Pesquisa Brasileira em Odontopediatria e Clínica Integrada

ISSN: 1519-0501

apesb@terra.com.br

Universidade Estadual da Paraíba

Brasil

Fregoneze, Andréa Paula; Guimarães, Lara Karolina; Walesco Zanelatto, Marcela; Luzzi, Tatiana; Lira Ortega, Adriana; Bönecker, Marcelo; Branche, João Armando

Analysis of Salivary Flow, pH, Buffer Capacity, and Creatinine in Individuals Undergoing Hemodialysis

Pesquisa Brasileira em Odontopediatria e Clínica Integrada, vol. 16, núm. 1, 2016

Universidade Estadual da Paraíba

Paraíba, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=63749588015

Brazilian Research in Pediatric Dentistry and Integrated Clinic 2016, 16(1):141-147
DOI: http://dx.doi.org/10.4034/PBOCI.2016.161.15
ISSN 1519-0501

Original Article

Analysis of Salivary Flow, pH, Buffer Capacity, and Creatinine in Individuals Undergoing Hemodialysis

Andréa Paula Fregoneze[1], Lara Karolina Guimarães[2], Marcela Walesco Zanelatto[2], Tatiana Luzzi[3], Adriana Lira Ortega[4], Marcelo Bönecker[5], João Armando Brancher[6]

[1]Senior Lecturer, Pontifical Catholic University, Curitiba, PR, Brazil.
[2]Undergraduate student in Dentistry, Pontifical Catholic University, Curitiba, PR, Brazil.
[3]Post graduation student in Dentistry, Positivo University, Curitiba, PR, Brazil.
[4]Senior Lecturer, São Paulo University, São Paulo, SP, Brazil.
[5]Professor, Department of Pediatric Dentistry, São Paulo University, São Paulo, SP, Brazil.
[6]Professor, Masters of Science Program in Clinical Dentistry, Positivo University, Curitiba, PR, Brazil.

Author to whom correspondence should be addressed: João Armando Brancher, Positivo University, Pedro Viriato Parigot de Souza Street, 5300, Curitiba, PR, Brazil. 81280-330. Phone: 0051413317-300. E-mail: brancher.a@gmail.com.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 20 March 2015 / Accepted: 28 July 2016 / Published: 02 August 2016

Abstract

Objective: To quantify salivary creatinine levels patients with end-stage renal disease (ESRD) before, during, and after hemodialysis sessions. **Material and Methods:** Sixty-eight individuals, aged between 4 and 25 years, were selected, and among these, 34 were diagnosed with ESRD and were undergoing hemodialysis (Group 1) and 34 were clinically healthy patients (Group 2). Saliva samples were collected before, during, and after hemodialysis sessions for Group 1 and compared with those of Group 2. Stimulated saliva flow rate (SSFR), buffer capacity (BC), pH, and salivary creatinine levels were determined. Shapiro-Wilk test, followed by the Friedman, Mann-Whitney and ANOVA tests were used to analyze the variables. **Results:** Mean SSFR values of Group 1 at the three stages of hemodialysis sessions did not differ from those of Group 2. Furthermore, BC and pH values were within their normal limits, and no difference was detected between the two groups. Mean salivary creatinine levels at baseline and during hemodialysis were significantly higher in Group 1 that in Group 2, but these values were not different between Groups at the end of hemodialysis. **Conclusion:** Salivary creatinine levels reduce significantly after hemodialysis sessions suggesting that saliva may be used to monitor the efficiency of hemodialysis or even indicate the moment at which it should start.

Keywords: Saliva; Creatinine; Renal Dialysis.

Introduction

Saliva provides several innate and acquired defense factors that can inhibit bacterial invasion, growth, and metabolism by different mechanisms [1,2] such as bacterial adherence and streptococci acid production [3]. Several studies have so far investigated many biological determinants that may influence biofilm cariogenicity [4,5], such as saliva flow and composition [6]. A constant salivary flow efficiently eliminates microorganisms from the oral cavity; conversely, a reduced flow may easily favor microbial growth, followed by teeth deterioration [7]. Some salivary proteins such as lysozymes, lactoperoxidase, immunoglobulins, agglutinines, mucins, and lactotransferrin have antibacterial effects [8] and hence help in preventing oral diseases.

