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A case - control study to evaluate the role of TNF– alpha as proinflammatory cytokine among chronic periodontitis women patients at tertiary care hospital in Tamil Nadu.

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AIM

This study was done to evaluate the role of TNF – α from gingival crevicular fluid among subjects with chronic periodontitis.

Materials and Methods

The study population was divided into two groups (Group 1 : Women with chronic periodontitis and Group 2 : Women without chronic periodontitis) Gingival crevicular fluid samples were taken for assessment based on clinical parameters like probing depth , bleeding on probing , OHI and PI . Data analysis was performed by using SPSS as software for statistics. Mean values were compared among different study groups by using Independent - Sample t-test.

Results

The results of this case-control study which was done among south Indian women population of age group 25-40 revealed a significant increase in the level of TNF - α in gingival crevicular fluid from patients with chronic periodontitis (Group 1 - 502.21pg/ml) when compared to the control group (Group 2 - 399.20pg / ml) and a positive correlation was found betweenmeanTNF - α levels and values of the OHI –I ,plaque index and probing depth (Group 1 Mean TNF – α - 502.21±211.64, OHI -2.85, PI -3.45 and PD - 20.60) in Group 1.

Conclusion

Based on these results, it can be concluded that GCF level of TNF – α was higher in patients with chronic periodontitis when compared to the subjects without periodontitis.

Keywords: Gingival Crevicular Fluid , Plaque Index , Periodontitis and TNF – Alpha.

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INTRODUCTION

Cytokines are a group of low molecular weight proteins used for communication between cells. Cytokines are released as a result of a variety of stimuli, and interact with their receptors to regulate cell function. They are closely related to inflammation. Among the cytokines, TNF - α is considered one of the main cytokines related to inflammation and immune processes, and operates in various parts of the body was discovered by Carswell et al 1975.[1]

TNF-α, along with its close relative lymphotoxin-α (LTα or TNF-β), was first isolated as the active principle causing tumor necrosis in bacterially infected animals Gray PW et al 19842, Pennica D et al 19843 . It is encoded by a single gene on human chromosome 6p within the major histocompatibility complex. The protein product can exist as a 26-kd membrane bound monomer or as a 17-kd secreted trimer .The TNF – α produced acts in local-, para-, juxta-, and/or autocrine effects of TNF- α include stimulation of expression of fibroblast-type collagenase-1 (or MMP-1), collagenase-2 (mesenchymal form of the classic "neutrophil" collagenase, or MMP-8), and collagenase-3 (MMP- 13). All these MMPs are able to degrade the periodontal ligament due to their unique collagenolytic activity.4,5,6,7,8The present study was done to evaluate the role of TNF – α from gingival crevicular fluid among subjects with and without chronic periodontitis.

Subjects and Methods

This case – control study was done among 40 female patients, aged 20–45 years. Thesample size was calculated by using the formula:Where n 1 (each group) = ($Z_{\alpha/2} + Z_{1-\beta}$) ^{2}pq(r +1)/ r ($p_1 - p_2$)^2 .According to the formula[9]the estimated sample size was 19 for caseand 19 for control and the total sample size was 38. Where Two – sided confidence level (1 – α) = 95, Power (% chance of detecting) =80, ratio of controls to cases is 1 , Hypothetical proportion of controls with exposure=40 Hypothetical proportion of cases with exposure = 85.[9]T he patients who have been included in this study have been chosen from among thepatients who are attending the outpatient Department of Periodontology S.R.M. Dentalcollege, Ramapuram for redressal of their periodontal problem. All subjects were systemically healthy. Periodontitis is defined as the inflammation of the supporting structures of the periodontitium which is accompanied by attachment loss and bone loss.Based on the clinical findings like clinical attachment level and probing depth the study population was further divided into 20 patients with periodontitis and 20 patients without chronic periodontitis. Women under hormonal therapy, who were pregnant lactating, patients who had periodontal therapy done six months prior to the study, patients under any medications within three months prior to this study, patients with lesser than 20 permanent teeth, and teeth with fixed or removable prosthetics were excluded from this study. Alcoholics, pan chewers, and drug addicts were also excluded from this study.

The study protocol was explained to the participants; Each subject completed a detailed medical questionnaire and received a complete periodontal examination, which included oral hygiene index (simplified),[10]plaque index (PI),[11]Community periodontal index of treatmentneeds (CPITN)[12]and probing depth (PD). Ethical clearance number SRMU/M&HS/SRMDC/2009/MDS-P.G Student/502 and written informed consent was also obtained from each patient.

The study population was divided into:
Group 1 – Women patients with chronic periodontitis:Presence of bleeding on probing, probing depth ≥5 mm and CPITN index >3 .Group 2 – Women patients without periodontitis: Absence of bleeding on probing, probing depth ≤3 mm and no evidence of clinical attachment loss.

