et al*, 2006). IL20 is a pro-inflammatory cytokine, which plays a role against the inflammatory diseases, such as rheumatoid arthritis (RA). IL-20 has many functions in human body. Reinforce inflammation, angiogenesis, chemotaxis and control osteoclast differentiation, for example. (Hsu YH *et al*, 2011). IL-20 plays a role in stimulating the body's immunity because it controls reproduction and differentiation of keratinocytes during inflammation. (Hofmann MA *et al*, 2016 and Albanesi C *et al*, 2005). There are many studies that have been conducted about deficiency of pro-inflammatory biomarkers production with smokers who suffer from chronic

periodontitis (Keelen D *et al* , 2011 and Zhong, H *et al* , 2007; Alfaqeeh SA *et al* , 2014; M.A. Palmer *et al* , 2005 and Matesanz-Pérez P *et al* 2013)). There is only one study that measured the level of IL-20 among non smoker patients with chronic periodontitis and the samples in this study were taken from the blood serum. Results of this study showed that IL-20 plays a role with moderate and sever chronic periodontitis patients (WANG Zuomin *et al*, 2010)**.** The treatment plan of chronic periodontitis are divide into non-surgical (patient education, motivation, scaling and root planning) and surgical treatment. (O'Leary TJ *et al*, 1972). According to our knowledge there are no available studies about the level of IL-20 present on the saliva and GCF fluids among non smoker who periodontally healthy and non smokers who have chronic periodontitis. Therefore, the present study was designed to investigate the levels of IL-20 in non smoker chronic periodontitis patients before and after non-surgical therapy.

Aims

The aims of the present study are to:

- Evaluate the level of IL-20 that is found in saliva and gingival crevicular fluid (GCF) in healthy and diseased chronic periodontitis patient.
- Investigate the effect of IL-20 present in saliva and GCF fluid that cause chronic periodontitis.
- Investigate the level of IL-20 after periodontal non-surgical therapy for chronic periodontitis patient.

MATERIALS AND METHODS

The study method was a control trial, to determine the levels of IL-20 in saliva and GCF of chronic periodontitis patients before and after one month of the non-surgical treatment. The patients on this study have been selected from screening unit of Umm Al-Qura dental teaching hospital after ethical approval was obtained from the Institutional Review Board of Faculty of Dentistry (IRB) in Umm Al-Qura University, and the consent for participation in the study was obtained from the patients. GCF and Saliva samples were obtained from the patients who met the specific inclusion criteria which are, systematically health patient, age between 20 – 60 years, periodontally diseased patients who have moderate to severe chronic periodontitis: PD $\geq$ 3, CAL $\geq$ 3 and non smokers. Immuno-compomised patients, Pregnant women, Smokers patients, gingivitis patients , aggressive periodontitis patientst , mild chronic periodontitis patients , patients on NSAID drugs and patients who received periodontal therapy in duration of one year or less were excluded from this study. The systemically healthy individuals were divided into two equals groups. Group one consist of ten patients with moderate and severe chronic periodontitis, and group two consist of ten periodontally healthy subjects. All individuals were informed about the course of periodontal treatment, and they signed on consent form. After patients signed on consent form, the researchers took full medical and dental history with full mouth probing depth (PD) at six surfaces around the teeth, Plaque index, clinical attachment level (CAL) and presence or absence of bleeding on probing measured by using a William's periodontal prop. Patients without a history of periodontal disease were included in the periodontally healthy groups. (Karam, T.A., 2013)Patients have PD $\geq$ 3, CAL $\geq$ 3 were selected to the chronic periodontitis group (Gerardo Mendoza-Azpur *et al*, 2015).

