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Original article

Chemometric evaluation of the efficacy of locally administered chlorhexidine in patients with periodontal disease

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ABSTRACT

The process of assessment of drug efficacy produces multivariate data which are difficult to interpret. The interpretation and extraction of relevant data requires application of chemometric algorithms for multivariate data analysis. The aim of our study was evaluation of the efficacy of local treatment with chlorhexidine (CHX) in patients suffering from periodontal disease by chemometric algorithms for multivariate data analysis. Several algorithms were used: principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal projection to latent structures discriminant analysis (OPLS-DA). The PCA models identified the examined variables as suitable for monitoring the periodontal disease progression at the same time revealing mutual relationship among them. The developed PLS-DA model successfully distinguished patients treated with CHX from non-treated patients. The OPLS-DA model revealed differences in the mechanism of action of the two widely applied treatments in periodontal disease, local administration of CHX and local administration of doxycycline (DOX). The approach presented in this study opens the possibility of application of chemometric algorithms for multivariate data analysis for assessment of treatment efficacy.

1. Introduction

The process of drug efficacy assessment encompasses conduction of large controlled clinical trials where relatively homogenous group of subjects are carefully evaluated with respect to predefined clinical and laboratory parameters and closely monitored with respect to unexpected effects (Rasmussen et al., 2010; Ren et al., 2012; Cleophas and Zwinderman, 2013). The massive amounts of data generated by these trials are traditionally analyzed using univariate approach *i.e.* considering mean result versus control (Liland, 2011; Cleophas and Zwinderman, 2013).

However, the univariate approach suffers from several disadvantages such as the need for large number of samples as well as the inability to compensate for the missing data points which may require important observations to be discarded from analysis (Helmy et al., 2012).

Compared to the univariate approach, the multivariate approach that uses chemometric algorithms for data analysis represents a powerful tool for exploring large datasets derived from biological systems which contain multiple variables, missing data points or relatively small number of observations (Eriksson et al., 2006; Helmy et al., 2012). Their application provides "multicomponent insight" into treatment effects and therefore the adoption of chemometric algorithms for multivariate data analysis for assessment of treatment efficacy is highly recommendable (Durcekova et al., 2011; Mocak, 2012; Jimenez et al., 2013; Mrazova et al., 2014).

Abbreviations: GCF, gingival crevicular fluid; ALP, alkaline phosphatase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; IL-1β, interleukin -1 beta; TNF, α- tumor necrosis factor-alpha; CAL, clinical attachment loss; PD, pocket depth; GI, index of gingival inflammation; CHX, chlorhexidine; DOX, doxycycline; PCA, principal component analysis; PLS-DA, partial least square discriminant analysis; VIP, variable influence on projection; OPLS-DA, orthogonal projection to latent structures discriminant analysis.

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Periodontal disease is an inflammatory condition initiated by a bacterial infection which affects the supporting structures of the teeth. The treatment for periodontal disease is known as scaling and root planning and is followed by local administration of antiseptics or antibiotics. The treatment effects can be assessed by: (a) determination of clinical indices: index of gingival inflammation (GI), periodontal pocket depth (PD) or clinical attachment loss (CAL); (b) determination of inflammatory biomarkers in gingival crevicular fluid (GCF), (enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH)) or cytokines (interleukin - 1β (IL-1β) and tumor necrosis factor - α (TNF-α)) and (c) determination of concentration of the applied drug in GCF (Perinneti et al., 2008; Aimetti et al., 2012; Ertugrul et al., 2013; Spruill et al., 2014). However, the conclusions regarding treatment efficacy are commonly derived using the conventional approach. The biggest drawback of the univariate approach in dentistry is multicollinearity – mathematical coupling of variables which may lead to greater uncertainty in the result and may require removal of certain variables. One of the possible solutions to the problem lies in the application of chemometric algorithms for multivariate data analysis such as principal component analysis, partial least square regression or extension of these algorithms (Tu et al., 2009).

In our study, we will assess the performance of chemometric algorithms for multivariate data analysis for evaluation of efficacy of local administration of chlorhexidine (CHX) in patients suffering from periodontal disease. Furthermore, the efficacy of local administration of CHX is compared to the efficacy of local administration of doxycycline (DOX), another commonly used drug in periodontal treatment.