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Gingival crevicular fluid (GCF) sampling

After isolation and preparation of the concerned tooth, calibrated microcapillary tubes were used to collect GCF sample from the site with greatest probing pocket depth as per the recommendation of Sueda et al. 1969.[13] A standardized volume of 2–3 microlitre of crevicular fluid was collected by placing the tip of the pipettes extracrevicularly. The sampling time was 5–15 mins. Samples of GCF contaminated by blood or saliva were discarded. GCF samples were then transferred into Eppendorf tube. A total of 60 GCF samples collected were stored at -80°C. The samples were then assayed for TNF-α concentration by using the human TNF-α ELISA kit.

Determination of TNF-α levels

The TNF-α level was evaluated using Diaclone TNF-α ELISA kit (Gen-Probe diagnostic, Cat no. 950.090.096) which is a solid phase sandwich ELISA kit. The detection limit of TNF-α obtained after preparing the standard curve according to the manufacturer's instruction as follows: TNF-α (concentration ranging from 25 to 800 pg/mL) and the TNF-α level in each group was measured according to the manufacturer's instructions.

Assay preparation

After preparing the standard curve according to manufacturer's instruction, add 100 ml of each, Sample, Standard, Control and zero (appropriate standard diluent) in duplicate to appropriate number of wells. Add 50 ml of diluted biotinylated anti TNF-α to all wells. Cover with a plastic plate and incubate at a temperature range of about (18 to 25°C) for three hour(s).

After three hours, remove the cover and wash the plates three times; then add 100 µl of Streptavidin-HRP solution into all wells, cover with a plastic plate, and incubate again at temperature range of about (18 to 25°C) for 30 minutes. After 30 minutes, again remove the plastic plate cover and wash the plates three times. Add 100 µl of ready-to-use TMB Substrate Solution into all wells and incubate in the dark for 12–15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil. Add 100 µl of H_2SO_4 (Stop Reagent) into all wells and immediately read the absorbance value of each well on a spectrophotometer using 450 nm as the primary wavelength and, optionally, 620 nm as the reference wave length. Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance of up to 2.0 O.D. Therefore the color development within individual microwells must be observed and the substrate reaction stopped before positive wells are no longer within recordable range. The levels of TNF-α in the samples were estimated using the standard curve. TNF-α concentration was obtained in pg/mL.

The data was collected for various parameters like oral hygiene index (simplified), plaque index, probing Depth andTNF-α levels . The data was analyzed statistically. The obtained data was presented as mean and standard deviations. Data analysis was performed by using SPSS as software for statistics. Mean values were compared among different study groups by using Independent- Sample test.

Results

The results ofthiscase- control studydone among40 patients who were further divided into 20 patients with chronic periodontitis (Group 1) and 20 patients without chronic periodontitis (Group 2) had an odd ratio of about 6.9 showed that the mean TNF-α levels in Group 1 - 502.2 pg/ml is higher when compared to Group 2 - 399.2 pg/ml as shown in figure 1.