The periodontal status of the patients are evaluated by using the following clinical parameters:

- ***Plaque index*** (O'leary TJ *et al*, 1972 and Ainamo, J. Bay I, 1975): simplified plaque index by using of disclosing agent that apply on all teeth, the positive staining sites will be count in relation to all examined periodontal sites to obtain the percentage (%) of plaque accumulation.
- ***Bleeding index*** (Ainamo, J. Bay I, 1975): is performed through gentle probing of the sulcular line of the gingival crevice. If bleeding occurs within 10 seconds a positive finding is recorded and the number of positive sites is recorded and then expressed as a percentage (%) of the number of sites examined.
- ***Probing pocket depth***: by using of William's periodontal probe, to measure the depth of periodontal pocket from six sites (mesio-buccal, disto-buccal, mid-buccal, miso-lingual and disto-lingual). The mean pocket depth by dividing the total accounts of all pocket depths on the number of examined sites in millimeters (mm).
- ***Clinical attachments loss (CAL)*** (Ramfjord S *et al*, 1968): recording of the clinical attachments loss will be based on the location of gingival margin as following:
- When the gingival margin on enamel: the CAL will be obtained by subtracting the cemento-enamel junction distance from the probing pocket depth.
- When the gingival margin on cemento-enamel junction: the CAL will be equal to the probing pocket depth.
- When the gingival margin on the cementum: the CAL will be recorded by adding the gingiva margin cemento-enamel junction distance and probing pocket depth.

The evaluation of clinical attachment loss by the followings:

- **The mean of CAL:** the result obtained by dividing the accounts of all CAL measurements on the number of all examined periodontal sites in mm.
- Severity of CAL: according to percent (%) of sites measurement; mild (1-2 mm), moderate (3-4 mm) and sever (≥5mm).

Samples collected: The GCF and saliva samples were collected from periodontally healthy subjects and chronic periodontitis patients before and after one month of periodontal therapy. Two samples before and after treatment have been taken from GCF from all periodontally diseased patients. Also, two samples before and after treatment have been taken from Saliva from each periodontally diseased patients. One sample has been taken from GCF and salaiva from healthy subjects. The way of taking GCF samples include 5 micro μL sample of GCF that have been collected by micropipette capillary tube from selected periodontal site, and the samples were placed in tube contain 300 μL phosphate buffer solutions as diluting solution. The way of taking saliva samples involve 5 ml un-stimulated saliva samples that have been collected by using fine syringe from the oral cavity. Then the sample has been placed in Ependorph tube. All GCF and saliva samples were incubated at -70°C.

Statistical analysis

All GCFand saliva samples were measured by using enzyme-linked immunosorbent assays (ELISA) kit for selected cytokine [IL-20 (pg/ml)] levels. Data was statistically analyzed using SPSS v.17. T-test is used to compare IL-20 levels between GCF and saliva samples of chronic periodontitis and periodontal healthy patients. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

There was a statistical difference of IL-20 level between patients with chronic periodontitis compared to healthy periodontally patients (T=2.712, P≤0.010). For patients with chronic periodontitis, there was a slight difference in readings of the IL-20 level before and after non-surgical periodontal treatment, but it was statistically insignificant (T=1.859, P≤0.079).

Table 1. Comparison between different groups to evaluate the level of IL-20

Groups	IL-20 M± SD	Comparison
Healthy	0.0±0.0	Independent
Vs.	Vs.	T-Test:
Chronic Periodontitis patients	0.93 ± 1.534	T=2.712
(Before treatment)		p=0.010
Chronic Periodontitis patients	0.93 ± 1.534	Depended T-
(Before treatment)	Vs.	test:
Vs.	0.805 ± 1.294	T=1.859
Chronic Periodontitis patients		p=0.079
(After treatment)		

DISCUSSION

Chronic periodontitis is one of the most common oral diseases that affect many people worldwide. There are many cytokines that play a role in the chronic periodontitis disease, and one of this cytokine is IL-20. This cytokine is a pro-inflammatory cytokine, which plays a role against the inflammatory diseases. According to our knowledge there is no previous studies that measured the level of IL-20 on the saliva and GCF among non smoker periodontally health and chronic periodontitis patients. The findings of the present study indicated that, there is a statistically difference in the level of the IL-20 among patients with chronic periodontitis when compared to a healthy periodontal patients (Tab1). The study also showed a slight difference in the level of the IL-20 among patients suffering from chronic periodontitis before and after non surgical periodontal treatment, but this difference is statistically insignificant. This insignificance may be due to insufficient number of samples and /or insufficient period of the following the patients after treatment (Table1).

Conclusion

Our results showed that there is an effect for the IL-20, and it may play a role in chronic periodontitis patients. We are advising on doing more research to discover the reasons that lead to the results in our research.

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