Saliva is also an extremely useful biological fluid for prognosis, laboratory, or clinical diagnosis and monitoring systemic diseases [9]. Salivary biomarkers are employed for screening purposes in epidemiological studies [10] and are being used to monitor and detect various oral and systemic diseases such as breast cancer [11], lung cancer [12], celiac disease [13], and chronic renal failure [14]. Recent studies on end-stage renal disease (ESRD) reported a series of salivary markers associated with ESRD. The list of markers included cortisol, nitrite, uric acid, sodium, chloride, pH, amylase, and lactoferrin [15].

In the case of ESRD, measurement of serum creatinine in individual patients provides an estimate of disease progression and may determine the effects of therapy. It may be used to predict when dialysis becomes necessary and also indicate progress of ESRD [16]. Since serum constituents may reach the saliva [17], the goal of this study was to quantify the salivary creatinine in patients with ESRD before, during, and after hemodialysis sessions.

Material and Methods

All individuals included in the study signed informed consent form, according to norms of the Ethical Committee on Research of the Center for Health and Biological Sciences of the São Leopoldo Mandic, Campinas, SP, Brazil, following Resolution 196/96 of the Health National Council, register nº. 05/452. If the individual was under age, their parents/guardians signed the informed consent form.

Thirty-four unrelated ESRD patients, (23 male and 11 female, age group 4-25 years, with more than 30 months of hemodialysis sessions at the Hospital Pequeno Príncipe, Curitiba PR Brazil, formed the group one (G1). The control group (G2) consisted of 34 healthy subjects, matched for age and gender, from the Health Unit Nossa Senhora da Conceição, Campo Magro, PR, Brazil. All individuals were examined by two dentists. The consistency of each examiner (inter- and intra-examiner reproducibility) was assessed by duplicate examinations conducted on 10% of the sample; the kappa test measured reliability at 95% probability.

Saliva samples from both groups were obtained by spitting [18]. For the G1, saliva was collected in the hemodialysis room before connecting the hemodialysis machine, during the hemodialysis, and after switching off the hemodialysis machine. Saliva samples for G2 were collected

before the dental clinical care at the Health Unit Nossa Senhora da Conceição, as they sat in the dental chair. Salivary flow was evaluated by means of stimulated saliva collection, or rather, by mechanical masticatory stimulation using a standard (1.5 cm) piece of sterile rubber tourniquet during continuous mastication by the patient for 6 minutes. Saliva produced during the first minute of stimulation was discarded. During the following 5 minutes, individuals expelled saliva into a sterile universal collector vial that had been previously weighed with Marte® analytical scales, model AL 500 (São Paulo SP, Brazil). Stimulated saliva flow rate (SSFR) was evaluated using the gravimetric method and expressed in mL/min [19]. Furthermore, pH was measured by a portable pH meter (Digimed Analytical Instrumentation Inc., São Paulo, SP, Brazil). Salivary buffering capacity (BC) was determined with Caritest®–SL kit (Technew Com. e Ind. Ltda., Rio de Janeiro, RJ, Brazil). Moreover, 1 mL of the collected saliva was added to a flask containing 3 mL of 0.005N HCl solution. Sample readings were performed following the manufacturer's instructions.

The remaining saliva samples were centrifuged (3,000 *g* for 10 min). Salivary creatinine was measured using the alkaline picrate colorimetric method, Creatinina K – Labtest Diagnóstica, on a Bioplus spectrophotometer (Hoffmann-La Roche, Basel, Switzerland). Creatinine reacts with picrate in an alkaline medium to form a red complex measured photometrically. The addition of an acidulant lowers pH to 5.0 and causes the decomposition of creatinine picrate. The reading of the samples takes place at 30 and 90 seconds and the difference between the two readings provides the true creatinine rate. All tests were performed three times.

