

VALIDATION OF AN ALTERNATIVE DEVICE FOR VOLUMETRIC QUANTIFICATION OF CREVICULAR FLUID

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ABSTRACT

The volume of crevicular fluid is directly proportional to the degree of periodontal inflammation. The Periopaper[®] system, with readings taken using the Periotron[®], has gained wide acceptance in the literature for measuring this volume. Alternative methods have also been proposed. This study investigated an alternative device for measuring the volume and compared it to the readings taken in Periotron[®] Units (PU). Strips of absorbent paper (PS) of the same size as Periopapers[®] (P) were obtained. Next, previously defined volumes were quantified in PU (R1: P and R2: PS) and also in millimeters (T1: P; T2: PS) using an alternative reading method (ruler and magnifying glass). The mean measurements for each volume were compared (Student's *t* test) and no differ-

ence was detected between them in PU (R1=66.8 and R2=72.5) or in millimeters (T1=2.83 and T2=2.96). The degree of correlation between groups was evaluated using Pearson's coefficient, with an intragroup *r* of 0.98 (R1xT1; R2xT2; R1xT2). Linear regression coefficients were also calculated, finding R1xT1=23.5; R2xT2=26.5 and R1xT2=23.3. The method's reproducibility was verified using intraclass correlation coefficients, with excellent results - all above 0.96. This being so, it was concluded that it is possible to measure the volume collected in millimeters, with a high degree of reproducibility.

Key words: Gingival crevicular fluid, validation studies, periodontics

VALIDAÇÃO DE UM INSTRUMENTO ALTERNATIVO PARA QUANTIFICAÇÃO VOLUMÉTRICA DO FLUIDO CREVICULAR

RESUMO

O volume do fluido crevicular é diretamente proporcional ao grau de inflamação dos tecidos periodontais. O sistema Periopaper[®]/Periotron[®] é amplamente aceito frente à sua capacidade de permitir a mensuração deste volume. Métodos alternativos para medição do volume do fluido, no entanto, têm sido propostos. O presente estudo investigou um instrumento alternativo (lupa e régua milimetrada) para medição do volume e comparou-o com a leitura obtida no Periopaper/Periotron[®] (R1) e tiras de papel absorvente (previamente validadas e nas mesmas medidas do Periopaper[®])/Periotron[®] (R2). Para tanto, volumes, previamente conhecidos, de saliva foram medidos em Unidades Periotrom (UP) para R1 e R2 e em milímetros: T1: Periopaper[®] e T2: papel absorvente. Todas as leituras foram realizadas por examinador treinado e cego para os volumes dispensados. As medidas, para cada volume, foram repetidas três vezes e, na seqüência, foram geradas as médias.

Estes valores foram, então, comparados (Student's *t* test). Nenhuma diferença entre as médias foi observada tanto para as medidas em UP (R1=66,8 e R2=72,5) quanto em milímetros (T1=2,83 e T2=2,96). O grau de correlação entre os grupos foi avaliado usando o coeficiente Pearson's, atingindo-se um valor intragrupo de *r*=0,98 (R1xT1; R2xT2; R1xT2). Coeficientes de regressão linear foram, também, calculados, atingindo-se valores de 23,5 (R1xT1); 26,5 (R2xT2) e 23,3 (R1xT2). A reprodutibilidade do método foi aferida, atingindo-se coeficientes de correlação intra-classe acima de 0,96. Pode-se concluir que é possível aferir volume por meio de medição em milímetros, com alto grau de reprodutibilidade, in vitro. Este método poderá reduzir custos de investigações periodontais.

Palavras-chave: fluido crevicular gengival, estudos de validação, periodontia.

INTRODUCTION

Gingival crevicular fluid is an inflammatory exudate that follows an osmotic gradient and is similar in appearance to blood plasma. It originates in connective tissues and picks up certain components, such as inflammatory cells produced by the host in

response to bacterial aggression and bacterial products during its passage through tissues¹⁻⁵.

