JOURNAL of
OSSEOINTEGRATION

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Levels of salivary cysteine in periodontitis patients with and without hopeless teeth: diagnostic validity of the assay

ABSTRACT

Aim Levels of salivary cysteine in periodontitis have been evaluated in previous studies. The aim of our study was to assess the diagnostic accuracy of salivary assays for the determination of cysteine levels in periodontal patients.

Materials and Methods Cysteine concentration was measured, by HPLC, in saliva samples of 15 patients with healthy periodontium (negative controls), and 35 periodontitis patients (tests) 18 with and 17 without hopeless teeth. The cysteine values were analyzed by Receiver Operating Characteristic (ROC) curve analysis in order to assess the diagnostic accuracy of salivary cysteine determination.

Results and conclusion Salivary levels of cysteine were significantly higher ($P<0.01$) in periodontitis patients than in controls. Levels were significantly higher ($P<0.01$) in periodontitis patients with hopeless teeth than in those without. The evaluation of salivary cysteine concentration by ROC curve analysis demonstrated diagnostic accuracy as a biochemical marker to identify periodontitis affected patients and distinguishes between patients with or without hopeless teeth.

KEYWORDS Cysteine; Periodontitis; ROC curve analysis; Saliva.

INTRODUCTION

Periodontitis is one of the main causes of tooth loss in adult subjects and it is the first cause in elderly people. When not treated it leads to jawbone resorption and tooth loss (1, 2).

In patients affected with periodontitis, several studies have shown a correlation between volatile sulphur compounds (VSCs) concentration in mouth air and increased pocket depth (3-6). VSCs, particularly hydrogen sulphide and methyl mercaptan, are a family of gases which are primarily responsible for halitosis. They had been identified as the main contributors to oral malodour and they have also been found in increased levels in bleeding pockets. Moreover, these compounds are highly toxic to tissues even at extremely low concentrations and, therefore, they may play a role in the pathogenesis of inflammatory conditions affecting the periodontium, such as periodontitis (7).

Zappacosta et al. (8) showed that the concentration of some sulphur compounds (cysteine, cysteinylglycine and glutatione) in saliva of patients suffering from periodontitis is significantly higher than that of control subjects. More recently, Zappacosta et al. (9) found that salivary cysteine, a direct precursor of hydrogen sulphide, could be considered a reliable marker for oral tissue damage severity in patients suffering for periodontitis. Advanced periodontal disease is often associated to teeth considered hopeless, according to the criteria of Becker et al. (10).

Laboratory assays do not often give a clinically useful diagnosis (11) as they should have a threshold with

Variable	Healthy subjects	P1 patients	P2 patients
N° of subjects	15	18	17
Cysteine(Ìmol/L)	1.16± 0.36	4.72±1.05*	10.78±3.13*°

All values are expressed as the mean±SD
**Mann-Whitney U-test, statistically significant difference between healthy subjects and periodontitis patients; P<0.01*
°Mann-Whitney U-test, statistically significant difference between P1 and P2 patients; P<0.01

Table 1 *Detection of salivary cysteine in clinically healthy subjects and periodontitis patients.*

discriminatory and predictive capabilities. Receiver Operating Characteristic (ROC) curve analysis has a long history in medical diagnostics (12, 13), and it identifies thresholds that discriminate between healthy subjects and diseased patients.
The goals of the present paper are to determine, by HPLC assay, salivary cysteine levels in periodontitis affected patients with or without hopeless teeth and to evaluate, by ROC curves analysis, the diagnostic accuracy of these measurements.

MATERIALS AND METHODS

Subjects

The study population included 15 systemically healthy subjects (mean age 33.6 years, range 23-44 years) with clinically healthy periodontium (controls) and 35 systemically healthy subjects (mean age 32.5 years, range 26-48 years) affected by periodontitis. The clinical criteria for the diagnosis of periodontitis consisted of standard measurements of clinical pocket depth and radiographic examinations. Particularly, periodontal patients should show at least two sites with probing depths greater than 4 mm. Periodontitis patients were divided into two subgroups based on the absence (n=18, P1 patients) or presence (n=17, P2 patients) of periodontally hopeless teeth, according to the criteria of Becker et al. (10). The mean number of teeth with hopeless prognosis per subject was 2.85±0.32.

