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Keywords

Saliva; Diagnosis; Periodontal diseases; Rheumatoid arthritis; Diabetes Mellitus.

Research Article

Potential Chair-side Test for Gingival Inflammation Screening of Patients With and Without Comorbidities

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Abstract

Objectives: To evaluate a diagnostic kit prototype for salivary total protein (TP), as a complementary clinical examination, for the diagnosis and monitoring of gingival inflammation during periodontal treatment.

Materials and Methods: Patients (n=57) were divided into six groups: systemically and Periodontally Healthy (H), Systemically Healthy with Periodontal Disease (PD), Periodontal Health and Diabetes (D), Periodontal Disease and Diabetes (PDD), Periodontal Health and Rheumatoid Arthritis (RA) and Periodontal Disease and Rheumatoid Arthritis (PDRA). Collection of non-stimulated saliva, oral hygiene instructions and basic periodontal treatment were performed. Clinical parameters, gingival index (GI), visible plaque index (PI), probing pocket depth (PPD), bleeding on probing (BOP) and clinical attachment level (CAL), were evaluated at days 0 and 45.

Results: Most groups showed clinical improvements with significant changes in H, PD and PDRA for PI, PD and PDRA for PPD and PD for BOP ($p<0.05$). Total protein reduction was significant only in the PD group ($p<0.05$). Correlations analysis between salivary TP with BOP or with GI, showed that the salivary test can detect the GI variation ($p<0.05$).

Conclusion: The salivary test has the potential to customize the therapeutic approach, as a complementary clinical examination, serving as a visual indicator for the patient to perceive the clinical signs of their evolution throughout the treatment, and to validate the improvement of the clinical parameters in a practical and accessible quantitative way.

Clinical Relevance: The use of saliva for disease monitoring has been consolidated as a versatile diagnostic tool, the results demonstrated its usage to monitor gingival inflammation, a potential visual test to present to patients, and a much less invasive method than the conventional Gingival Index.

Introduction

Periodontal Disease (PD) is an inflammatory disease that occurs on the tissues around the tooth and may destroy the periodontal ligament, alveolar bone and root cementum. Periodontal infections promote a systemic inflammatory state, spreading inflammatory mediators and pathogenic oral microorganisms to other organs increasing the risks of developing other diseases [1]. Periodontopathogenic bacteria produce endotoxins which have great potential for the development of systemic diseases. Also, periodontal pathogens, after entering the bloodstream, can be a source of systemic infections and inflammation (bacteremia) due to the frequent activation of the body's line of defense. Some studies have observed that the treatment of periodontitis is associated with preventing the systemic diseases [2-5]. There are several similarities between Rheumatoid Arthritis (RA) and PD, such as the increased release of pro-inflammatory mediators, the activation of RANKL, and the frequent infiltration of immuno-inflammatory cells. In addition, there is a high prevalence of PD in patients with RA and, after periodontal therapy, studies have demonstrated that RA has its activity reduced [6-9].

Studies have demonstrated that the association between Diabetes Mellitus (DM) and PD is considered bidirectional, as DM acts in the deposition of advanced glycation end products (AGEs) in periodontal tissues and, when interacting with their receptors (RAGEs), end up stimulating inflammatory responses that are related to bone resorption, exacerbating PD. In addition, patients with DM have higher levels of glucose in their saliva, worsening the oral microbiota imbalance, exacerbation of inflammatory with impaired signaling and insulin resistance [10-12]. Preshaw and Bisset [10] demonstrated a reduction in pro-inflammatory mediators and improved metabolic control in diabetic patients after periodontal treatment.

