Archives of Oral Biology 60 (2015) 1784–1788

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

# Salivary markers in patients with chronic renal failure

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ARTICLE INFO

*Article history:*
Received 8 May 2015
Received in revised form 15 August 2015
Accepted 10 September 2015

*Keywords:*
Saliva
Chronic renal failure
Inflammation
Markers

ABSTRACT

*Introduction:* Chronic renal failure (CRF) is a progressive loss of renal function over a period of months or years. The major function of the kidneys is the removal of metabolic waste products, electrolytes and water. When this function is impaired, systemic changes, oral complications and alterations in salivary composition may occur.
*Objective:* This study aimed to compare the levels of immunological and inflammatory components in the saliva samples from patients that undergo to hemodialysis treatment (HD), without HD and control.
*Design:* This study evaluated IgA, IgG, C reactive protein (CRP) and nitric oxide (NO) in saliva samples from 119 patients, who were divided into the control group (C), chronic renal failure (CRF) patient group and CRF patients on hemodialysis treatment (HD) group. IgA and IgG levels were analyzed by ELISA. Nitric oxide levels were determined indirectly by the nitrite concentration using Griess reagent; CRP by agglutination tests; and total proteins, by Bradford assay.
*Results:* The HD group showed significantly higher levels of IgG, IgA and CRP compared with the control and CRF groups. The CRF group presented the same amounts of IgG, IgA and CRP as the C group but significantly higher levels of NO similar to the HD group.
*Conclusion:* Renal disease, particularly hemodialysis treatment during renal disease, seems to alter salivary immunological and inflammatory components. Thus, analyzing the levels of IgA, IgG, NO and CRP in saliva may be beneficial for monitoring renal disease.



## 1. Introduction

Chronic renal failure (CRF) is a structural change that involves kidneys with limited or reduced capacities for glomerular filtration and that is characterized by the accumulation of substances in the blood that should be filtered and excreted by the kidneys. Regular monitoring of creatinine levels allows physicians to predict the need for hemodialysis or kidney transplantation (Tomás et al., 2008). The clinical signs and symptoms of CRF are dependent on the disease stage, affect most body systems and are collectively called uremia (De Rossi & Glick, 1996; National Kidney Foundation, 2002; Proctor, Kumar, Stein, Moles, & Porter, 2005).

Patients on hemodialysis frequently present chronic inflammation, which is the most important cause of cardiovascular disease in these individuals. C-reactive protein (CRP), a major biomarker of inflammation, is elevated in the serum of a large proportion of patients on hemodialysis (Blicharz et al., 2008).

In contrast, an increase in toxic substances in the bloodstream due to renal failure can cause immunodeficiency. CRF patients can have suppressed humoral and cellular immune responses and subnormal serum IgA, IgM and IgG concentrations.

Saliva is an oral fluid mixture that is derived from the major and minor salivary glands and that is composed of non-salivary source constituents, including a variety of microorganisms and their products, blood cells, and desquamated epithelial cells. Saliva also contains components derived from serum resulting from passive

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http://dx.doi.org/10.1016/j.archoralbio.2015.09.008
0003-9969/

diffusion through crevicular fluid. Thus, saliva has been proposed to be a good source for diagnostic purposes (Blicharz et al., 2008; Nagler, 2008; de Almeida, Grégio, Machado, de Lima, & Azevedo, 2008; Humphrey & Williamson, 2001). Several chronic diseases, autoimmune disorders such as Sjögren's syndrome, cardiovascular diseases, infectious diseases (viral and bacterial), renal diseases and cancer can be diagnosed by saliva (Streckfus & Bigler, 2002). Tomás et al. (2008) found higher salivary pH and higher salivary urea, sodium and potassium concentrations in CRF patients than in healthy controls. Additionally, the salivary calcium concentration values were significantly lower. The salivary creatinine, urea and potassium conditions are conditioned by the degree of renal failure and/or by treatment with dialysis.

Because few studies have quantified the levels of immunoglobulins (Igs) IgA and IgG, nitric oxide (NO), and CRP in patients with renal disease, this study aimed to evaluate these salivary markers in chronic renal failure patients with varying stages of disease.

## 2. Materials and methods

### 2.1. Subjects

For this cross-sectional study, 119 individuals between 24 and 82 years old (67 females and 58 males) that were treated previously at different nephrology clinics in the municipalities of Taubate and Pindamonhangaba—SP were analyzed. The participants were informed regarding the purpose and methodology of the study and signed a consent form that had been approved previously by the Ethics Committee of the University of Taubaté —UNITAU (Ref: 0485/07).