There are authors who believe that these components, or a combination of them, may come to serve as markers predictive of disease and of periodontal healing⁶⁻⁹. Many studies of crevicular fluid and its

components have been carried out, prompted by the need for early diagnosis and of assessing the risk of the site/patient losing insertion and to uncover the process of periodontal disease pathogenesis⁶⁻¹⁵.

Collection and volumetric quantification of CF is a sensitive method for determining the degree of periodontal inflammation. This is because variations in its volume are directly proportional to the inflammatory expression^{3,5,16,17}. Furthermore, measurements of the composition of CF may be expressed as concentrations, units per volume or absolute values per sample or against time^{2,6,10,11}. In many cases, therefore, it is necessary to measure the volume in order to report the composition.

This volume can be quantified when collection is carried out by microcapillaries or micropipettes or when strips of absorbent paper are used in combination with the Periotron[®] electronic reading machine^{1,2,10,16}. Some authors have reported their results in millimeters, using ninhydrin to achieve this¹⁸. However, using this stain limits the analysis of the composition of the CF¹⁶. It is important to develop methods for the quantification of CF which meet the clinical requirements for ease of use and reproducibility. Although many collection methods have been proposed¹⁹, none of them has been compared to the reference method. Therefore, the objective of this study was to validate a method for reading the volume collected, in millimeters, which would be easy to use, low-cost and which correlates with the reference method.

MATERIALS AND METHODS

Type of Study: *In vitro* study.

Construction of the standard curve: Three different examiners, blind to the volumes they were testing, carried out the readings in millimeters.

A - Reference Group

Reference Group 1 (R1: Periopaper[®] and Periotron[®] 8000)

A standard curve was constructed based on known volumes of saliva, starting at 0.1 microliters (μl) and increasing in 0.1 μl increments up to 0.8 μl . Each of these volumes was pipetted three times by a trained examiner using a Hamilton[®] syringe. Another examiner, blind to the volumes, then measured each of the volumes on three different pieces of Periopaper[®], taking the readings in a Periotron[®] 8000 (R) immediately after soaking up the liquid.

Reference Group 2 (R2: Paper strips (PS) and Periotron[®] 8000):

Initially, standardized strips of absorbent paper were produced (20). A standard curve was then constructed based on known volumes of saliva, starting at 0.1 microliters (μl) and increasing in 0.1 μl increments up to 0.8 μl . Each of these volumes was pipetted three times onto separate PS by a trained examiner using a Hamilton[®] syringe. Another examiner, blind to the volumes, measured each of the volumes on the PS, taking the readings in the R immediately after soaking up the liquid.

B - Group test

Group Test 1 (T1: Periopaper[®] with measurement in millimeters)

Volumes of saline were pipetted by a trained examiner using a Hamilton[®] syringe (0.1 μl , with 0.1 μl increments, up to 0.8 μl) onto a glass plate. Another examiner, blind to the volumes, soaked up the volumes using Periopapers[®] and then took a reading in millimeters using the alternative reading method. The method employed a magnifying glass (Adeck[®], 4 times magnification, 60 mm diameter) and an endodontic ruler (Prisma[®]). Each of three repetitions of each volume was measured using a separate Periopaper[®], making 24 in total.

Group Test 2 (T2: paper strips (PS) and measurement in millimeters)

Once more, the same volumes of saline were pipetted with a Hamilton syringe[®] (0.1 μl with 0.1 μl increments, up to 0.8 μl) onto a glass plate. An examiner blind to the volumes, soaked up the liquid using PS (80 gram qualitative paper) and, immediately afterwards, took readings using the same alternative method described for T1. Each of the three repetitions of each volume was also measured using a separate PS.