Saliva is a fluid that has many advantages over other types of collection, such as convenience, greater acceptance by patients, absence of problems related to asepsis, easy to collect and non-invasive. The composition of saliva undergoes alterations according to changes arising from environmental and genetic factors, such as total protein, mucins and immunoglobulins and, therefore, its components have been extensively studied as biomarkers for various alterations [13,14].The main composition of salivary total protein (TP) is mucin, proline-rich proteins, statherins, immunoglobulins, antimicrobial factors and amylase, which are responsible for most functions of saliva [15,16]. Studies have analyzed the direct relationship between the alteration of salivary TP and oral dysbiosis, since the microbiota existing in PD leads to an increase in salivary levels of TP, stating that this fluid allows a good diagnosis and monitoring of PD progression [17,18]. Also, Justino et al (2017) [19] compared the effects of oral hygiene on biomarkers and noticed that increased salivary TP concentration is associated with inflammation in PD [15,20,21].

Purpose

This study aims to evaluate a prototype diagnostic kit based on the presence of proteins in saliva as a non-invasive auxiliary chair-side diagnostic method with the potential to visually and quantitatively present the health or disease status and to monitor the periodontal treatment response.

Methodology

The present study is an applied research, as it seeks to generate knowledge for practical application, in addition to having a quantitative and qualitative disposition, as it allows the understanding of the complexity and details of the information obtained, data collection and their statistics treatment. This research also has an explanatory disposition in view of the applied experimental method, being classified as a practical study. Regarding the technical procedures that were used, the intention was to guide the study in an experimental way, since the object of study (periodontitis), the variables that can influence it (comorbidities) and forms of control and observation of the correlation between both were established.

The sample calculation was performed based on the results from the study by Kejriwal et al. (2014) [15]. Based on a β power of 0.80 and α power of 0.05, a minimum sample of 8 patients per group was estimated.

The inclusion criteria were patients with a minimum of 14 natural teeth (at least 10 posterior teeth), periodontitis diagnosis (clinical attachment loss, periodontal pocket ≥ 4mm with bleeding on probing), systemically healthy or diagnosed with DM and/or RA

The exclusion criteria were smoking, pregnancy, recent periodontal treatment, recent use of antibiotics and patients who refused to sign the Consent Form.

57 patients were selected and divided into the following groups:

a. Health Group (H) (n=14): Systemically healthy patients without PD (control)
b. Periodontitis Group (PD) (n=11): Systemically healthy patients with PD
c. Diabetes with Periodontal Health Group (D) (n=5)
d. Diabetes with Periodontitis Group (PDD) (n=10)
e. Arthritis with Periodontal Health Group (RA) (n=5)
f. Arthritis with Periodontitis Group (PDRA) (n=12)

All groups underwent saliva collection, clinical examinations, basic periodontal treatment (BPT) and oral hygiene instructions; reassessment and new collection after 45 days (Figure 1). All patients were instructed not to drink or eat food and to brush their teeth at least one hour before the appointment.

