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Research paper

Assessment of tumor necrosis factor- alpha in gingivitis and periodontitis patients

Sajeev Shrestha [1]*, Manisha Neupane [2], Shivalal Sharma [3], Madhab Lamsal [4]

[1] *Assistant Professor, Department of Periodontology and Oral Implantology, BP Koirala Institute of Health Sciences, Dharan, Nepal*
[2] *Private Dental Practitioner (Periodontist), Trishakti Dental Clinic, Basundhara Chauki, Kathmandu, Nepal*
[3] *Professor, Department of Periodontology and Oral Implantology, BP Koirala Institute of Health Sciences, Dharan, Nepal*
[4] *Madhab Lamsal, Professor, Department of Biochemistry, BP Koirala Institute of Health Sciences, Dharan, Nepal*
**Corresponding author E-mail: sajeevshrestha@gmail.com*

Abstract

Background: Tumor necrosis factor-α, one of the cytokines, is released in various chronic inflammatory diseases including periodontitis.
Aims: To estimate the level of Tumor necrosis factor- alpha (TNF-α) in gingivitis and periodontitis patients
Settings and Design: A cross-sectional study was conducted. A total of 75 patients were recruited by purposive sampling technique among the patients visiting the Department of Periodontology and Oral Implantology during the period one year from August 2014 to July 2015.
Material and methods: The recruited samples were divided into gingivitis and periodontitis groups based on clinical attachment level (CAL). A periodontitis subject was defined as having at least 4 sites with pocket depth (PD) >3mm and at least 4 sites with CAL>3 mm, while gingivitis group included the subjects having no CAL measurements greater than 3 mm with signs of inflammation. The TNF-α level was measured using the RayBio Human TNF-alpha ELISA (Enzyme-linked Immunosorbent Assay) kit.
Results: The Median TNF-α interquartile range (IQR) (minimum-maximum) in gingivitis and periodontitis was 58.37 (32.63 – 198.77) and 111.89 (39.27 – 215.0) pg/ml, respectively.
Conclusion: The level of TNF-α was found to be higher in periodontitis group compared to gingivitis group, although the result was not statistically significant.

Keywords: *Clinical Attachment Level; Gingivitis; Periodontitis; Pocket Depth*

1. Introduction

Periodontal disease is regarded as the most common disease that affects 80 % of the worldwide population and a major cause of tooth loss (Albandar & Rams 2002, p. 7). Plaque-induced gingivitis and periodontitis are two most common forms of periodontal diseases. Gingivitis is a reversible inflammation of the gingiva caused by bacterial plaque and may progress to affect deeper periodontal structures if not treated (Ranney 199, p. 15). In Nepal, it was reported that 29% of 35-44 years population are suffering from deep periodontal pockets (Van Palenstein et al. 1998, p. 58).
Periodontal diseases can be considered as the sixth complication of diabetes mellitus (Loe 1993, p. 334). In another way, the periodontal diseases have also been considered as a risk for a variety of systemic conditions including cardiovascular disease, diabetes mellitus, rheumatoid arthritis and respiratory disorders. According to the most accepted hypothesis, the relationship between acute myocardial infarction and periodontitis depends on risk factors common to both diseases (Syrjänen 1990, p. 499), with tobacco as the main confounding factor (Müller 2001, p. 286). Another hypothesis point to the direct action of the periodontal pathogen that produces endotoxins and the release of pro-inflammatory mediators by the host monocytes, causing the local and systemic destruction of connective tissue, and favoring platelet aggregation and thromboembolic events (Herzberg & Weyer 1998, p. 153).
Therefore, evidence suggests a bi-directional relationship between periodontitis and systemic diseases (Kim & Amar 2006, p. 18).
Tumor necrosis factor alpha (TNF-α) is the most important cytokine produced by the host against the active component of Gram-negative bacteria (endotoxin). It causes an increment of prostaglandin E2 (PGE2) concentration and activation of osteoclasts. Consequently, along with interleukin-1 (IL-1), it causes bone resorption, resulting in the release of the matrix metalloproteinases (MMPs) and destruction of the extracellular matrix (Nikolopoulos et al. 2008, p.764). Noh et al. (2013) had shown that higher plasma levels of TNF-α were significantly associated with the periodontal disease. Although there is a plausible biological mechanism that could link the level of TNF- α in the development and progression of periodontal disease, a number of studies (Bastos et al. 2009, p 82, Yousefimanesh et al. 2013, p. 739) do not show a significant association between periodontitis and concentration of TNF-α. Therefore, the present study aims to estimate the levels of TNF-α in gingivitis and periodontitis patients to observe the relationship between them if present.

