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Impact of Non-Surgical Periodontal Therapy on the Salivary Levels of Tissue Inhibitor of Metalloproteinse-1 (TIMP-1) in Patients with Chronic Periodontitis: A Third World Experience

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Abstract

Chronic periodontitis is a disease of public health concern due to its high prevalence globally, especially in the elderly. The aim of this study was to determine the impact of non-surgical periodontal therapy on salivary levels of TIMP-1 among patients with chronic periodontitis in Nigeria. In this experimental study, unstimulated whole saliva (2 mL) was collected from participants in the experimental and control groups, coded (SP1-40 and SH1-40) respectively and assays for salivary TIMP-1as well as clinical measurements such as plaque (PI), probing depths (PD), and CAL were recorded before and 4 weeks after periodontal treatment. Assay was done using Quantikine human TIMP-1 ELISA kit. Data were presented using frequency tables, means and standard deviation. Paired-T Test assessed association between salivary TIMP-1 before and after treatment. Pearson correlation coefficient was used to correlate salivary TIMP-1 levels with clinical parameters of periodontal disease and levels of statistical significance were set at $p < 0.05$. A total of 80 respondents participated in the study of which 43.80% were females. Age range was 18 - 60 years with a mean of 35.8 ± 12.46 years. Salivary TIMP-1 levels were lower in the case group (13.58 ± 6.53 ng/mL) than the control (15.27 ± 6.10 ng/mL) at baseline but this was not statistically significant ($p = 0.13$). There was a statistically significant increase in the salivary levels of TIMP-1 in the case group after phase-one periodontal therapy from 13.58 ± 6.53 ng/mL to 17.24 ± 8.44 ng/mL ($p = 0.001$). Negative correlations were observed between TIMP-1 and clinical parameters of periodontitis. This was not statisti-

cally significant. Therefore, TIMP-1 may not be an ideal biomarker for periodontal diagnosis but may be useful in treatment monitoring of the disease.

Keywords

TIMP-1, Periodontal Treatment, Saliva, ELIZA

1. Introduction

Chronic Periodontitis was defined by the consensus committee of the American Academy of Periodontology as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment and bone loss [1] [2]. The disease is highly prevalent globally especially among the elderly [3] [4]. Globally, the prevalence of periodontal disease is 5% - 15% [5]. In Europe, severe periodontal disease is found in 5% - 20% of middle-aged (35 - 44 years) adults and up to 40% of older people (65 - 74 years) [6]. In the United State of America, over 47 percent of the adult population aged 30 years and above has mild, moderate or severe periodontitis, according to the Centers for Disease Control and Prevention (CDC) [7]. According to CDC, periodontitis is more prevalent in men (56% versus 38% for women), non-Hispanic Black and Mexican-Americans (58% and nearly 67% respectively), current smokers (64%), and adults below 100% federal poverty levels (65%) or with less than a high school education (nearly 67%). [6] These findings underscore the notable oral health disparities among racial and ethnic groups, and persons with lower education and income [6]. In Africa, 35% are affected by varying degrees of periodontitis [8].

In developing countries such as Nigeria, periodontal disease with deep pocketing occurs at an early age, the prevalence is 15% - 58% in those aged above 15 years [9]. The rate of progression of periodontal disease is determined by a complex interplay between pro- and anti-inflammatory mediators, making some individuals more susceptible to the disease than others [10]. For healthy condition to be maintained, there are the counteracting effects of the tissue inhibitors of metalloproteinases (TIMPs) which are naturally occurring endogenous inhibitors to the activities of matrix metalloproteinases (MMPs) preventing their hyperactivity. MMPs are a family of genetically distinct but structurally related host-derived proteolytic enzymes which are involved in the physiological and pathological degradation of extracellular matrix and basement membrane proteins [10]. These enzymes have been shown to play a key role in periodontal tissue destruction seen in chronic periodontitis. In health, there's a balance between MMPs and TIMPs, which when destabilized, will predispose to the destruction of collagen in periodontal tissues manifesting clinically as periodontitis. There are four identical types of TIMPs namely TIMP-1, TIMP2, TIMP-3 and TIMP-4 [10].