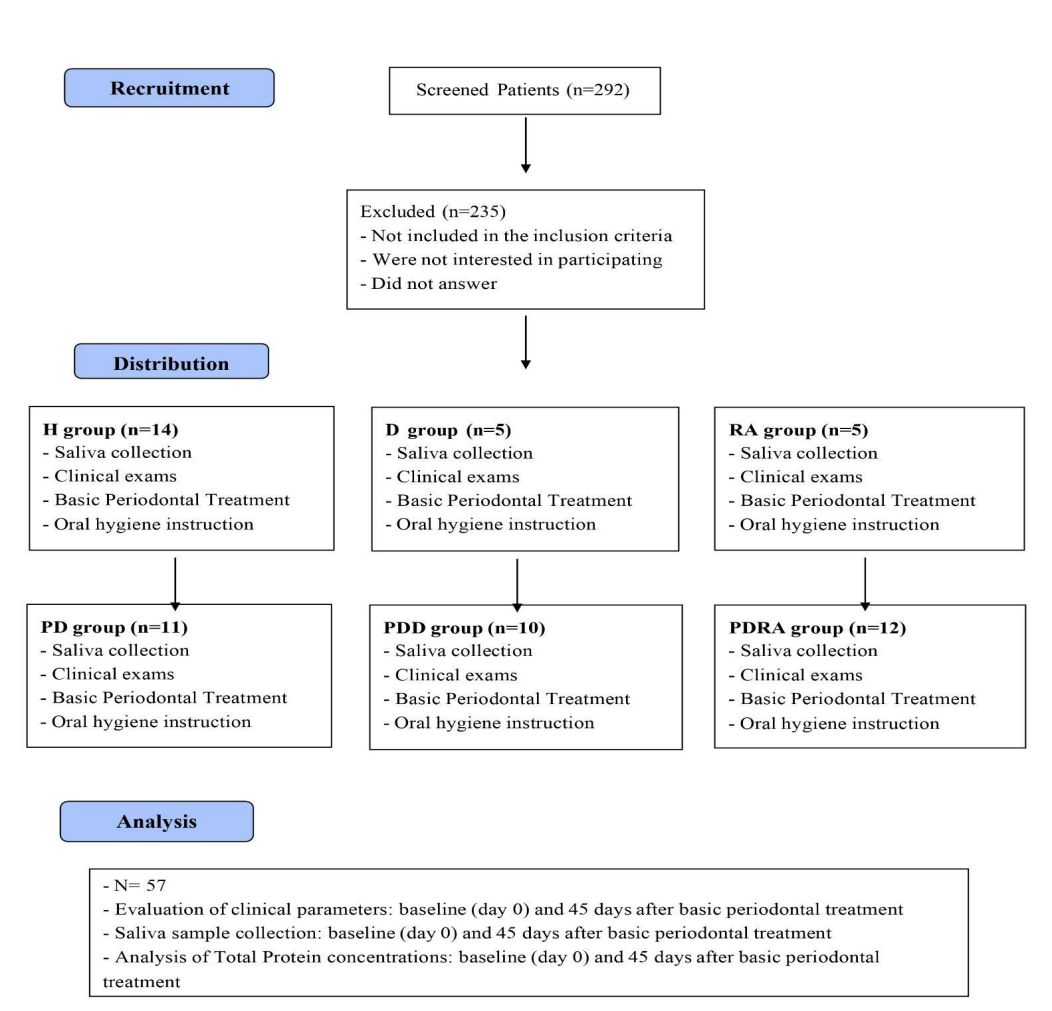

Figure 1: Flow diagram.

Saliva test preparation

A Biuret reagent was prepared by dissolving 1.5g of copper sulfate pentahydrate and 6g of double sodium and potassium tartrate in 500mL of distilled water. 300mL of 10% NaOH solution were added and the volume was completed to 1L with distilled water. 1g of potassium iodide was added to inhibit the copper reduction reaction. The solution was stored in an amber plastic container at 2-8°C.

Casein protein preparation (positive control)

For positive control, 2g of casein were added to 20Ml of distilled water and stored at 2-8°C.

Salivary test and register of Positive and Negative Control and Saliva

Patients were asked to rinse their mouth with water prior to saliva collection, then 5mL of unstimulated saliva were collected and 1mL was mixed with 1mL of biuret solution for colorimetric reaction and test of the kit from the modification of the methodology described by Anson-Hagihara [22] (Casein-tyrosine). The remaining 4mL were refrigerated at -80°C for laboratory analysis.

Three eppendorf tubes with 1mL of the biuret solution were separated during all appointments.

For the positive control, 1mL of casein was deposited in one of the eppendorfs, generating a purple hue that simulates the saliva from a patient with PD.

For the negative control, 1mL of distilled water was deposited in another eppendorf, generating a characteristic transparency of a patient with periodontal health.

Finally, for colorimetric saliva analysis, 1mL of unstimulated saliva collected at each study time was added to the third eppendorf, separately, and its resulting color was photographed for comparison with the other tubes (Figure 2). The resulting color scale was processed by the ImageJ software (Wayne Rasband, Wisconsin, United States), generating a quantitative result for later comparison.