2. Material and methods

A cross-sectional study was conducted at the Department of Periodontology and Oral Implantology in collaboration with Depart-

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ment of Biochemistry, BP Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal. A total of 75 patients were recruited who fulfilled the inclusion and exclusion criteria. The study included OPD patients visiting the Department of Periodontology and Oral Implantology from August 2014 to July 2015. Subjects were divided into gingivitis and periodontitis groups based on pocket depth and attachment level measurements as per study was done by Craig et al. (2003). A periodontitis subject was defined as having at least 4 sites with pocket depth (PD) >3mm and at least 4 sites with clinical attachment level (CAL) >3 mm, while gingivitis group included the subjects having no attachment level measurements greater than 3 mm with signs of inflammation (Craig et al. 2003, p. 1009).

The criteria for inclusion included patient attending Periodontology OPD who met our criteria for case definition, patients whose age was between 30 to 60 years, and patients with at least 20 natural teeth within both jaws. The exclusion criteria included patients with known systemic disease, patients on antibiotic treatment preceding 3 months, patients receiving treatment with any medications known to affect the serum levels of inflammatory markers, pregnant or lactating females, patients receiving periodontal treatment within the preceding 6 months and smokers.

Clinical examination was performed after taking written informed consent. A thorough oral examination was carried out by the single examiner. Periodontal examinations included Plaque Index (PII) (Löe 1967, p. 41), Gingival Bleeding Index (GBI) (Ainamo & Bay 1975), pocket depth (PD) and clinical attachment level (CAL). All periodontal examinations PI, GBI, PD, and CAL were evaluated on four sites/tooth (mesiobuccal, buccal, distobuccal and lingual/palatal) and means of all parameters were calculated except for gingival bleeding index where the percentage of gingival bleeding were calculated. A periodontal probe {University of North Carolina-15 (UNC-15) Hu-friedy, IL, USA} and a mouth mirror were used for periodontal examination.

After periodontal examination, 2 ml of venous blood sample was taken from all subjects using 2 ml syringe and transferred into a plain vial. Samples were allowed to coagulate for 30 minutes at room temperature. Serum was then separated by centrifugation at 2000 x g for 10 minutes and stored in 2 ml plastic tube at -20 ℃ until assayed.

The TNF-α levels were measured using the RayBio Human TNF-alpha ELISA (Enzyme-linked Immunosorbent Assay) kit in the Department of Biochemistry, BPKIHS. The value was expressed in pg/ml. The assay employs an antibody specific for human TNF-alpha coated on a 96-well plate. Standards and samples were pipetted into the wells and TNF-alpha present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human TNF-alpha antibody was added. After washing away unbound biotinylated anti-human TNF-alpha antibody, Horseradish peroxidase (HRP)-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution (3,3′,5,5′-Tetramethylbenzidine) was added to the wells and color developed in proportion to the amount of TNF-alpha bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm, and the concentration was calculated using the standard plot. The minimum detectable dose of TNF-alpha is 30 pg/ml typically. As per the manufacturer's certification, this ELISA kit shows no cross-reactivity with any of the cytokines tested.

A study done by Gokul et al. (2012) was used for sample size calculation. Collected data was entered in Microsoft Excel 2007 and converted it into Statistical Package for Social Science (SPSS) version 11.5 for statistical analysis. Descriptive statistics was calculated (mean, median, standard deviation, percentage, and proportion). For inferential statistics, an independent t test was applied for parametric data whereas Mann-Whitney U test was applied for non-parametric data. The study was carried out after approval by the Institutional Review Committee of BPKIHS.

3. Results

Out of 75 patients, 40 patients (53.3%) were in gingivitis group and 35 patients (46.7%) were in periodontitis group. The socio-demographic characteristics which included age, gender and marital status of the gingivitis and periodontitis patients were shown in Table 1.