Collection of saliva samples

At clinical examination, whole saliva samples were collected using paraffin wax-stimulation (14). Samples were stored at –80°C till cysteine evaluations. For chemical analysis cysteine of the highest purity available (Sigma, St. Louis, MO, USA) was used. For biochemical analysis salivary cysteine levels were evaluated by HPLC (15).

Statistical analysis

Statistical analysis was carried out using statistical software packages (MedCalc, demo version, and GraphPad Software, Version 4.02). Evaluations were undertaken using a non-parametric test Mann-Whitney U-test. Receiver Operating Characteristic (ROC) curve analysis was used for selecting the cut-offs that provide the best combination of sensitivity and specificity. The areas under the ROC curves and their 95% confidence intervals (CI) were also calculated (16). Significance was set at P<0.05.

RESULTS

The comparison between healthy and periodontitis patients shoved statistically significant differences in the salivary cysteine concentration (P<0.01) (Table 1). The results of ROC analysis with confidence interval of 95% are summarized in Table 2. Sensitivity of 100%, specificity of 100% and diagnostic accuracy of 100% were obtained at a cut-off of 1.85 µmol/L for controls and P1 patients. Similar results were obtained for controls and P2 patients at the same cut-off value. Sensitivity of 94.1%, specificity of 100.0% and diagnostic accuracy of 97.2% were obtained at a cut-off of 6.75 µmol/L for P1 compared to P2 patients.
Figure 1 illustrates ROC curve correlating sensitivity

Correlation	Technique	Value
P1 vs CS*	ROC**	
	AUC	1.000
	Cutoff	1.85 µmol/L
	95% confidence interval	0.893 a 1.000
	Sensitivity	100%
	Specificity	100%
	Diagnostic accuracy	100%
P2 vs CS*	ROC**	
	AUC	1.000
	Cutoff	1.85% µmol/L
	95% confidence interval	0.890 a 1.000
	Sensitivity	100%
	Specificity	100%
	Diagnostic accuracy	100%
P1 vs P2*	ROC**	
	AUC	0.990
	Cutoff	6.75 µmol/L
	95% confidence interval	0.881 a 1.000
	Sensitivity	94.1%
	Specificity	100%
	Diagnostic accuracy	97.2%

**Diseased patients (P1 and P2), healthy control subjects (HC)*
*** Receiver operating characteristics analysis: area under the curve (AUC) and standard error (SE), cutoff for sum of sensitivity and specificity maximized*

Table 2 *Statistical assessment for diagnostic accuracy of the salivary cysteine assay.*

and specificity associated with the cysteine concentration in saliva samples from P1 compared to P2 patients. Two decision levels of salivary cysteine are evident:

- at 1.85 µmol/L of salivary cysteine (to differentiate periodontitis patients from controls);
- at 6.75 µmol/L of salivary cysteine (to differentiate P2 from P1 patients) (Fig. 2).

DISCUSSION AND CONCLUSION

A variety of biological substances including xenobiotics, enzymes, hormones, immunoglobulins, and other molecules have already been successfully quantified in saliva (14). Recently, the use of saliva as a viable alternative source of human genomic DNA for genetic epidemiological studies has been explored (17) and studies on salivary 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a marker of oxidative stress have been carried out (18).

In the present study, the analysis of salivary cysteine levels of healthy and periodontitis patients with or without hopeless teeth has been undertaken by HPLC assay. Salivary cysteine levels from control subjects were in agreement with those reported by Zappacosta et al. (8, 9), with only small differences, possibly due to the different techniques used for saliva samples collection. Moreover, salivary cysteine level in periodontitis patients were significantly higher than that in controls ($P<0.01$); as supported by Zappacosta et al. (8, 9). It has been suggested that the significant increase of salivary cysteine showed by patients suffering for periodontitis could likely depend on both the typical oral tissues damage and a modification of the oxidant-antioxidant balance (19, 20).

Salivary cysteine in P2 was statistically greater ($P<0.01$) than in P1 patients. Therefore, the present findings suggested that hopeless teeth are a source of salivary cysteine. Previous studies reported that in hopeless teeth destructive episodes of periodontal tissue damage induced by oxidative stress might be increased (18).