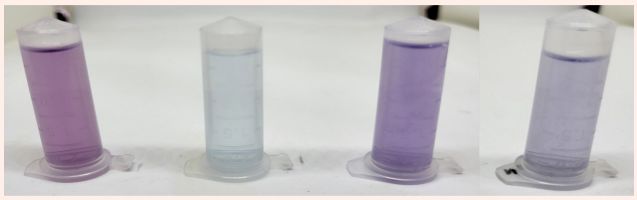

Figure 2: Diagnostic Kit salivary test, from left to right the eppendorf tubes contents illustrate: positive control (first tube with biuret reagent + protein), negative control (second tube with biuret reagent + distilled water), baseline saliva (third tube with biuret reagent + saliva), 45 days saliva (fourth tube with biuret reagent + saliva).

Clinical examination and Basic Periodontal Treatment

Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD) and Bleeding on Probing (BOP) were performed. For the PI and GI, a WHO probe was used, and 4 surfaces of each tooth were evaluated (buccal, lingual, mesial and distal). For PPD, BOP and CAL, a North Carolina probe was used and each tooth was divided into six surfaces, three buccal and three lingual. Each surface was recorded as an individual site. After clinical examinations, BPT of supragingival scaling and oral hygiene instruction were performed. After 45 days, the patients returned and the entire protocol performed at baseline was repeated.

Total Protein Concentration Assessment Protocol

Saliva samples were defrosted and analyzed using the Pierce™ Rapid Gold BCA Protein Assay Kit (manufacturer: Pierce Biotechnology, Illinois, USA). The plates were read with an absorbance of 480nm using the Gen5 software (manufacturer: Agilent Technologies, Santa Clara, United States).

Statistical analysis

The collected data were grouped and presented in tables in the format of averages and standard deviations. Data were submitted to statistical analysis using the JASP 0.16.4 software (Eric-Jan Wagenmakers, Amsterdam, The Netherlands, Windows 64-bit version; Released October 3rd, 2022). Normality was verified with the Shapiro-Wilk test and, according to the results, the appropriate tests were applied. For the analysis between groups, the ANOVA or Kruskal-Wallis test was applied and, respectively, the Tukey or Dunn post-test for multiple comparisons between pairs. For analyzes between study times, the paired Student's t-test or Wilcoxon was applied. For correlation analysis, Pearson's test was applied. In all analyses, the significance level used was 0.05.

Results

Table 1 presents the mean and standard deviation of the results obtained in the groups H, PD, D, PDD, RA, PDRA, referring to the clinical parameters evaluated (PI, GI, PPD, BOP) both in baseline and time 45.

Plaque Index

In intra group evaluations, the PI at baseline and time 45 after BPT and oral hygiene instruction were compared. In this analysis, it was observed that the H, PD and PDRA groups had a significant reduction in PI ($p<0.05$), while D and PDD groups had a reduction, but it was not significant ($p>0.05$) and the RA group increased the PI, but it was not statistically significant ($p>0.05$). When evaluating between groups, PI analysis of all groups at baseline was performed and, after, another analysis of all groups at time 45. It was observed that there was a significant difference between the groups both at baseline and at time 45 ($p<0.05$), and, after analyzing multiple comparisons, differences were observed between RA x PD ($p=0.004$) and also PD x H groups ($p<0.001$) at baseline and in the comparison between the PD x H groups ($p=0.011$) at time 45 (Table 1).

Gingival Index

When evaluating both intra groups and between groups, all groups showed a reduction in GI, but this difference was not statistically significant (p>0.05) (Table 1).

Table 1: Periodontal Clinical Parameters.