Table 1: Socio-Demographic Characteristics of Gingivitis and Periodontitis Cases

Socio-demographic characteristics	Category	Diagnosis		p value	Remarks
		Gingivitis	Periodontitis		
Age	30-39	36(67.9)	17(31.2)	-	-
	40-49	4(26.7)	11(73.3)		
	>50	0	7(100)		
Mean age in years ± SD		33.35 ± 4.023	42.03 ± 8.631	<0.001	Significant*
Gender	Male	24	21	<0.001	Not significant ¶
	Female	14	16		
Marital status	Married	15	29	<0.001	Significant ¶
	Unmarried	25	6		
Total		40	35	-	-

*Independent t-Test, ¶Chi Square Test

Similarly, the periodontal parameters (plaque index, gingival bleeding index, pocket depth and clinical attachment level were tabulated in Table 2.

Table 2: Periodontal Parameters of Gingivitis and Periodontitis Cases

Periodontal parameters	Category	Diagnosis		p value	Remarks
		Gingivitis	Periodontitis		
Plaque index	Fair	28	15	-	-
	Poor	12	20		
Mean plaque index ±SD		1.669 ± 0.4655	1.935 ± 0.4776	0.017	Significant*
Gingival bleeding index	<25	22	21	-	-
	25 – 50	15	12		
	≥50	3	2		
Mean gingival bleeding index ±SD		23.36 ± 12.34	21.96 ± 11.36	0.613	Not significant*
Mean Probing depth ±SD		0.9712 ± 0.274	2.394 ± 0.304	<0.001	Significant*
Mean CAL ±SD		1.319 ± 0.274	3.261 ± 0.589	<0.001	Significant*
Total		40	35	-	-

*Independent t-Test

As the data obtained from the measurement of TNF-α was not normally distributed, Man Whitney test was applied to test the significant difference between the gingivitis group and periodontitis as showed in Table 3.

Table 3: Biochemical Parameter of Gingivitis and Periodontitis Cases

Biochemical parameters	Category	Diagnosis		p value	Remarks
		Gingivitis	Periodontitis		
Median TNF-α IQR(min-max) pg/ml		58.37 (32.63 – 198.77)	111.89 (39.27 – 215.0)	0.477	Not significant †

†Man-Whitney 'U' test

4. Discussion

Periodontal diseases are inflammatory diseases affecting the surrounding and supporting tissues of teeth—the periodontium. Gingivitis is an inflammatory reaction induced by the pathogens residing in dental plaque (biofilm) which forms on the adjacent tooth surfaces, is highly prevalent and readily reversible by simple, effective oral hygiene (Pihlstrom et al. 2005, pg. 1809). Periodontitis results in an apical migration of epithelial attachment along the tooth root with the destruction of periodontal soft and hard tissues (Flemmig 1999, p. 32). Unlike gingivitis, which is cured following the removal of local etiological factors, periodontitis is irreversible. Recent advances in periodontal research suggest that periodontal immunity is a double-edged sword, with one side fighting invading pathogens and the other side triggering tissue damage in the host (Teng 2004, p. 476). The present study estimated and compared TNF-α in 40 gingivitis and 35 periodontitis subjects.

Many case definitions have been proposed and used in epidemiological and clinical studies. No consensus has been reached on the threshold values for PD and CAL or on the number of sites or teeth that must be affected to constitute disease (Page & Eke 2007, p. 1395). In our study, we use the definition of "periodontitis" as having at least 4 sites with PD >3mm and at least 4 sites with attachment loss >3 mm. Subjects with gingivitis were defined as having no attachment level measurements greater than 3 mm. The reason behind using this criterion was because a histological study of Gargiulo & Wentz (1961) found that the mean distance between the CEJ and the bone crest was 1.08 mm with normal values ranging from 0.04 mm to 3.36 mm. Thus, a distance greater than 3.36 mm is required to include the patients with true periodontal attachment loss.

