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LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN PERI-IMPLANT CREVICULAR FLUID (PICF) OF SMOKERS VERSUS NON SMOKERS (CLINICAL AND BIOCHEMICAL STUDY)

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ABSTRACT

Background: Peri-implant infections occur in response to pathogenic bacterial flora in addition to release of inflammatory markers similarly to periodontal infection, but faster and more intensely. As the smoking is a critical modifying factor for periodontal disease intensity, it can also be considered as a risk factor of periimplantitis.

Subjects and methods: Fourteen participants with endo-osseous root form implant; seven of them are non smokers (group 1) and the other seven are smokers (group 2).Clinical parameters as plaque index (PI), gingival index (GI) and probing pocket depth (PPD) are recorded for each participant and also periimplant crevicular fluid (PICF) samples are collected from all participants to biochemically investigate levels of vascular endothelial growth factor (VEGF).

Results: Clinical parameters as plaque index (PI), gingival index (GI) and probing pocket depth (PPD) were higher in smoker group compared to non smoker group .however, The VEGF levels in PICF were higher in the non smoker group compared to the smoker group.

Conclusion: VEGF is not a highly sensitive pathogenesis maker and can not be used alone to predict periimplantitis.

KEYWORDS: PICF, smokers, VEGF

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INTRODUCTION

The last years has witnessed considerable increased use of dental implants and implant supported fixed restorations like all on four and all on six restorations. Periimplantitis can be described as an inflammatory reaction caused by bacteria which affects peri-impant tissues and that can lead finally to supporting bone loss [1].

Similar to the gingival crevicular fluid, the peri-implant crevicular fluid (PICF) is considered as a collectable fluid that might show health status of tissues around dental implant [2]. Patients suffering from periimplantitis usually express an increase in quantity of PICF together with increased inflammatory markers which may indicate active periimplantitis [3,4].

Since periimplantitis might not be clearly obvious in its initial phases, patient vulnerability depends mainly on presence of specific inflammatory markers in PICF which helps to early detect possibility of future periimplantitis in such patients. Presence of these mediators or markers can also help in detecting usefulness of different treatment options which can direct the implantologists to continue with a certain treatment option or shift to another [5].

Angiogesis and vasculogenesis are two important continuously occurring biologic processes which are controlled strategically by many mediators such as cytokines and growth factors .Vascular endothelial growth factors (VEGFs) are some of these strategic controllers [6].

New blood vessel formation, endothelial cell proliferation, regulation of vascular permeability, maintenance of bone homeostasis, monocyte/ macrophage alignment, neuroprotection and preservation of tissue barrier defensive functions are among the physiologic roles of VEGFs [7, 8]. It was shown by ***Wang et al.*** [9] that VEGF increases in the peri-implantitis affected implants due to its role in the formation and attraction of osteoclasts which are important cells in periimplantitis.

Aim of the work:

To detect levels of VEGF in PICF of smokers versus non smokers.

SUBJECTS AND METHODS

Ethical approval:

In agreement with Helsinki declaration, [10] and also the SPIRIT guidelines for reporting clinical trials [11]. The current study protocol was accepted by the ethics committee for scientific research Faculty of Oral and dental medicine, Future University in Egypt with approval number (FUE.REC(14)/10-2021) and registered in the US national institute of health clinical trials registry (clinicaltrials.gov, identifier: NCT05325918).

Subject recruitment:

The present clinical trial included 14 participants; each participant received a single endo-osseous root form implant 6 months prior to clinical measurements and PICF collection. Participants were chosen from the outpatient clinic of the Oral Medicine, Diagnosis and Periodontology Department, Faculty of Oral and Dental Medicine, Future University in Egypt. The detailed procedures were clearly described in details to the participants after their approval of written informed consents.

The patients were categorized in 2 groups as following:

Group 1: Non smokers.

Group 2: Smokers.