EXPERIMENTAL GROUPS							
VARIABLE	**PERIOD**	**H**	**PD**	**D**	**PDD**	**RA**	**PDRA**
Plaque Index	Baseline	17,6 ± 10,5 A§	51,3 ± 24,9 B§	27,7 ± 20,9 AB§	29,0 ± 21,2 AB§	13,0 ± 6,5 A§	34,8 ± 18,9 AB§
	Day 45	13,1 ± 8,0 A†	32,8 ± 16,4 B†	19,5 ± 11,7 AB†	27,2 ± 11,6 AB†	15,3 ± 11,8 AB†	24,5 ± 18,9 AB†
	p-value	0,007*	0,005#	0,188	0,706	0,452	0,018#
Gingival Index	Baseline	11,6 ± 8,4	23,6 ± 16,9	13,3 ± 9,5	10,4 ± 9,5	8,9 ± 6,0	16,2 ± 8,1
	Day 45	10,0 ± 5,7	18,7 ± 8,5	11,6 ± 10,6	9,4 ± 8,6	7,7 ± 5,0	13,7 ± 7,0
	p-value	0,597	0,107	0,739	0,76	1	0,369
Probing Pocket Depth	Baseline	1,7 ± 0,3 A§	2,8 ± 1,0 B§	2,0 ± 0,2 ABC§	2,2 ± 0,3 ABC§	2,0 ± 0,1 AC§	2,5 ± 0,3 BC§
	Day 45	1,8 ± 0,1 A¶	2,5 ± 0,7 B¶	2,0 ± 0,2 ABC¶	2,3 ± 0,4 BC¶	1,9 ± 0,2 AC¶	2,3 ± 0,4 BC¶
	p-value	0,082	0,029#	0,735	0,116	0,341	0,008#
Bleeding on Probing	Baseline	0,12 ± 0,08 A※	0,41 ± 0,28 B※	0,13 ± 0,12AB※	0,22 ± 0,21AB※	0,13±0,049AB※	0,28 ± 0,16AB※
	Day 45	0,1 ± 0,06	0,26 ± 0,22	0,1 ± 0,07	0,15 ± 0,09	0,14 ± 0,06	0,24 ± 0,18
	p-value	0,324	0,032#	0,519	0,26	0,88	0,399

§ Significant difference when comparing between groups on baseline (ANOVA, Tukey, p<0,001)
※ Significant difference when comparing between groups on baseline (Kruskal-Wallis, Dunn, p<0,05)
† Significant difference when comparing between groups on day 45 (ANOVA, Tukey, p<0,05)
¶ Significant difference when comparing between groups on day 45 (ANOVA, Tukey, p<0,001)
* Significant difference when comparing baseline and day 45 (Wilcoxon)
# Significant difference when comparing baseline and day 45 (Student)
Different letters represent significant differences (p<0,05). Capital letters represent comparisons in the evaluated time (line).

Probing Pocket Depth

In intra group comparisons, there was a reduction in PPD in PD, RA and PDRA groups, but there was only a significant difference in PD and PDRA groups (p<0.05). In groups H, PDD and D, there was an increase in PDD, but without statistical significance (p>0.05). When evaluating between groups, it was observed that there was a statistical difference both at baseline and time 45 (p<0.05), being evident at baseline between PDRA x H (p=0.001), RA x PD (p=0.044) and PD x H (p<0.001) and at time 45 when between PDRA x H (p=0.028), RA x PD (p=0.037), PDD x H (p=0.037) and PD x H (p<0.001) (Table 1).

Bleeding on Probing

Regarding intra group comparisons, there was an improvement in BOP in H, PD, D, PDD and PDRA groups, but this change was statistically significant only in the PD group (p<0.05), while there was a worsening in the RA group, but without statistical significance (p>0.05). There was a difference between the groups when comparing PD x H groups (p=0.003) at baseline, but there was no statistical difference in time 45 analysis (p>0.05) (Table 1).

Analysis of Total Protein Concentrations

For the intra group evaluation, it was analyzed that the TP concentration values (μg/mL) were reduced in the PD, D, PDD groups and PDRA, with statistical difference only in the PD group (p<0.05). The H and RA groups had their TP concentrations increased, however, this increase was not significant (p>0.05). As for the evaluation between groups, there was a statistical difference in the results at both times (p<0.05), and this difference was found in the comparison between PDRA x H (p=0.02) and PDD x H groups (p=0.002) at baseline and between PDRA x H (p=0.02) and PDD x H (p=0.006) groups at time 45 (Table 2).