2. Materials and methods

2.1. Chemicals and materials

Chlorhexidine digluconate and chlorpheniramine maleate (internal standard, IS) were purchased from Sigma Aldrich (Germany) and Supriya Lifescience Ltd. (India), respectively. HPLC grade methanol and acetonitrile (ACN) were supplied by Carlo Erba (Italy). Sodium phosphate, triethylamine (TEA) and phosphoric acid (p.a grade) were also purchased from Sigma Aldrich (Germany). Throughout the entire chromatographic analysis, HPLC water was used. Whatman 3MM chromatography paper strips, 2 × 5 mm (Whatman Lab sales Ltd., UK) were used for GCF collection. Chlorhexamed 1% gel (0.5 g chlorhexidine digluconate/50 g gel) was purchased from GlaxoSmithCline, GmbH, Buehl, Germany while ATRIDOX 10% gel (45 mg doxycycline hyclate/0.5 g gel) was supplied by TOLMAR Inc. Fort Collins (USA). Protease inhibitor cocktail for cytokine determination was purchased from Sigma Aldrich (Germany). The assay kits for ALP, LDH and AST activity determination were purchased from Biosystems (Spain). The concentration levels of IL-1β and TNF-α in GCF samples were analyzed using commercial ELISA kits (Booster Immunoleader, Fremont, CA).

2.2. Subjects and protocol

The subjects who participated in the study were divided in three groups: experimental group of 34 patients who received local treatment with CHX gel (conventional release gel formulation), experimental group of 25 patients who received local treatment with DOX gel (controlled release gel formulation) and a control group of 9 healthy volunteers, with no previous history of periodontal disease. All patients were recruited from the Department of Periodontology, Faculty of Dentistry in Skopje. The study protocol was approved by the Ethics Committee at the Faculty of Dentistry and all the patients provided written informed consent before attending the study.

2.3. GCF sample collection

GCF samples were collected from quadrants consisting of five periodontal pockets before and after the local periodontal treatment. The patients who received the local treatment with CHX were administered 330 mg of gel containing 2 mg of CHX and GCF samples were taken 30 min after the gel application. The patients treated with DOX gel were administered 115 mg gel containing 10 mg of DOX and the GCF samples were taken 7 days after the local treatment. GCF from the selected periodontal pockets was collected using the method proposed by Koss et al. In brief, the paper strips were placed in selected periodontal pockets until mild resistance was felt and left in place for 30 s (Koss et al., 2009).

2.4. Chromatographic conditions for determination of CHX in GCF samples

The HPLC analysis was conducted on Shimadzu Nexera HPLC system with UV diode array detector. The chromatographic separation was achieved using Discovery C18 chromatographic column, 250 × 4.6 mm, 5 μm (Supelco, USA) at 25 °C. The mobile phase consisted of ACN, 0.01 mol L^{-1} phosphate buffer adjusted to pH = 3.0 using phosphoric acid and TEA (33: 66:1, *V/V/V*). The mobile phase flow rate was set at 1 mL/min and the injection volume was 50 μL. The wavelength of detection was 253 nm and the total runtime for the analysis was 10 min. The method was validated according to EMA Guideline on bioanalytical method validation (EMA, 2011).

2.5. Measurement of clinical indices

The degree of periodontal inflammation/health was assessed by the following clinical indices: pocket depth (PD), clinical attachment level (CAL) and index of gingival inflammation (GI). The indices were recorded by a single examiner, before and after local administration of CHX and DOX. GI was expressed using values from 1 to 3, where value of 1 expresses low inflammation and the value of 3 means high inflammation. PD and CAL were measured in mm. PD value larger than 3 mm indicates existence of periodontal pockets and CAL value larger than 3 indicates periodontal disease.

2.6. Determination of inflammatory biomarkers in GCF samples

For determination of inflammatory biomarker activity/concentration in GCF, the total of five paper strips were placed in 500 μL PBS (phosphate buffer saline, pH = 7.4) and the tube was centrifuged for 5 min at 1000 × *g* (4 °C) in a microcentrifuge (Centurion Scientific K3 Series) to elute the GCF component. The solutions containing the GCF component were divided in two aliquots of 250 μL. The aliquot for determination of enzyme activity was assayed immediately after collection whereas the aliquot for determination of cytokine concentration was added proteinase inhibitor cocktail and kept at −80 °C until analysis.

ALP, LDH and AST activity in GCF sample solutions were determined using semiautomatic photometer (HymaLizer Primus, Germany). The determination was performed using commercial kits according to the International Federation of Clinical Chemistry (IFCC) recommendations. The final results were expressed as total enzyme activity per sample (IU/sample).

The concentration levels of IL-1β and TNF-α in GCF sample solutions were analyzed using commercial ELISA kits, according to manufacturer's instructions. The absorbance was measured by

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