Previous studies have been able to analyze TIMP-1 in gingival crevicular fluid to assess its relationship with periodontitis [11] [12]. However, there is paucity

of data on the levels of this biomarker in the saliva and the effect of periodontal therapy on these levels. The initial periodontal therapy consisting of oral hygiene instructions, scaling and root planing as well as identification and correction of stagnation areas are usually the first line of treatment for many periodontal diseases. This involves subgingival scaling and root planing to remove bacterial endotoxins bound to the contaminated root surface [13]. Attention has recently been drawn to saliva as a potentially valuable diagnostic specimen because it is acceptable to both patients and doctors [14]. Contents of saliva which include proteins and enzymes can serve as biomarkers of periodontal disease since qualitative and/or quantitative changes in salivary components can be diagnostic [14]. This can be assessed using commercially available ELIZA kits such as the Quantikine Human Total TIMP-1 Immunoassay. This ELIZA kit utilizes a 4.5-hour solid-phase ELISA designed to measure total TIMP-1 (pro- and active-TIMP-1) in cell culture supernates, serum, plasma, and saliva. It contains NS0-expressed recombinant human TIMP-1, and antibodies raised against the recombinant protein. The ELISA kit is all-inclusive supplied with optimized reagents ready for use or by simple reconstitution as specified in the manuals provided. R&D Systems is a well-referenced ELISA manufacturer in various studies on salivary TIMP-1 [10].

However, active surveillance and treatment monitoring of the disease is practically non-existent in many developing countries including Nigeria [6]. This study, therefore, set out to assess the effect of non-surgical periodontal treatment on salivary levels of TIMP-1 so that it can serve as a useful tool for treatment monitoring of periodontal disease.

2. Materials and Method

This was an experimental study conducted at the University College Hospital, Ibadan for duration of 6 months. Sample size was calculated from a previous similar study using the formula for comparison of two means and was found to be 80. A representative sample of 80 consecutive, newly registered, dentate, adult patients who had presented for dental treatment/checkup were recruited into the study by simple random sampling (ballot). Forty patients with clinical signs of periodontal disease and 40 healthy controls were recruited into the test and control groups respectively.

Only consenting patients who were ≥18 years and in good general health with at least 20 erupted teeth, had not undergone periodontal treatment or used any antibiotics in the last 6 months, without any systemic conditions (such as cardiac disease, diabetes mellitus), were included in the study. Also, those that will require antibiotic prophylaxis before treatment and those with disorders that could affect periodontal treatment outcomes were excluded from the study. The medical and dental history of all participants was taken and patients who did not meet the inclusion criteria were excluded from the study. All the participants received full-mouth periodontal examination by one calibrated examiner (AO).

For the purpose of this study, a patient with chronic periodontitis was defined as any individual who had two or more teeth with probing pocket depth (PPD) and or clinical attachment loss (CAL) of more than 4mm with or without gingival recession [6].

Using the CDC criteria for periodontal disease, the experimental group were participants having at least 2 interproximal sites with bleeding on probing, probing pocket depth and or clinical attachment loss of greater than 4 mm involving two or more teeth, while participants for the control group had good periodontal health with no bleeding on probing, no periodontal pathologic pocket and no clinical attachment loss [15]. All clinical findings were recorded on the modified WHO oral health assessment form 53 modified to exclude other aspects not relevant to the study.

All the teeth were assessed for bleeding on probing, periodontal pathologic pocket and clinical attachment loss using a Williams's periodontal probe.

Participants in the experimental group received non-surgical periodontal treatment (scaling, root planing and polishing) while only polishing of teeth was done for those in the control group using bristle brushes (placebo).

Unstimulated whole saliva (2 mL) was collected from each participant about 5 minutes before treatment each group and coded (SP1-40) and (SH1-40) for the experimental and control groups respectively. The samples were labeled and transported immediately in ice-packs to the laboratory (University College Hospital Virology laboratory) where they were stored at −20˚C till the time of analysis.