Table 2: Evaluation of Total Protein Concentration and Salivary Test Quantification

EXPERIMENTAL GROUPS							
VARIABLE	**PERIOD**	**H**	**PD**	**D**	**PDD**	**RA**	**PDRA**
Total Protein (µg/mL)	Baseline	1285,8 ± 777,7 A§	1855,6 ±656,8AB§	1857,6±944,3AB§	3413,3 ± 2473,4B§	1846,1 ±739,1AB§	3293,3 ±3143,6B§
	Day 45	1444,3±1249,1A†	1312,9±580,1AB†	1350,6±586,6AB†	2797,6 ±1986,5B†	1963,9±965,4AB†	2272,3 ± 907,1 B†
	p-value	1	0,03#	0,45	0,13	0,77	0,30
Salivary Test	Escore baseline	6,1 ± 10,6	-1,9 ± 13,5	11,8 ± 14,3	-1,1 ± 8,4	2,4 ± 7,7	0,75 ± 18,8
	Escore 45	2,7 ± 14,8	-1,45 ± 7,2	1,2 ± 11,7	5,4 ± 8,3	7 ± 6,8	1 ± 15,7
	p-value	0,5	0,9	0,2	0,1	0,2	0,9

§ Significant difference when comparing between groups on baseline (Kruskal-Wallis, Dunn, p<0,05)
† Significant difference when comparing between groups on day 45 (Kruskal-Wallis, Dunn, p<0,05)
# Significant difference when comparing baseline and day 45 (Student)
Different letters represent significant differences (p<0,05). Capital letters represent comparisons in the evaluated time (line).

Salivary test

To represent the patients' worsening and improvement degrees through the salivary kit prototype, first, a color scale resulted from the reaction saliva x biuret solution (test), casein x biuret solution (positive control) and distilled water x biuret solution (negative control) was obtained, and a colorimetric evaluation was processed by ImageJ software for quantitative results. Once the pixel analysis was done, the numerical difference obtained between the test pixel and the positive control pixel was used as a representation of the status of each patient at baseline (baseline score) and at time 45 (score 45). In the intra group evaluation, it was observed that as the value decreases when comparing the baseline score with the score 45 within the same group, it means that there has been a worsening in the patient's oral health and, if there is an increase in the value, it means that there has been improvement, therefore, in the colorimetric reading, the healthier the patient, the more transparent the resulting color, because more light passes through the processing, increasing the resulting value.

Thus, the analysis showed that there was an improvement in the PD, PDD, RA and PDRA groups, while there was a worsening in the H and D groups, however, this difference was not significant (p>0.05). There was also no significant difference when comparing between groups at baseline and time 45 (Table 2).

Pearson's correlations

Aiming a more detailed analysis, correlations were made between the differences obtained with Score, BOP and GI in groups with and without PD and, subsequently, all groups together. In PD groups, it was observed that the difference in the BOP variation does not concur with the difference in the variation in the salivary test, that is, the variation of BOP was not detectable in the salivary test (p> 0.05). On the other hand, the difference in the GI variation had a statistical difference when compared with the difference obtained from the score, that is, the GI variation was detected in the salivary test (p<0.05). Finally, the difference in the GI variation compared to the BOP difference also had a significant difference, therefore, the GI influences the BOP (p<0.05) (Table 3).

Table 3: Pearson's Correlation, groups with periodontitis.

Variable		ΔScore	ΔBOP	ΔGI
ΔEscore	Pearson's r	-		
	p-value	-		
ΔSS	Pearson's r	0.026	-	
	p-value	0.887	-	
ΔIS	Pearson's r	-0.431§	0.515†	-
	p-value	0.012	0.002	-

Table referring to the comparison between the differences (Δ) obtained from the evaluated groups with periodontitis.

§ Significant difference in the comparison between the GI and Score group differences (p<0,05)
† Significant difference in the comparison between the GI and BOP group differences (p<0,005)

In the groups without PD, there was no statistically significant difference in any of the comparisons (p>0.05), which would be expected from periodontally stable or healthy patients (Table 4).

Table 4: Pearson's Correlation, groups without periodontitis.

Variable		ΔScore	ΔBOP	ΔGI
ΔEscore	Pearson's r	-		
	p-value	-		
ΔSS	Pearson's r	-0.318	-	
	p-value	0.13	-	
ΔIS	Pearson's r	-0.074	0.264	-
	p-value	0.73	0.212	-

Table referring to the comparison between the differences (Δ) obtained from the evaluated groups without periodontitis.