The subjects were divided into three groups according to the presence of CRF and/or stage of the disease following the guidelines of the National Kidney Foundation (2002):

- Control group (C): 47 individuals without CRF (creatinine clearance>60 mL/min/1.73 $m^2$)
- Chronic renal failure group (CRF): 34 individuals with moderate/severe CRF in pre-dialysis (creatinine clearance 15–60 mL/min/1.73 $m^2$)
- Hemodialysis group (HD): 38 individuals in the terminal stage of CRF on hemodialysis (creatinine clearance <15 mL/min/1.73 $m^2$)

Blood pressure, diabetes, and laboratory (creatinine, urea and albumin) data were obtained from the medical records of patients followed up for renal disease, and when unavailable, from blood samples collected for laboratory analysis.

Criteria for inclusion: present available time for work execution and exclusion criteria: antibiotic therapy in the last 72 h before the saliva collection; being seropositive for HIV, hepatitis C virus (HCV) or hepatitis B (HBV).

With the data obtained from laboratory tests, the estimating creatinine clearance (CrCl) was calculated using the Cockcroft–Gault equation as follows: CrCl (mLl/min) = (140 − age) × BWI/(Scr × 72) × (0.85 for females) (Cockcroft & Gault, 1976).

### 2.2. Sample size calculation

Due to the unknown population size with kidney disease, three unknown parameters were defined: (1) the desired level of confidence, (2) the sampling error allowed, and (3) the actual success rate p: The confidence level used in determining the size of the sample was 95% (where $Z = 1.96$), because it provides good balance between accuracy and reliability. The sampling error allowed was 18%, ie, $e = 0.18$, which is the error of volume we are willing to accept to study the proportion of the population, that is, the maximum likely difference between the sample proportion and the true population proportion.

$$N = \frac{1.92^2 \times 0.5(1 - 0.5)}{0.18^2} = 30$$

As the true proportion of successes is unknown, and we have no past experience or relevant information, we used $p = 0.5$ as the most conservative way to determine the sample size. Therefore, for each study group we should have at least 30 individuals.

### 2.3. Saliva collection

Saliva was collected at the Clinic of Dentistry of the University of Taubaté. Subjects refrained from brushing for 12 h and from drinking, eating or chewing gum for 1 h before sample collection. Whole saliva that had accumulated for five minutes was collected into a labeled, identified sterilized plastic tube, which was stored at −80 °C.

### 2.4. IgA and IgG analysis

Immunoglobulin analysis was performed by ELISA. The wells of the plates were sensitized with anti-Ig/Fab′2 at a concentration of 1 μg/ml. The saliva samples, which were diluted 100 times in PBS-TG, were added in duplicate. The plates were washed with PBS-T, and then the conjugate anti-IgA or anti-IgG was added to each well along with peroxidase (1/5000) in PBS-TG. Peroxidase activity was revealed using the substrate with orthophenylenediamine (OPD, Sigma) in 0.1 M acid citrate buffer (pH 5.5). After the reaction was incubated at room temperature for 15 min, it was stopped with 2.5 N sulfuric acid, and the optical density was measured at 490 nm.

### 2.5. Nitric oxide (NO) analysis

The NO levels were determined indirectly by measuring the nitrite concentration detected by Griess reagent, which is composed of equal volumes of three solutions (A, B and C). Solution A: 0.6 g of sulfanilic acid dissolved in 70 mL of hot distilled water, 20 mL of concentrated hydrochloric acid and distilled water to a final volume of 100 mL. Solution B: 0.6 g of alpha-naphthylamine dissolved in 20 mL of distilled water, 1 mL of hydrochloric acid and distilled water to a final volume of 100 mL. Solution C: 16.4 g of CH3COONa 3H2O dissolved in 100 mL of distilled water. After mixing equal parts of the three solutions, Griess reagent was added to the wells of a microplate. Then, the same volume of samples was added. Reading was performed using an ELISA reader at 520 nm.

### 2.6. C-reactive protein (CRP) analysis

The agglutination test was used for the determination of CRP. The reagent, a suspension of polystyrene latex particles sensitized with anti-human CRP immunoglobulin, was added to the same sample volume. When the reaction was positive, the sample was titrated to calculate the concentration of CRP.

### 2.7. Statistical analysis

Statistical analyses and graph generation were performed using GraphPad Prism version 4.0 and BioEstat version 5.0. First, a normality test (Kolmogorov–Smirnov) was performed. Then, comparisons between groups (control/CRF and HD) at each trial were performed using the Mann–Whitney test. A significance level of 5% was used for all analyses.