Statistical analysis

The means of the measurements of each volume were compared using Student's *t* test for independent samples, using the Periotron[®] Unit (PU) as the standard measurement in groups R1 and R2, and millimeters in groups T1 and T2. Data are presented as total means for each group and 95% confidence intervals. Additionally, the mean differences between the two devices, in millimeters and in PU, with their respective 95% confidence intervals,

were calculated in order to estimate the general variability between them. Scatter plots were created in order to illustrate the correlation between the mean results for the two devices when the Periotron® 8000 was used for readings and when readings were taken in millimeters. Simple linear regression models were constructed in order to estimate to what extent T1 and T2 were capable of predicting the reference group. Therefore, linear regression coefficients (β) and 95% confidence intervals were calculated. Additionally, in order to test the hypothesis that the volume reading methods correlated, Pearson correlation coefficients (r) were calculated. Inter-observer reproducibility was assessed by means of intraclass correlation coefficients (ICC). The data analysis was carried out using the statistical package *Stata SE 10.0 (Stata Corp.)*. The significance level adopted was 5%.

RESULTS

Table 1 lists the means of the measurements taken in millimeters and of the values read from the Periotron®, both for the PS and the Periopaper®. In general, the differences between the groups were small and without statistical significance. For the measurements in Periotron® units, the difference found

between R1 and R2 was 5.7 (2.45 – 8.96). The difference between the measurements taken in groups T1 and T2, in millimeters, was 0.13 (-0.52 – 0.26). The Pearson correlation coefficients (r) for the comparison between the reference groups (R1 and R2) and the test groups (T1 and T2) are given in Table 2. As can be observed, the correlation coefficients were high, 0.99 for PU and 0.94 for the measurements taken in millimeters. We also observed a correlation coefficient of 0.98 for groups R1xT1; R2xT2 and R1xT2. The linear regression coefficients (β) correlated significantly (Table 2).

The intraclass correlation coefficients (ICC) between examiners demonstrated there was an excellent correlation between them: E1x E2 = 0.99; E2x E3 = 0.96; E3x E1 = 0.97 (Table 3).

The correlations between the standard curves for groups R1 and R2, Periotron® units (Fig.1,A) and between groups T1 and T2, in millimeters (Fig.1,B) demonstrated excellent results ($r= 0.99$; $\beta = 0.94$) e ($r= 0.94$; $\beta 0.93$) respectively. The correlations between the standard curves for groups R1 and T1 (Fig.2,A), groups R2 and T2 (Fig.2,B) and groups R1 and T2 (Fig.2,C) also demonstrated excellent results ($r= 0.98$ $\beta=23.5$; $r= 0.98$ $\beta =26.5$ and $r= 0.98$; $\beta= 23.3$ respectively).

Table 1: Means (confidence intervals), and differences between the means, of the figures in Periotron® units for the reference group (R1 and R2) and in millimeters for the test groups (T1 and T2).

	R1	R2	T1	T2
Means	66.8 (41.9-91.7)	72.5 (49.1-96.0)	2.83 (1.87-3.80)	2.96 (1.98-3.94)
Differences	5.7 (2.45-8.96)		0.13 (-0.52-0.26)	
Intergroup p*	0.70		0.83	

Table 2: Pearson correlation coefficients (r) and linear regression coefficients (β) for reference groups (R1 and R2), in Periotron® units, and for the test groups (T1 and T2), in millimeters.

	R1 X R2	T1 X T2	R1 X T1	R2 X T2	R1 X T2
R	0.99	0.94	0.98	0.98	0.98
β	0.94 (0.83-1.05)*	0.93 (0.54-1.33)*	23.5 (18.8-28.1)*	26.5 (20.5-32.5)*	23.3 (16.3-30.3)*

p<0.05*

Table 3: Intraclass correlation coefficients (ICC) for means of different examiners, (E1, E2 and E3).