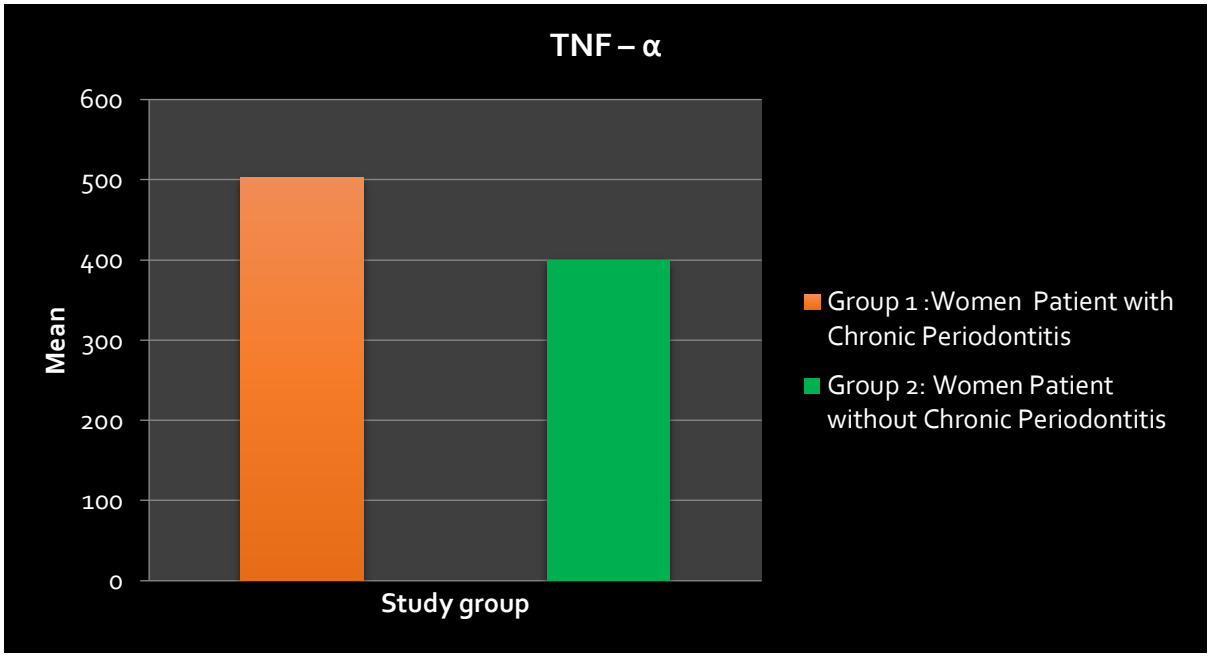

Figure 1 Comparison of mean TNF – α level between study groups.

On comparing the mean values using Independent - Sample t-test found that there is statistically significant difference between the groups in regard with the clinical parameters as shown in Table 1. This positive relationship with the parameters in group 1 could be due to higher number of inflammatory cells at the site of infection leads to the release of TNF-α upon stimulation by the bacterial products.

Table 1 : Comparison of the parameters between two groups

Mean values of Parameter	Women patients with chronic periodontitis	Women patients without chronic periodontitis.	t-test	p-value
OHI	2.85	1.25	12.42	<0.001
PD	20.60	17.10	1.73	<0.001
PI	3.45	1.90	10.21	<0.001
TNF – Alpha	502.21	399.20	1.66	<0.001

Discussion

Human periodontal diseases are predominantly caused by a complex interplay between bacteria and the host immune system inducing inflammatory conditions that result in the loss of the collagenous structures that support the teeth.[14,15] A number of inflammatory mediators, such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor-α (TNF-α), prostaglandins and matrix metalloproteinases (MMPs) are involved in periodontal diseases.[16,17] These mediators may affect the activities of leukocytes, osteoblasts and osteoclasts and promote the tissue

remodeling process systemically and locally.[18,19,20].TNF-α, secreted predominantly by monocytes and macrophages, is a potent inflammatory cytokine that up regulates the production of collagenases, prostaglandin (PG) E2, chemokines and cytokines, cell adhesion molecules and bone resorption-related factors.[21,22]Cytokine synthesis and release by cells in the affected tissues possesses bioactivities, which are consistent with a causative or contributory role in the destruction of bone and connective tissue in periodontitis.[23] Several cytokines have been detected in GCF and in gingival tissues of patients with periodontitis, reflecting the possibility of evaluating the contents of GCF as "Indicators" or "Markers" of periodontal disease.[24] In the present study, a total of 40 subjects were examined. The subjects were grouped into control and periodonititis based on bleeding on probing, probing depth and evidence of clinical attachment loss.

The result of the present study shows significant difference in TNF-α levels in GCF between the 2 groups. The meanTNF-α levels in Group 1 - 502.21 pg/ml and in Group 2 -399.20pg/ml .Highest level belongs to group – 1 indicating a positive relation with inflammatory changes. Increase in level of TNF-α in group 1 could also be due to higher number of inflammatory cells in the connective tissue and gingival crevice can lead to the release of TNF-α upon stimulation by the bacterial products.[25]It was not surprising that some amount of TNF-α could also be detected in sites of group 2 subjects as Stashenko and Jandinski [23] demonstrated by immunofluroscent technique that, TNF-α positive staining cells were also present in normal gingival tissues, but are much lesser than that found in the inflamed tissues. The role of cytokines in normal sites may be related to the physiological activities. The presence of low number of macrophages and mononuclear cells in the gingival tissues and neutrophils in GCF in clinically normal tissues could also account for the presence of TNF-α in GCF in group I subjects.[25]

The limitation of this study is that the male patients have to be included in this study in order to rule out the influence of gender on the level of TNF-α. Major limitations posed by the present study were the grouping of patients based on the clinical parameters and not on the microbiological assessments. Hence, quiescent and active states of the disease could not be differentiated. Other limitations include a small sample size and the lack of an intervention group.

CONCLUSION

Within the limits of the study it can be concluded that the increased levels of the inflammatory cytokinesTNF-α in periodontitis may have diagnostic and prognostic potentials for monitoring the progression of the disease and severs as a tool to take the therapeutic decisions. Future work to analyze the level of TNF-α at the onset of the disease and during the progression of the disease and to compare it with the clinical and microbiological assessment so that they can be used as biomarker in the early diagnosis of periodontal disease

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