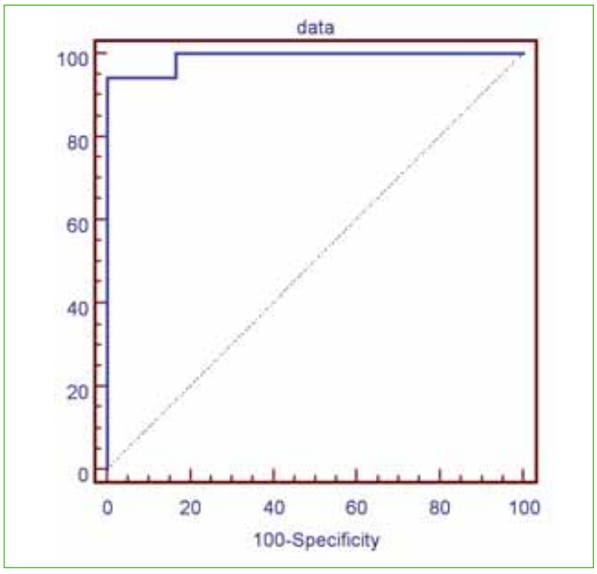

Fig. 1 *Receiver Operating Characteristic (ROC) curve analysis of salivary cysteine in P1 ad P2 patients. The true positive rate (sensitivity) is plotted as a function of the false rate (100 – specificity). Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. AUC (area under curve) value represents the combined effects of both sensitivity and specificity of cysteine assay in diagnosing P2 patients.*

The diagnostic accuracy of salivary cysteine measurements has been evaluated by the ROC plot method, which proved to have very high specificity, sensitivity and diagnostic accuracy. Indeed, it has been suggested that a test should have a diagnostic accuracy not lower than 80% to be considered valid and suitable for diagnostic purposes (21). In ROC analysis, an AUC (area under curve) value represents the combined effects of both sensitivity and specificity for an assay system. We determined the

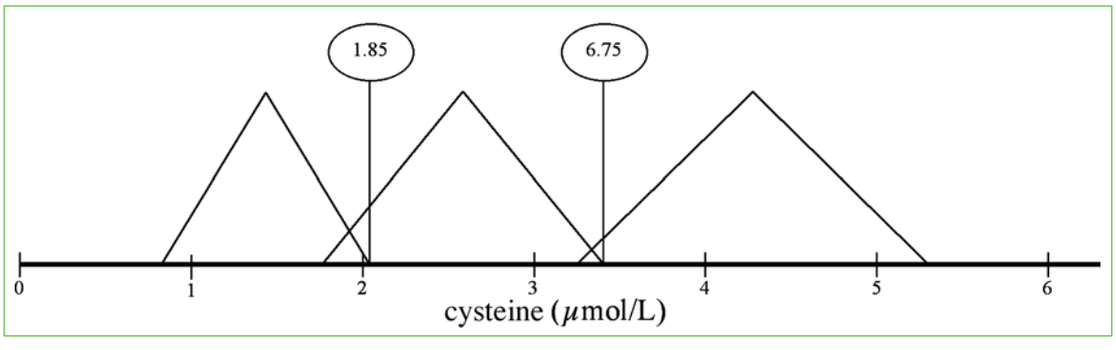

Fig. 2 *Decision levels of salivary cysteine (Ìmol/L). Decision levels (encircled values) and reference intervals are reported (reference intervals are triangle shaped).*

AUC values as 1.000, 1.000 and 0.990, respectively. An AUC value of 0.9 or higher indicates an excellent diagnostic marker (13).

The determination of clinical decision levels (DL) or "cut-offs" for laboratory parameters involves the analysis of sensitivity and specificity at varying levels of the predictor variable (PV). Commonly, ROC curves are used for this purpose. Two decision levels have been proved, thus improving the diagnostic usefulness of salivary cysteine measurements obtained from laboratory test. Indeed, ROC curves enable to detect a specific clinical category by comparing salivary cysteine values with these decision levels. It seemed that the 6.75 µmol/L decision level was particularly useful as it allowed to discriminate between P1 and P2 patients.

In conclusion, the present findings indicate that the measurement of salivary cysteine has revealed useful for identifying patients with hopeless teeth. Future perspectives of the present investigation should be to assess salivary cysteine assay suitability to detect alterations of peri-implant tissue, implant loss and peri-implantitis progression.

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