	E1 X E2	E2 X E3	E1 X E3
ICC	0.99	0.96	0.97

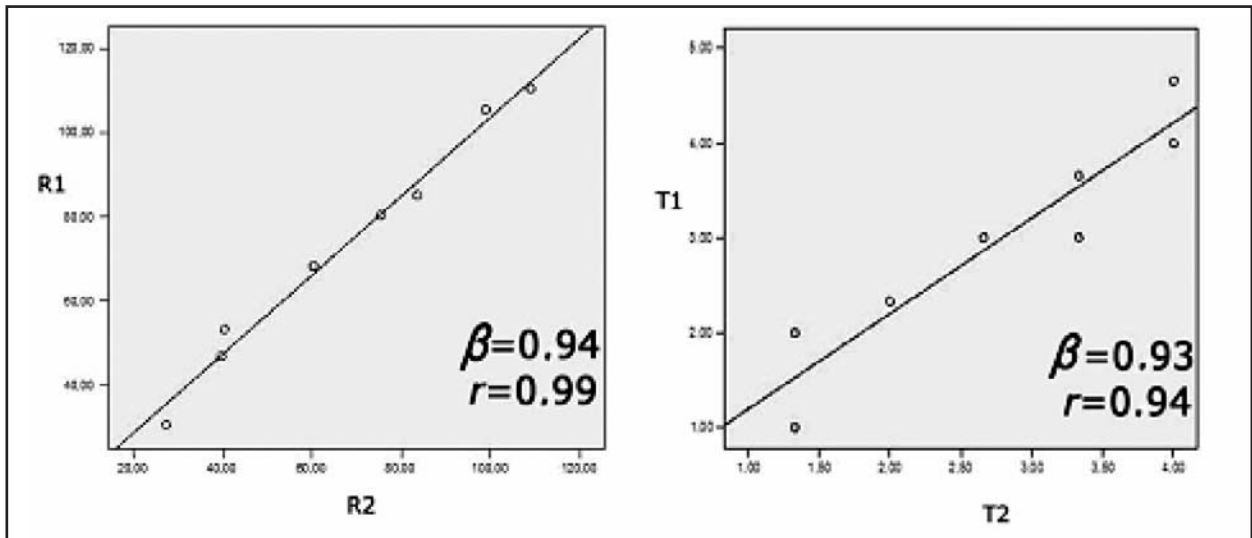


Fig. 1: A: Correlation between the standard curves constructed for the reference groups (R1 and R2) in Periotron® units and B: for the test groups (T1 and T2) in millimeters.

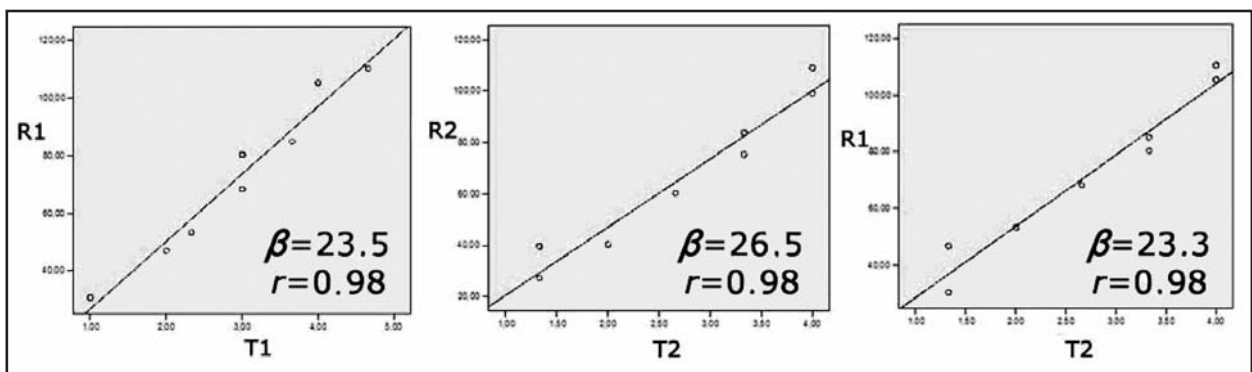


Fig. 2: Correlations between the standard curves constructed for the reference group (R) in Periotron® units and for the test groups (T) in millimeters, using Periopapers® (R1 and T1) and strips of absorbent paper (R2 and T2) illustrated in A (R1 x T1); in B (R2 x T2) and in C (R1 x T2).