A second set of saliva samples were collected from each group 4 weeks after treatment and coded (SPA1-40) and (SHA1-40). Salivary TIMP-1 level was determined in the samples using the Quantikine human total TIMP-1 ELISA kit from R&D systems® Europe [16]. Data was analyzed using SPSS version 22 and presented as frequency tables, graphs, means and standard deviation. T-test was used to compare mean salivary TIMP-1 between the two groups. Correlation between clinical parameters of periodontal disease was measured using Pearson correlation coefficient and the level of significance was set at $p < 0.05$. Ethical approval was obtained from the UI/UCH ethical review board with reference number NHREC/5/01/2008a. Informed consent was taken from the patients prior to inclusion in the study.

3. Result

A total of 80 respondents participated in the study with female preponderance (43.80%). Age range was 18 - 60 years with a mean of 35.80 ± 12.46 years. A large percentage of the respondents (52.50%) had skilled employment, 28.70% had non-skilled employment while 18.80% were dependent. Many of the participants (67.50%) were married, (25%) were single while (7.50%) were divorced (**Table 1**).

Chronic periodontitis as measured by the CAL was worse in the males but this was not statistically significant ($p = 0.69$). Participants in the older age group (43

years and above) had the most severe disease with mean CAL of 3.16 and this was statistically significant (p = 0.016). Also, patients with low educational status had the most severe disease and this was statistically significant (p = 0.017, Table 2).

Table 3 illustrates the effect of periodontal treatment on the clinical parameters of periodontitis. All the clinical parameters of periodontitis improved significantly after non-surgical periodontal treatment (p = 0.001).

Table 1. Socio-demographic characteristics of respondents.

Socio-demographic characteristics	Frequency	Percentage
Age [Years]		
18 - 30	35	43.7
31 - 43	14	17.5
>43	31	38.8
Gender		
Male	45	56.3
Female	35	43.7
Marital status		
Single	20	25
Married	54	67.5
Divorced	6	7.5
Occupation		
Skilled	42	52.5
Unskilled	23	28.7
Dependant	15	18.8
Education		
Tertiary	30	37.5
Secondary	19	23.8
Primary	22	27.5
None	9	11.2

Majority were young adults, there were more females than males and many had tertiary education.

Table 2. Relationship of age group, gender and level of education with clinical attachment loss.

Variables		Periodontal Index (CAL)	Test	p-Value
Gender	Male	2.82 (2.82SD)	t = 0.163	0.688
	Female	2.57 (2.69SD)		
Age Groups (yrs):	18 - 30	2.31 (2.70SD)	F = −2.465	0.016
	31 - 43	2.71 (2.73SD)		
	43 and above	3.16 (2.83SD)		
Level of Education	No	4.67 (2.29SD)	F = −2.430	0.017
	Primary	2.79 (2.76SD)		
	Secondary	2.27 (2.83SD)		
	Tertiary	2.40 (2.66SD)		

Periodontitis as measured by CAL was worse in the older age groups and this was statistically significant relationship between age and level. Participants with no education had the worst periodontal status.

Table 3. Impact of periodontal treatment on the parameters of periodontal disease.

Periodontal parameters		Mean	Student t-test	Degree of freedom	p-Value
Oral hygiene index	Baseline	4.20 ± 0.50	30.65	39	0.001
	After	1.59 ± 0.52			
Gingival index	Baseline	2.10 ± 0.47	28.43	39	0.001
	After	0.98 ± 0.49			
Probing pocket depth	Baseline	5.87 ± 0.91	10.78	39	0.001
	After	3.50 ± 0.84			
CAL	Baseline	5.4 ± 0.74	16.40	39	0.001
	After	3.52 ± 0.71			

All the clinical parameters of periodontitis improved after non-surgical periodontal therapy and this was statistically significant.

Figure 1 is a bar-chart showing the different concentrations of salivary TIMP-1 before and after treatment in the experimental and control groups.

Although salivary TIMP-1 levels were lower in the experimental group (13.58 ± 6.53 ng/mL) than the control (15.27 ± 6.10 ng/mL) this was not statistically significant (p = 0.13) (Table 4). However, there was a statistically significant increase in the salivary levels of TIMP-1 in the experimental group after non-surgical periodontal therapy from 13.58 ± 6.53 ng/mL to 17.24 ± 8.44 ng/mL (P = 0.001) (Table 5). Negative correlations were observed between TIMP-1 and clinical parameters of Periodontitis which were not statistically significant (Table 6).