Evaluating all groups together, it was observed again that the difference in the BOP variation does not coincide with the difference in the variation in the salivary test (p>0.05), however, there is a correlation between the difference obtained between the GI and the score, proving that the variation was detected in the salivary test and there was also a correlation between the GI variation and the BOP difference (p<0.001) (Table 5).

Table 5: Pearson's Correlation, all groups.

Variable		ΔScore	ΔBOP	ΔGI
ΔScore	Pearson's r	-		
	p-value	-		
ΔBOP	Pearson's r	-0.072	-	
	p-valor	0.593	-	
ΔGI	Pearson's r	-0.300§	0.432†	-
	p-value	0.023	< .001	-

Table referring to the comparison between the differences (Δ) obtained from the evaluated groups with and without periodontitis.

§ Significant difference in the comparison between the GI and Score group differences (p<0,05)

† Significant difference in the comparison between the differences of the GI and BOP groups (p<0,001)

Discussion

The diagnosis of periodontitis is determined through clinical and radiographic examinations that have limitations in presenting the patient's current biological state of health. Thus, in the present study, a prototype test for salivary total protein was evaluated as a complementary, non-invasive clinical test to aid in the diagnosis and monitoring of the effect of BPT. The ability to detect changes in total protein levels by the salivary test and its correlation with GI was evaluated in patients with and without comorbidities before and after BPT. When evaluating the correlations of the differences obtained with the salivary test score, BOP and GI, it was observed that the variation of GI promoted color changes in the kit prototype according to the increase or reduction of gingival bleeding. One of the focuses of the present study was the evaluation of the response to BPT in patients with DM and with RA. Regarding DM, there are studies such as the one by Sanz et al [11] proving that these patients are more likely to develop PD, especially with altered blood glucose. Regarding RA, De Molon et al [7] explain that these patients have a greater tendency to develop PD, as they have a greater load of pathogenic species associated with periodontal diseases. This is also observed in the present study, where all patients with DM or RA had some level of gingival inflammation when compared to systemically healthy individuals.

In the present study, after periodontal clinical examinations (PI, GI, PPD and BOP) biofilm and supragingival calculus were removed, and the patient was instructed, motivated and directed to dental treatment in the Perio Clinic of the university, when necessary. This protocol follows studies such as Tonetti et al [23], Sanz et al [24] and Haas et al [25]. Ximénez-Fyvie et al [26] and Gomes et al [27] agree that BPT with a focus on supragingival scaling is of great importance for the success of periodontal treatment, and may bring beneficial changes to the subgingival microbiota, provided there is constant maintenance of the periodontal stability. However, DeMarco et al [28] and Tonetti et al [23] point out that, for the therapy to be effective, caution is needed when evaluating the biofilm retaining factors, as they can disrupt the treatment. Therefore, authors such as Tonetti, Greenwell and Kornman [29], Loos and Needleman (2020) [30] and Haas et al (2021) [25], agree that an effective periodontal treatment has the benefits of reducing bleeding indexes, gaining clinical attachment, adequate plaque control, absence of suppuration and reduction of periodontal pockets depths.

Considering the expected benefit of periodontal therapy, in the present study, it was observed that, just as there was an improvement in the parameters of several groups, there was also a worsening in other groups and this difference was significant in just a few analyzes (see Table 1). Thus, the groups that resulted in significant improvement are in agreement with the cited studies that cover the effectiveness of BPT, while the groups that showed no significant improvement or worsening in clinical parameters are in disagreement with all the cited studies. To justify these results, many patients returned at time 45 with some deficiencies in oral hygiene techniques for various reasons, such as lack of adaptation or difficulty moving the hand or arm in cases of RA, pain in dental elements (which restrained hygiene habits), in addition to cases of very deep pockets. All of these reasons could worsen the parameters on time 45.