DISCUSSION

The objective of this study was to validate an alternative method for volumetric quantification of crevicular fluid (CF), and the results demonstrate that it was possible to measure previously established volumes, in millimeters, using either Periotron® or strips of absorbent paper.

The volume of CF is measured in order to determine periodontal inflammation levels. This is due to the fact that the volume collected is directly proportional to the degree of inflammation present. Furthermore, according to the literature, it is important to calculate the volume of CF so that its components can be described in terms of their concentrations^{6, 21}

This volumetric measurement of the CF can be carried out if it has been collected using microcapillaries/micropipettes or when strips of absorbent paper are used in combination with the Periotron® electronic reading machine^{16,20, 21}.

There are inconveniences related to the suction method: time, technique sensitivity, trauma to tissues^{16,24}. The electronic device (Periotron®) is a machine that is easy to operate, offers consistent calibration and was created in order to quantify CF. According to Chapple et al.²⁵ and Griffiths¹⁶, the Periotron® also offers other advantages when compared with other volumetric quantification methods: it also quantifies saliva and has computer software that accurately converts Periotron® unit readings into vol-

umes. Furthermore, it takes little time to measure the volume, about 30 seconds per site¹⁶. However, price may be an issue, since the machine is expensive.

In response to this, alternative methods for the collection and volumetric quantification of this fluid have been proposed in the literature¹⁹. Medlicott et al.²⁷ validated mass (weighing) as a unit for volume analysis, but this method also demands the acquisition of specialized high-precision electronic apparatus. Ninhydrin has also been used and is a simple and low cost method. However, its use prevents analysis of the components of the crevicular fluid¹⁶, and it is limited to simple confirmation of the presence (increase or decrease) of CF at a given site. A study carried out by Weidlich, Souza and Oppermann¹⁸ employed ninhydrin with readings taken in millimeters, measuring the purple-colored area. However, volumetric quantification presupposes prior construction of a standard curve. It is only after construction of the standard curve that the relationship between the number of millimeters and the volume can be determined.

The decision was taken to measure volume in millimeters for this study. An alternative system for volumetric quantification was therefore designed, consisting of a magnifying glass (Adeck[®], 4 times magnification, 60 mm diameter) and an endodontic ruler (Prisma[®]). Standard curves were constructed in Periotron[®] units for Periopaper[®] (R1) and for the strips of absorbent paper (R2) and also in millimeters for Periopaper[®] (T1) and the strips of paper

(T2). Great care was taken when standardizing the collection strips, repeating measurements and constructing the standard curve, since these are essential to obtaining accurate results^{24-26, 28-30}.

Different solutions were used to construct the standard curves. Almeida et al.³¹ have shown that there is no difference between standard curves constructed using saline or saliva. The volumes were measured using the Periotron[®] 8000 and the magnifying glass and ruler method (millimeters).

After correlating the standard curves generated for the different volumetric quantification methods (Periotron[®] units x millimeters), excellent results were observed, both for Pearson's correlation coefficient (r greater than 0.90) and for the linear regression coefficient. It can therefore be inferred that there is an excellent correlation between the groups and that it is possible to predict the changes in one group on the basis of the other.

This study also tested the reproducibility of the method. An excellent correlation among the three different examiners was observed, since all of the coefficients (ICC) were greater than 0.96. According to Steward³², reproducibility is an indispensable part of validation of a measurement instrument. Medlicott et al.²⁷ validated mass (weighing) as a unit for fluid measurement, but did not report the method's reproducibility.

It can therefore be stated that the method of volumetric quantification by millimeters is valid, and can be used to measure CF collected with Periopaper[®] or strips of absorbent paper.

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