The Shapiro-Wilk Test was employed for the normality analysis. Friedman test, followed by nonparametric multiple comparisons, at 0.05 significance level, was used. The Mann-Whitney and ANOVA tests were employed when statistically significant rates ($p \leq 0.05$ and confidence interval [CI] 95%) occurred.

Results

Sixty-eight participants, 34 patients with ESRD (Group 1) and 34 healthy subjects (Group 2), participated in the current analysis. Twenty-three subjects were male (67.6%) and 11 were female (32.4%) in each group. The average age was 13.4 years in both groups. Five of the 34 patients in the study did not participate in the analysis due to the following causes: two of the patients were unable to produce a salivary flow with stimulation, two displayed behavioral resistance, and one died before the collection date.

Mean SSFR rates of ESRD patients at the three stages of hemodialysis sessions did not differ significantly from those of healthy patients (Group 2). In addition, BC was normal in the three stages of hemodialysis sessions and did not differ between both groups. Initial salivary pH of the ESRD group was higher at the start of the hemodialysis sessions and decreased during and after the sessions. Nonetheless, it remained higher than the salivary pH of the Group 2, albeit without statistically significant differences.

Mean salivary creatinine levels before and during hemodialysis were significantly higher in ESRD patients compared to healthy subjects ($p = 0.0063$ and $p = 0.0409$, respectively), but these values were not different between groups at the end of hemodialysis ($p = 0.4569$). ANOVA test showed a difference between the three sessions of hemodialysis for creatinine ($p = 0.0531$). Descriptive statistics of the variables analyzed according to the groups are given in Tables 1 and 2.

Table 1. Descriptive statistics of the variables in the two groups.

	ESRD Patients	Mean Value	Median	Standard Deviation	Standard Error
SSFR (mL/min) Initial	29	0.93	0.77	0.67	0.12
SSFR (mL/min) During	29	0.96	0.85	0.69	0.13
SSFR (mL/min) After	29	0.97	0.95	0.60	0.11
pH Initial	29	8.01	8.03	0.34	0.06
pH During	29	7.69	7.73	0.31	0.06
pH After	29	7.75	7.77	0.40	0.07
	Control Group				
SSFR	34	0.89	0.84	0.42	0.07
pH	34	7.39	7.41	0.25	0.04

Ssfr: Stimulated Salivary Flow Rate; Cre: Creatinine.

Table 2. Distribution of average creatinine values for each group according to the time of saliva collection.

Groups	Creatinine								
	Initial			During			After		
	Mean Value (mg/dl)	CI 95% LL	CI 95% UL	Mean Value (mg/dl)	CI 95% LL	CI 95% UL	Mean Value (mg/dl)	CI 95% LL	CI 95% UL
ESRD Patients	1.26 aA	0	3.23	0.28 abA	0.22	0.34	0.23 bB	0.17	0.28
Control	0.20 B	0.15	0.24						

CI: Confidence Interval; LL: lower limit; UL: Upper limit; The same small letters in the lines and the same capital letters in the columns show no statistical difference; Mann–Whitney U and ANOVA tests.

Discussion

The complex oral fluid called saliva has long been acknowledged as a clinically informative biological fluid that is highly useful for novel approaches in the diagnosis of diseases. The many advantages of saliva comprise good cooperation with patients, non-invasive collection, low cost, easy transportation and storage, early detection of disease [20], and correlation with levels of the same metabolites in blood [21].

Biochemical analysis of saliva and blood have two main objectives: to identify individuals with disease and evaluate the prognosis of the same during treatment [22]. Salivary fluid composition may be affected by different forms of stimulation, time of day, diet, age, gender, a variety of disease conditions, and several pharmacological agents [17,23]. Whole saliva is a mixed fluid that is derived predominantly from major and minor salivary glands. It contains gingival crevicular fluid, mucosal transudations, expectorated bronchial, and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, cellular components, and food debris [8,21] besides urea, ammonia, uric acid, glucose, cholesterol, fatty acids, triglycerides, phosphorus, neutral lipids, glycolipids, amino-acids, and steroid hormones [24].