Other patients needed the removal of biofilm retaining factors, as well as the extraction of dental elements. These patients were directed for treatment at the university at baseline, some returned within 45 days with the treatment completed and a decrease in inflammation, but others could not get treatment, which may have influenced the non-significant improvement or worsening of periodontal parameters. These justifications are in accordance with the study by Sanz et al [24] who stated that patients with a lot of inflammation and high probing depth with bleeding on probing may end up not responding well to BPT. Perhaps a longer follow-up would lead to more positive results, still in agreement with Gomes et al [27] and Sanz et al [24], who emphasized that this primary stage of treatment must be constantly reassessed for a greater guarantee of effectiveness. Another possibility of improving the results would be to perform subgingival instrumentation when necessary.

Regarding TP in the present study, it was observed that there was a reduction in concentration values (µg/mL) between baseline and time 45 in most groups (Table 2), however, this difference was significant only in the PD group, which is in disagreement with the study by Lorenzo-Pouso et al [17] who demonstrated that periodontal treatment brings significant reductions in the concentration of TP. Another observation in the present study was that TP concentrations in groups without PD were lower than in groups with PD, which is in accordance with studies by Shaila, Pai and Shetty [31], Kejriwal et al [15] and Lorenzo-Pouso et al [17], with the exception of group H at time 45, which had its value increased after baseline.

Shaila, Pai and Shetty [31] point out that TP is a potential source of energy for Treponema denticola, which is found in greater abundance in individuals with PD. So, TP can be considered a potential biomarker of PD. Kejriwal et al [15] complement emphasizing that TP concentrations are higher in patients with PD probably due to the activation of the inflammatory process in order to increase the synthesis and secretion of proteins as a way to increase saliva's protective capacity.

Regarding the salivary test, there was improvement in most groups and worsening in some, however, this difference was not significant (see Table 2). The justification found for there being no statistical difference in these results was the need for other treatments, such as tooth extraction, endodontics, and biofilm retaining factors removal, as well as the other reasons explained before, since the salivary test would have its variation according to the inflammatory alterations in the patients. However, visually, it was possible to observe that there was a change in color when saliva was deposited in the biuret solution, as illustrated in Figure 2. In the present study, in view of all the limitations explained so far, the absolute value of the test did not present a discriminatory capacity between groups. Perhaps more accurate and within expectation results would have been obtained with a larger sample size, patients demographics and clinical outcomes more homogeneous and by including more intensive treatment and follow-ups in the study design. Despite the limitations, when evaluating the correlation of the differences obtained with the score (salivary test), BOP and GI in groups with and without PD, it was possible to conclude, in groups with PD, that the difference in the GI variation was correlated with the salivary test and this may explain the color changes when the test was applied. In the groups without PD, there was no significant difference in any of the comparisons, but this result would already be expected from patients with periodontal health. These same results were obtained when comparing all groups together, confirming the correlation between GI and the salivary test.

Interestingly, the result offered a similar indication to the clinical sign of GI. This behavior confirms the findings that the expression of proteins in saliva demonstrates a relationship with the biological event of gingival inflammation [17,31], but, just like GI, it does not allow to differentiate the stage of the disease that involves other periodontal parameters that are more invasive. Both the GI and the kit score provide visual information on the alteration of normality that can be used to monitor treatment progress. In this sense, we can highlight that, unlike conventional clinical methods that require the use of a periodontal probe, the kit provides a quick and non-invasive assessment of gingival inflammation, which can be performed with the patient at the clinic and even by the patient at home. From a practical point of view, the use of the kit as an auxiliary salivary test can be perceived as a ludic way of motivating the patient through the visual stimulus of treatment improvement. Still, it can be compared to the stained plaque index, which also does not have a discriminatory diagnostic capacity, but is an auxiliary tool for patient guidance with the advantage of being a practical, low-cost and non-invasive alternative for detecting gingival inflammation.

Conclusion

Despite the limitations faced throughout the study, the salivary test has the potential to help customize the therapeutic approach, as a complementary clinical examination, offering a visual demonstration for the patient to follow their evolution in the treatment, and to validate the improvement of clinical parameters in a quantitative way.

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