-Inclusion criteria:

Age range between 30 and 60 years old; American Society of Anesthesiologists class1 (ASA-1) patients who received a single endo-osseous root form implant 6 months prior to

clinical measurements and PICF collection and not complaining from any systemic disease; no current use of medication or alcohol that could jeopardize results; absence of periodontitis or other mucosal and bone tissue lesions in addition to absence of signs of periimplantitis.

- Exclusion criteria:

Pregnant and lactating females.

Patients with periodontal inflammations next to the implant site.

Periodontal clinical parameters

Clinical measurements like full-mouth plaque index (PI), gingival index (GI) and probing pocket depth (PPD) measurements were recorded for six sites per implant (mesiobuccal, midbuccal, disobuccal, mesiolingual/palatal, mid lingual/palatal and distolingual/palatal aspects). A William graduated periodontal probe was used to assess PPD (Hu-Friedy Co., Chicago, IL, USA).

PICF collection

To avoid contamination with blood, Clinical recordings were taken after PICF samples' collection (Figure 1) from the mesiobuccal site following the careful isolation with cotton rolls, and gentle air drying using an air tip to limit salivary contamination. The collection was done using Periopaper strips (Interstate Drug Exchange, Amityville, NY, USA), where the strips have been introduced into peri-implant sulcus till mild resistance was sensed for duration of half minute. Samples were then directly transferred to an Eppendorf containing 200μL phosphate buffer saline for elutionin which they are kept frozen at -80°C for further analysis. Blood contaminated samples were discarded.

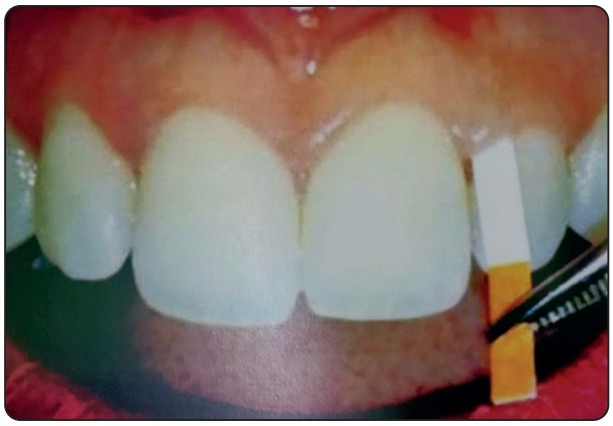

Fig. (1): PICF sample collection from mesiobuccal aspect of upper left lateral implant site using periopaper strip

Biochemical analysis

Following the manufacturer's instructions, enzyme-linked immunosorbent assay (ELISA) test kits were used to investigate VEGF levels (Figure 2).

The PICF testers and standards were added to microtiter plate precoated with an analogous antibody and then incubated and the residuals were washed away. Antibodies labelled with horseradish peroxidase (HRP) were then added for binding and the excess was removed. Repeated washing and addition of the substrate for HRP; tetramethylbenzidine (TMB) were done which produced a bluish material which turns yellow upon adding of the end solution. Finally, the resultant yellow color intensity was proportional to the concentration of VEGF levels.

Statistical analysis

The results are shown as mean ± standard deviation (SD). All statistics were done using Windows version 6.01 of GraphPad Prism software (San Diego, California, USA). To confirm normality, Shapiro-Wilk normality test was used and to compare normally distributed continuous data, unpaired student t-test was used. P-values lower than 0.05 were considered significant.