Earlier researchers had reported a series of salivary markers associated with ESRD. The list included cortisol, nitrite and uric acid, sodium, chloride, pH, amylase, and lactoferrin [15]. Salivary phosphate has also been widely used as a clinical biomarker for hyperphosphatemia and these levels correlated well with serum creatinine and glomerular filtration rate [25]. Some authors reported that salivary creatinine concentration in individuals with chronic renal failure was associated with increased serum creatinine levels, which is not observed in healthy subjects [26]. Therefore, salivary creatinine may be a marker for kidney diseases.

The current assay showed that initial salivary creatinine of the ESRD patients was significantly higher before hemodialysis. In the course of the session and after that there was a significant decrease in creatinine. At the end of the hemodialysis session, salivary creatinine concentration remained higher but near the values observed in healthy individuals. The hemodialysis session clearly caused a significant decrease in the amount of salivary creatinine in ESRD patients.

Previous studies tested colorimetric test strips to monitor salivary nitrate and uric acid before and after hemodialysis [27]. At this point, the development of test strips to monitor the salivary creatinine may be interesting to indicate when hemodialysis session must begin or end, saving the patient time and providing great benefits to health services, particularly in primary health care.

Since the current research is related to SSFR, BC and pH, an inert material was employed to avoid interfering with the patient's salivary flow. This is why a piece of latex hose was used. During the acquisition of saliva samples, the patients from the two groups were seated. ESRD patients were sitting on the same chairs used for the hemodialysis sessions. Results on SSFR, BC, and pH revealed that the variables at the three sessions of hemodialysis presented no significant difference from the results of healthy subjects. These findings were unexpected as they are in direct contrast with the findings from most studies in the literature [28,29], which have reported a significantly reduced mean salivary flow in uremic patients. According to these authors, the quantitative change in salivary flow is directly associated with uremia involving salivary glands and a lower degree of hydration due to the restriction of fluid ingestion.

Finally, salivary research is a dynamic field. General and oral health will largely benefit from the development and improvement of new research techniques in this field.

Conclusion

Mean rates for SSFR, BC, and pH were not different between ESRD patients and healthy subjects. Salivary creatinine decreased significantly after hemodialysis sessions suggesting that saliva may be used to monitor the efficiency of hemodialysis or even indicate the moment at which it should start.

