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Salivary levels of calcium, phosphorus, potassium, albumin and correlation with serum biomarkers in hemodialysis patients

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ABSTRACT

Objectives: Evidences suggest that hemodialysis patients have reduced salivary flow and changes in the composition of salivary secretion. These changes may reflect local and systemic disorders. The objectives of this study were to compare the salivary levels of calcium (Ca), phosphorus (P), potassium (K) and albumin in hemodialysis patients and healthy subjects, and to investigate a possible correlation between their serum and salivary levels.

Design: A case–control study was conducted with 60 hemodialysis patients (HD group) and 37 systemically healthy individuals (control group). Stimulated saliva samples were collected for biochemical analysis (Ca, P, K and albumin). Serum data were collected in the HD group. Statistical analysis included *t*-test, Pearson correlation and simple linear regression.

Results: The HD group exhibited higher salivary levels of Ca, P, and albumin ($p < 0.05$). There was a significant positive correlation between serum PTH and salivary phosphorus ($r = 0.342$, $p = 0.009$), and between serum PTH and salivary potassium ($r = 0.306$, $p = 0.020$). An increase of 100 pg/dL in serum PTH was associated with an elevation of salivary P levels (0.34 mg/dL, $p = 0.009$), and salivary K levels (0.20 mmol/dL, $p = 0.02$), in the HD group.

Conclusions: The findings suggest that HD patients present increased levels of salivary components (Ca, P, and albumin), and changes commonly observed in HD patients, such as hyperparathyroidism, appear to have an influence on salivary composition.

1. Introduction

Kidney failure is regarded as the most serious clinical outcome of chronic kidney disease (CKD). Its symptoms are usually caused by complications of the progressive reduction in glomerular filtration rate (GFR) and consequent imbalance in body homeostasis (Levey & Coresh, 2012; Eckardt et al., 2013; Awuah, Finkelstein, & Finkelstein, 2013), steep decline in quality of life (Awuah et al., 2013; Feng, Yap, & Ng, 2013), and increased risk of morbidity and mortality because of cardiovascular outcomes (Gansevoort et al., 2013; Block, Kilpatrick, Lowe, Wang, & Danese, 2013). At this stage, which is defined as end-stage renal disease (ESRD), GFR does not exceed 15 mL/min for 1.73 m^2 (KDOQI Working Group, 2002). Consequently, affected individuals must be subjected to dialysis and/or kidney transplant for ensuring survival (Eckardt, Berns, Rocco, & Kasiske, 2009). Worldwide epidemiological projections suggest a substantial increase in the incidence rates and prevalence of ESRD, particularly in developing countries, such as China, India, and Brazil, where the number of elderly people is rapidly increasing (Jha et al., 2013).

Foremost among the endocrine changes resulting from ESRD is the deficiency in synthesizing calcitriol, a derivative of vitamin D, which reduces the intestinal absorption of calcium (Ca) (Wolf et al., 2007; Tangpricha & Wasse, 2014) and subsequent hypocalcemia in patients undergoing hemodialysis (Zimmerman et al., 2013; Kim et al., 2014). Meanwhile, high phosphorus (P) plasma levels are observed because of the failure to excrete this element via the renal tubules (Kim et al., 2014; Collinson, McMullan, Tse, & Sadler, 2014). As a mechanism to compensate for the homeostasis between Ca and P, the body increases the synthesis of parathyroid hormone

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(PTH) (Patel, Barron, Mirzazedeh, Gallagher, Hyer, & Cantor, 2011), leading to secondary hyperparathyroidism associated with extra-osseous calcifications, osteoporosis, and increased rates of mortality from cardiovascular diseases (Palmer et al., 2011). Therefore, serum components should be routinely measured for optimizing clinical interventions and monitoring possible adverse outcomes in patients undergoing hemodialysis (Eckardt & Kasiske, 2009; Locatelli et al., 2013; Evenepoel, Rodriguez, & Ketteler, 2014).

Biological indicators can also be monitored by measuring other bodily fluids, such as saliva, which has the advantages of being easy to collect, non-invasive, and inexpensive to test (Yoshizawa et al., 2013). Studies have been conducted for investigating salivary factors that may reflect local and systemic disorders (Lee & Wong, 2009; Kawas, Rahim, & Ferguson, 2012).

Evidence indicates that patients undergoing hemodialysis have a reduced salivary flow (Bossola & Tazza, 2012; Dirschnabel et al., 2011; Kawas et al., 2012). Moreover, the composition of the saliva may be modified, including increased viscosity; increased urea, sodium (Na), P, potassium (K), and total protein concentrations; decreased Ca levels; higher pH; and reduced buffer capacity (Gavalda et al., 1999; Tomás et al., 2008; Savica et al., 2008; Kaushik et al., 2013). However, no previous studies have investigated the association between the biochemical composition of saliva and possible reflections of the concentration of serum biomarkers, such as PTH, in order to test the feasibility of using saliva for identifying mineral bone disorders associated with ESRD as well as to generate data for understanding the association between ESRD and oral changes.