4. Discussion

Oral health (including periodontal health) has been described as an important aspect of general health and a bi-directional relationship has been described between periodontal disease and Non-Communicable Diseases (NCDs) [17]. Therefore, early detection and treatment of periodontal disease have become ever more important [18]. Periodontal health is maintained by the stability of the periodontal tissues and tissue integrity is maintained by a balance between matrix degradation and production, which is regulated mainly through the action of matrix metalloproteinases (MMPs) in both normal and pathological tissues as well as in tissue remodelling states [18] [19]. It is usually accepted that the balance between activated MMPs and TIMPs controls the extent of extracellular matrix remodeling, and tissue degradation is caused by the disruption of this balance in favor of proteinases [19]. Under pathological conditions associated with unbalanced MMP/TIMP activity, changes in TIMP-1 levels could be important in the regulation of the destruction of periodontal tissues by affecting the MMP levels in periodontal tissues [10]. TIMP-1 levels in periodontal tissues are important because these endogenous regulators of MMP activities are involved in the extracellular control of excessive extracellular matrix degradation. It has been previously

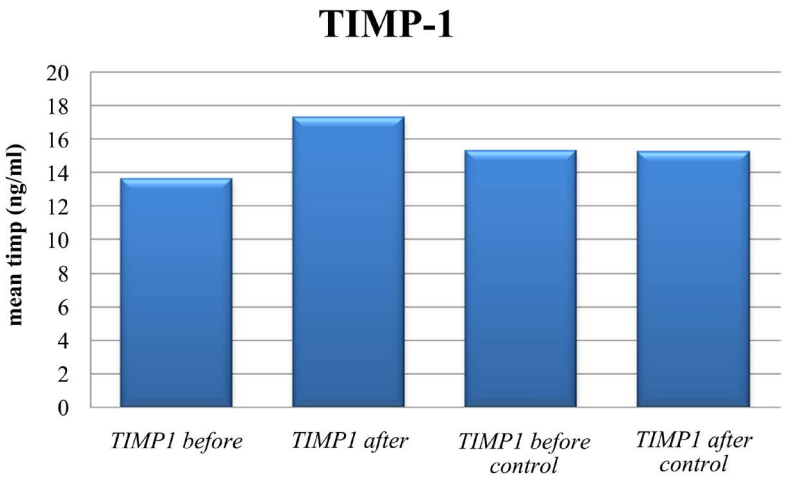

Figure 1. Comparison of salivary TIMP-1 among study groups. Salivary levels of TIMP-1 improved after periodontal treatment in the experimental group.

Table 4. Comparison of the salivary TIMP-1 between experimental and control groups.

		Experimental group	Control group	Student t	Deg of freedom	p-Value
TIMP-1 (ng/mL)	Baseline	13.58 ± 6.53	15.27 ± 6.10	1.55	39	0.13
	After	17.24 ± 8.44	15.20 ± 5.64	7.47	39	0.001

The salivary TIMP-1 levels in the control group is higher than that in the experimental group and it increase significantly in the experimental group after treatment.

Table 5. Impact of periodontal treatment on the salivary level of TIMP-1 (ng/mL) in the experimental group.

	Baseline ng/mL	Post-treatment	Student-t-test	p-Value
TIMP-1 (ng/mL)	13.58 ± 6.53	17.24 ± 8.44	7.34	0.001

TIMP-1 increased significantly in the experimental group after treatment.

Table 6. Correlation between post-treatment salivary TIMP-1 and clinical parameters of the experimental group.

Variable/characteristic	Mean values	Correlation	p-Value
Age	35.78 (±12.464)	−0.060	0.596
Gingival Index	1.32 (±0.888)	−0.121	0.285
Oral Hygiene Index	1.44 (±0.694)	−0.380	0.736
Clinical Attachment Loss	2.71 (±2.743)	−0.124	0.273
Probing Pocket Depth	3.65 (±1.424)	−0.089	0.432
Bleeding on Probing	0.38 (±0.487)	−0.028	0.803

Negative correlations were observed between all the clinical parameters of periodontitis and salivary TIMP-1.

demonstrated that TIMP-1 could inhibit the effects of MMP-1, -3, -8, and -9 in inflamed periodontal tissues [10]. TIMP-1 is widely synthesized by many cells

and tissues [10]. Transcription of the TIMP-1 gene is induced by pro-inflammatory cytokines (IL-1, IL-6, OSM, LIF, and TNF-α), TGF-β1, and phorbol esters [10]. Many physiological functions of TIMP-1 are closely tied to the functions of MMPs, and an improper balance of MMP and TIMP production correlates with pathological conditions such as periodontitis, arthritis, tumor growth, and metastasis [20].