References

1. Kidd EAM, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004; 83:C35-C38.
2. Dawes C, Pedersen AM, Villa A, Ekström J, Proctor GB, Vissink A, Aframian D, McGowan R, Aliko A, Narayana N, Sia YW, Joshi RK, Jensen SB, Kerr AR, Wolff A. The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. Arch Oral Biol 2015; 60(6):863-74.
3. Tenovuo J. Clinical applications of antimicrobial host proteins lactoperoxidase, lysozyme and lactoferrin in xerostomia: efficacy and safety. Oral Dis 2002; 8(1):23-9.
4. Singh PK, Parsek MR, Greenberg EP, Welsh MJ. A component of innate immunity prevents bacterial biofilm development. Nature 2002; 417(6888):552-5.
5. Díaz-Garrido N, Lozano C, Giacaman RA. Frequency of sucrose exposure on the cariogenicity of a biofilm-caries model. Eur J Dent 2016; 10(3):345-50.
6. Nariyama M, Shimizu K, Uematsu T, Maeda T. Identification of chromosomes associated with dental caries susceptibility using quantitative trait locus analysis in mice. Caries Res 2004; 38(2):79-84.
7. Amerongen AVN, Veerman ECI. Saliva - the defender of the oral cavity. Oral Dis 2002; 8(1):12-22.
8. Jentsch H, Beetke E, Göcke, R. Salivary analyses and caries increment over 4 years: an approach by cluster analysis. Clin Oral Investig 2004; 8(3):156-60.
9. Malamud D, Rodriguez-Chavez IR. Saliva as a diagnostic fluid. Dent Clin North Am 2011; 55(1):159-78.
10. Nilminie R, Sigvard A, Bjorn K, Nina L, Henrik J, Ylva T, et al. Salivary biomarkers for detection of systemic diseases. PLoS ONE 2013; 8(4):1-5.
11. Agha-Hosseini F, Mirzaii-Dizgah I, Rahimi A. Correlation of 19 serum and salivary CA15-3 levels in patients with breast cancer. Med Oral Patol Oral Cir Bucal 2009; 14:521-4.
12. Xiao H, Zhang L, Zhou H, Lee JM, Garon EB, Wong DT. Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. Mol Cell Proteomics 2012; 11(2):111-2.
13. Bonamico M, Nenna R, Luparia RPL, Perricone C, Montuori M, Lucantoni F, et al. Radioimmunological detection of anti-transglutaminase autoantibodies in human saliva: a useful test to monitor coeliac disease follow-up. Aliment Pharmacol Ther 2008; 28:364-70.
14. Fregoneze AP, Ortega AL, Brancher JA, Vargas ET, Bönecker MJS. Sialometric analysis in young patients with chronic renal insufficiency. Spec Care Dentist 2013; 33(3):118.
15. Arregger AL, Cardoso EM, Tumilasci O. Diagnostic value of salivary cortisol in end stage renal disease. Steroids 2008; 73(1):77-82.
16. Pimentel R, Couto M, Laszczyńska O, Friões F, Bettencourt P, Azevedo A. Prognostic value of worsening renal function in outpatients with chronic heart failure. Eur J Intern Med 2014; 25(7):662-8.
17. Mittal S, Bansal V, Garg S, Atreja G, Bansal S. The diagnostic role of Saliva - a review. J Clin Exp Dent 2011; 3(4):e314-e320.
18. Busato IMS, Ignácio SA, Brancher JA, Moysés ST, Azevedo-Alanis LR. Impact of clinical status and salivary conditions on xerostomia and oral health related quality of life of adolescents with type 1 diabetes mellitus. Community Dent Oral Epidemiol 2012; 40:62-9.
19. Annette Milton B, Bhambal A, Nair P. Evaluation of sialometric analysis of patients suffering from depressive disorders. Kathmandu Univ Med J, 2015; 13(50):134-9.
20. Herenia PL. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. J Can Dent Assoc 2002; 68(3):170-4.
21. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: current state and future applications. Clin Chem 2011; 57(5):675-87.
22. Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. Oral Dis 2002; 8(2):69-76.
23. Lee JM, Garon E, Wong DT. Salivary diagnostics. Orthod Craniofac Res 2009; 12(3):206-11.
24. Dodds MW, Johnson DA, Yeh CK. Health benefits of saliva: a review. J Dent 2005; 33(3):223-33.
25. Nagler RM. Saliva analysis for monitoring dialysis and renal function. Clin Chem 2008; 54(9):1415-17.
26. Lasisi TJ, Raji YR, Salako BL. Salivary creatinine and urea analysis in patients with chronic kidney disease: a case control study. BMC Nephrol 2016; 16:17-10
27. Blicharz TM, Rissin DM, Bowden M. Use of colorimetric test strips for monitoring the effect of hemodialysis on salivary nitrite and uric acid in patients with end-stage renal disease: a proof of principle. Clin Chem 2008; 54(9):1473-80.

28. Bayraktar G, Kazancioglu R, Bozfakioglu S. Stimulated salivary flow rate in chronic hemodialysis patients. Nephron 2002; 91(2):210-14.
29. Bayraktar G, Kazancioglu R, Bozfakioglu S. Evaluation of salivary parameters and dental status in adult hemodialysis patients. Clin Nephrol 2004; 62(5):380-3.