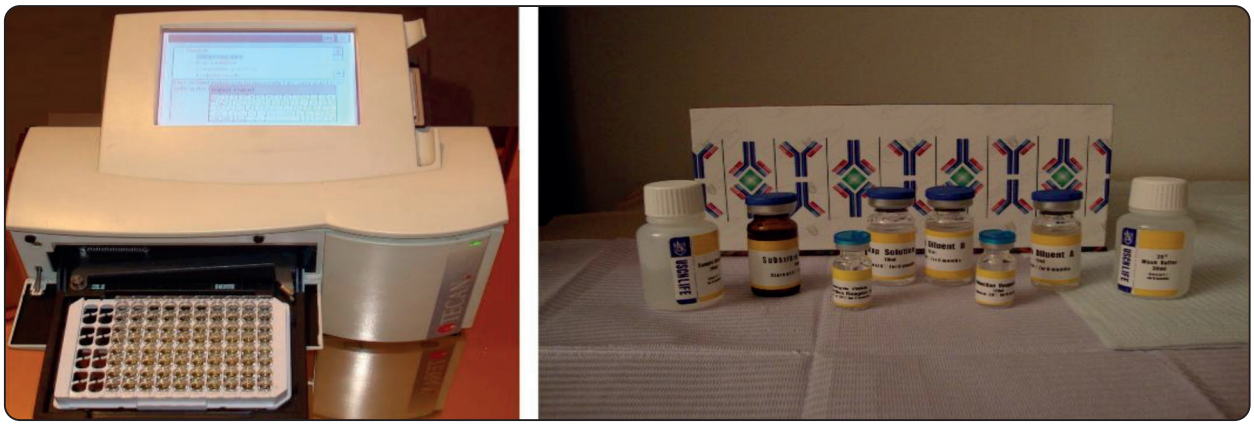

Fig. (2): ELISA reader system and test kits

RESULTS

Although smoker group showed higher clinical parameters more than the non smoker group Table (1), the non-smoker group showed higher statistically significant levels of VEGF as compared to the smoker group (Figure 3).

TABLE (1) Clinical parameters in the study groups

Parameter	Non-smoker	Smoker
PI, mm (mean ± SD)	1 ± 0	2 ± 0.82
GI, mm (mean ± SD)	0.71 ± 0.49	1.57 ± 0.53
PPD, mm (mean ± SD)	2.57 ± 0.79	3.43 ± 0.79

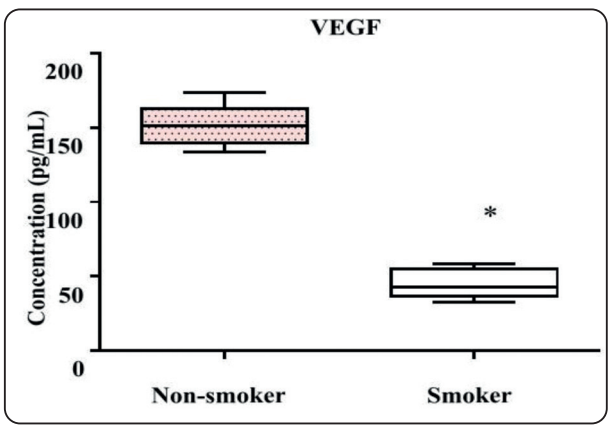

Fig. (3): Vascular endothelial growth factor alpha (VEGF) levels among the study groups.

(*: *p*-value <0.05)

DISSCUSSION

Peri-implant infections comprise peri-implant mucositis and periimplantitis which affect peri-implant soft tissue and bone respectively. Bone remodeling with its resorption and formation phases depend indirectly on angiogenesis. This angiogenesis is controlled to large extent by an important mediator called vascular endothelial growth factor (VEGF) that also encourages endothelial cell proliferation and capillary permeability. This mediator is released locally in a lot of highly vascularized tissues and appears to be a precondition for growth and spread of tumor cells [12].

The current study was attempting detect levels of VEGF in PICF of smokers versus non smokers and it revealed higher levels of VEGF in PICF of non-smokers compared to its levels in smokers.

Although a former study of ***Akbulut. et al.,*** [13] documented similar GCF levels of VEGF in both healthy and diseased periodontal tissues, a study of ***Yılmaz et al.,*** [14] revealed that smoking has decreased levels of VEGF in smoker patients with periodontitis.

In accordance with current study results, study by ***Naresh et al.,*** [15] showed that all clinical measurements; (PI, GI and PPD) were higher in smoker group compared to non smoker group.

In conclusion and within the limitations of the current study, our data confirmed that VEGF can not be used as a sole periimplantitis marker as its levels were lower in smokers. Follow-up studies with a larger number of participants are required to prove whether this molecule could be used as a PICF biomarker of periimplantitis.

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