In the present study, the mean age of the patient with gingivitis and periodontitis was 33.45 ± 4.023 and 42.01 ± 8.631 years, respectively, which was statistically significant (p= <0.001). This result was in agreement with numerous previous studies (Abdellatif & Burt 1987, p. 16, Ababneh et al. 2012, p. 3) that had reported higher prevalence and severity of periodontal disease with increasing age.

Periodontal disease, in population studies, is often reported to be more prevalent or severe in males than in females at comparable ages (Grossi et al. 1994, p. 264, Shiau & Reynolds 2010, p. 1387). In our study, gender was not found as a risk indicator of periodontal disease (p = 1.000) but was in agreement with the study done by Yousefimanesh et al. (2013) who too did not find any difference in the prevalence of periodontitis in either sex. One reason for this might be that the sample size of the study was less while the another reason might be that the total number of female subjects in our study was less as compared to male (46 male and 30 female).

It was shown by the present study that periodontitis was more common in married subjects, consistent with the study done by Marcenes & Sheiham (1992). In their study, they found that work-related mental demand and marital quality were statistically significantly related to periodontal disease after taking into account the other variables assessed in the study.

The mean plaque index scores in gingivitis and periodontitis were 1.669 ± 0.4655 and 1.935± 0.4776 respectively, which were statistically significant (p value=0.017). Similarly, the mean gingival bleeding percentage was not statistically significant between gingivitis and periodontitis groups (p = 0.613). Similar to the study done by Craig et al (2003), the present study showed the statistically significant difference in the probing depth and clinical attachment level measurement between gingivitis group and periodontitis group.

TNF-α is considered to be a major cytokine involved in the pathogenesis of periodontal disease, affecting the consequence of tissue destruction (Boström et al. 1998, p. 767). The normal level of TNF- α is 53.6pg/ml. According to Cair et al. (2010), the level of TNF- α may reach to 482 pg/ml in chronic periodontitis patients. Similar to the study done by Cairo et al., the concentration of TNF- α in our study was measured in venous blood. In the present study, the median TNF-α level in gingivitis group and periodontitis group was 58.37pg/ml and 111.89pg/ml, respectively. This showed that the level of TNF-α was found to be higher in periodontitis group compared to gingivitis group, although the result was not statistically significant. These results were in agreement with the studies done by Gokul et al. (2012) and Noh et al. (2013) which revealed that as the disease severity increased, the level of TNF-α also increased. These observations suggest a positive association between periodontal disease and increased levels of TNF-α. It was concluded that there was a possibility that absence or low level of TNF- α in gingival crevicular fluid (GCF) might indicate a stable site and elevated level might indicate active site. However, it was also recommended that more longitudinal studies are required before using TNF-α in GCF as an "Indicator" of periodontal disease (Gokul et al. 2012, p. 352). It was also suggested that IL-6, IL-8, and TNF-α might be relevant in the pathophysiology of periodontitis, and the measurement of these cytokines might be beneficial in the identification of patients with periodontitis (Noh et al. 2013, p. 847).

The finding of the study was inconsistent with various other studies (Gorska et al. 2003, Solhjoo et al. 2014) which revealed no association between the concentration of TNF- α and periodontitis. Twenty-five severe chronic periodontitis patients and 25 periodontally healthy persons were recruited to assess the relationship between clinical periodontal parameters and concentrations of the key cytokines (IL-1β, TNF-α, IL-2, IFN-γ, IL-4, and IL-10). Serum samples from both groups showed the high individual variability of cytokine profiles, and no association between cytokine concentrations and clinical parameters of periodontitis was found (Gorska et al. 2003, p.1046). Similarly, a case-control study also found no significant difference in the frequencies of genotypes and alleles, between healthy and periodontitis groups. The study also indicated that there was no association between TNF-α (-308 G>A) polymorphism and chronic periodontitis in that population(Solhjoo et al. 2014, p.13).

The limitations of the present study were the smaller sample size, measurement of TNF-α in blood rather than GCF, use of the manual periodontal probe and cross-sectional nature of the study. Further prospective studies with larger sample size are recommended to clarify the role of TNF-α in initiation and progression of periodontitis.

5. Conclusion

The level of TNF-α was found to be insignificantly higher in periodontitis group compared to gingivitis group.

Conflict of Interest: Nil

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