This study confirms the higher prevalence of periodontal disease in males than females because many of the participants were males. Also, severity of periodontal disease as measured by the CAL was worse in the male gender than the females as shown by the higher mean CAL (2.82 ± 2.62). This was however not statistically significant (p = 0.69) (**Table 2**). Various studies have demonstrated better oral health seeking behavior and periodontal health in the females as compared with males [21] [22]. The mean clinical attachment loss was worse with the increasing age of respondents, which some studies have reportedly claimed to be due to increased susceptibility of periodontal tissue with increasing age (**Table 2**) [17] [18]. However, other studies have reported that the increased attachment loss seen with the increasing age could be due to the individual host susceptibility to periodontal disease rather than the length of time the plaque is present (age effect) [23]. The greater the age of an individual, the slower the rate of wound healing and this increases susceptibility to periodontal disease [24].

In this study, the salivary TIMP-1 in the periodontally healthy group was found to be higher (13.58 ± 6.53 ng/mL) than that in their controls (15.27 ± 6.10 ng/mL) but this was not statistically significant (p = 0.13). This was in agreement with an earlier study done by Hayakawa *et al.* in 1994 where the salivary TIMP-1 levels were seen to be 273 ± 145 ng/mL in clinically healthy subjects in contrast to periodontally diseased subjects with TIMP-1 levels of 137 ± 67 ng/mL [24] [25]. Other similar studies have also substantiated these findings [10] [24]. MMP-8, also called neutrophil collagenase-2, is one of the important collagenases that have a major part in the destruction of connective tissue and alveolar bone in periodontitis [20]. The activity of MMP is controlled by TIMP-1 which forms 1:1 non-covalent complex with MMPs, blocking access of substrates to the MMP catalytic site. In healthy individuals, the TIMP-1 levels were found to be higher reflecting the capability of these individuals to antagonize the activity of MMPs as evidenced by healthy periodontium with normal clinical parameters. Individuals, who are prone to periodontal diseases, were discovered to have greater amount of MMP activity and this was reflected through a decreased or less potent TIMP activity, as also evidenced by the destruction of periodontal tissues and altered clinical parameters. The present study also revealed that the salivary levels of TIMP-1 in the experimental group increased after non-surgical periodontal therapy from 13.58 ± 6.53 ng/mL to 17.24 ± 8.44 ng/mL and this was statistically significant (P = 0.001). This is in tandem with previous studies which have shown that there is actually an increase in the TIMP-1 levels at 3 and 6 months after phase I therapy. Increase in TIMP-1 levels may be attributed to a

fall in the MMPs and attempts at resolution of the destructive inflammatory process which occurs immediately after non-surgical periodontal therapy [18].

This suggests that TIMP-1 may be effective in treatment monitoring of periodontal disease. Studies have also reported the potential utility of saliva as a diagnostic fluid as well as in monitoring therapeutic interventions.

All the assessed periodontal parameters improved following non-surgical periodontal therapy (NSPT). This is expected because the therapy leads to the resolution of the inflammatory condition followed by healing. Supportive periodontal therapy has been reported to be effective in the long-term preservation of teeth and improvement in the health of periodontal tissue among patients with advanced chronic periodontitis. This is more important when there is a good patient selection as the success of most periodontal therapy depends on compliance with strict home care by the patient.

However, negative correlations were observed between the clinical parameters of periodontal disease and salivary levels of TIMP-1 pre-treatment suggesting that TIMP-1 may not be reliable in periodontal diagnosis.

5. Conclusion

TIMP-1 may not be an ideal biomarker for periodontal diagnosis. It may however be useful in treatment monitoring of the disease.

Limitation of the Study

Larger sample size could not be used due to the high cost of the ELIZA kits.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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