Considering that salivary measurements can be an alternative strategy for the clinical monitoring of patients undergoing hemodialysis and that they also reflect imbalances in oral components (Nagler, 2008), the objectives of this study were as follows: (1) to compare Ca, P, K, and albumin concentrations in the saliva in patients undergoing hemodialysis with systemically healthy individuals and (2) to examine the linear correlation between the electrolytes (Ca, P, K, and albumin) in saliva and serum markers in the hemodialysis patients.

2. Materials and methods

2.1. Design and study groups

This present case–control study was conducted in São Luís, Maranhão, Brazil between January and August 2014. The project was approved by the research ethics committee of the University Hospital at the Federal University of Maranhão, São Luis, Brazil (case number 388/10). All participants were informed about the aims and procedures of the study and signed the terms of free and informed consent.

The hemodialysis group (HD group) included patients of both sexes aged >18 years, who presented a GFR of <15 mL/min per 1.73 m^2 and were undergoing conventional hemodialysis (three times per week for 4 h) for at least 6 months in a local hemodialysis center. Exclusion criteria included previous kidney transplant, decompensated diabetes mellitus, presence of neoplasia (verified in the medical records), current smokers or ex-smokers for <10 years, body mass index (BMI) >30 kg/m^2, clinical presence of candidiasis or stomatitis lesions in the buccal mucosa, presence of nodules and/or swelling in the region of the major salivary glands (verified by visual examination and digital palpation).

The control group included individuals of both sexes aged >18 years who had visited the dental clinic at the School of Dentistry at the Federal University of Maranhão, São Luís, Maranhão, Brazil for undergoing prophylactic, periodontal, and/or restorative procedures. The initial screening comprised anamnesis and a general physical examination, which involved measuring systolic blood pressure (SBP) and diastolic blood pressure (DBP) and calculating body mass index (BMI) as the ratio of weight (kg) to height squared (in m^2). Further, 20 mL of blood was collected for determining the hematological and biochemical analysis markers (complete blood count, white cell count, albumin, iron, ferritin, transferrin saturation, glutamic-pyruvic transaminase, C-reactive protein, fasting glucose, total cholesterol, triglycerides, creatinine, and uric acid). The following individuals were excluded: those reporting chronic systemic or infectious disease, users of orthodontic appliances; those with a history of anti-inflammatory use and/or antibiotic therapy in the past 3 months; individuals with arterial hypertension (SBP of >139 mm Hg and/or DBP of >89 mm Hg); obesity (BMI of >30 kg/m^2); serum parameters outside the reference values; and GFR calculated using the serum creatinine, age, and weight variables (or a GFR outside the range 90–120 mL/min for men and 80–110 for women). The same exclusion criteria used for HD group were also applied to the control group. This careful screening allowed the creation of a group with a low probability of significant systemic changes.

2.2. Interview and collection of serum markers

Data on demographic characteristics, smoking habits, and general health were obtained from a semi-structured questionnaire. In the HD group, hemodialysis time (in months) was recorded, and serum variables (PTH, Kt/V, glutamic-pyruvic transaminase, iron, ferritin, transferrin saturation, total iron binding capacity, hemoglobin, and hematocrit) were extracted from the blood samples collected for a routine monitoring of dialysis/ESRD. All serum testing processes were conducted in the same laboratory.

2.3. Saliva sample collection

Participants were told to refrain from eating, drinking, or performing oral hygiene procedures within an hour before collection. Stimulated saliva samples were collected by masticating a 5 × 5 cm sheet of paraffin (Parafilm®). Individuals were instructed to tilt their heads slightly forward and to not speak or swallow the saliva in their mouths. Every minute, the participant spit the accumulated volume of saliva into a 50 mL graduated cylinder until a volume between 5 and 10 mL was obtained.

The samples were stored on ice and transported for processing within 2 h. Saliva was collected from the control group in a second session (after the initial screening). In the HD group, samples were obtained during the first hour of the hemodialysis session on the same day that the blood sample was collected in order to measure the serum markers. In the laboratory, an aliquot of 10 μL of protease inhibitor solution (Protease Inhibitor Cocktail, Sigma–Aldrich, St. Louis, MO, USA) was added to each 5 mL of saliva. The samples were centrifuged for 15 min at 4300 rpm in a tube centrifuge (at 4 °C). After this procedure, the supernatant volume was stored in 1.5 mL microtubes and frozen at −80 °C for subsequent biochemical analysis.

2.4. Biochemical analysis of the saliva

Ca (mg/dL), P (mg/dL), K (mmol/L), and albumin (g/dL) in the saliva were quantified in triplicates using the colorimetric method and reagents provided by the manufacturer (Doles Chemistry, Goiânia, Goiás, Brazil). Subtraction of the control solution (without the sample) was used as a reference for calculating the converted measurement units, followed by multiplying with the factor of the standard solution. The absorbance reading for Ca at a 670 nm