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ORIGINAL ARTICLE

Salivary Changes in Patients Hospitalized for Complications of HIV Infection/AIDS: A Study Using Inductively Coupled Plasma Optical Emission Spectrometry

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ABSTRACT

Objective: The aim of this study was to analyze the salivary flow and levels of major salivary ions of HIV-infected individuals. **Methods:** One hundred and two participants (18 - 74 years of age) were divided into two groups (51 HIV - infected patients and 51 controls) and had a saliva sample collected. Salivary flow rate was measured gravimetrically. Levels of salivary sodium, potassium, magnesium, calcium and phosphorus were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). **Results:** The mean salivary flow of subjects with HIV infection was significantly higher when compared to controls (Case group = 1.0 mL/min $\pm$ 0.63 / Control group = 0.7 mL/min $\pm$ 0.46; p = 0.012). Sodium, potassium and calcium levels were significantly higher in the saliva of the patients HIV infection when compared to controls. There was no difference between the salivary levels of magnesium and phosphorus ions between groups. **Conclusion:** Based on these findings, it can be concluded that individuals with HIV / AIDS develop salivary changes. Thus, these patients need a greater attention to oral health by dentists, since salivary changes induced by the disease can lead to impairment in the oral condition.

Key words: acquired immunodeficiency syndrome, hospitalization, saliva, salivation, spectrometry

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INTRODUCTION

Saliva is a seromucous exocrine secretion constantly produced by the salivary glands. It is defined as a complex fluid that is composed of water, cells, debris and organic and inorganic molecules.[1] Once produced, saliva is excreted in the mouth where it performs several important functions, such as: oral digestion, ingestion and perception of food flavors, lubrication of mucous membranes, maintenance of tooth integrity and protection against microorganisms.[2,3] Saliva can reflect the physiological state of the body, including emotional, endocrine, nutritional and metabolic variations. Consequently, this fluid provides a source of information that serves to monitor oral and systemic health.[4]

Several factors may alter saliva production, such as: i) medications, ii) psychological conditions, iii) head and neck radiotherapy, and iv) salivary gland disorders.[5] HIV infection can alter the functioning of the salivary glands and, consequently, saliva. Salivary dysfunction after infection ranges from a complaint of xerostomia to a condition in which there is persistent glandular enlargement and hyposalivation.[6]

Several studies have been conducted with saliva since the recognition that HIV infection can alter the functioning of the salivary glands.[7-10] In 1992, Schiødt *et al.*[11] performed a biochemical analysis of saliva from patients with HIV infection. At that time,

the researchers observed a significant reduction in salivary protein levels, with elevation in salivary IgA, lysozyme and albumin when compared to negative controls. Patients with HIV / AIDS infection develop hyposalivation throughout the disease.[9,12] In 2020, our group developed a study and observed that the saliva of individuals with HIV /AIDS infection suffer no interference in levels of amylase and total salivary proteins. On the other hand, they have a significant decrease in salivary flow.[13]

Many debilitating diseases arise during the evolution of HIV infection to AIDS. Therefore, many patients with HIV infection need to be hospitalized for treatment. The average length of hospital stay varies, and many individuals may develop anxiety and stress at the hospital.[14] In addition, many patients need to take several medications and are reintroduced to antiretroviral therapy during hospital treatment. Some medications may interfere with the functioning of the salivary glands and, consequently, contribute to hyposalivation.[8]

The analysis of salivary components can be done by different analytical techniques. However, there are still doubts in the literature whether electrolyte levels in saliva change due to HIV/AIDS infection or the use of HAART.[15,16] Inductively coupled plasma optical emission spectrometry (ICP-OES) is a powerful analytical tool for the determination of metals, semi-metals and non-metals in various types of samples which is routinely used by modern chemistry.[17] Thus, the objective of this study was to investigate salivary flow and levels of the major electrolytes in the saliva of patients suffering from AIDS by ICP-OES.

METHODS

Ethics
This study was approved by the Committee of Ethics in Research of the Universidade Federal do Paraná (Number. 1.627.826).

Sample
The sample of this study consisted of 102 adult individuals of both genders with and without the diagnosis of HIV infection. The patients were divided into two groups: i) Case group – Composed of patients with a confirmed diagnosis of HIV infection; and ii) Control group – Healthy individuals without HIV infection.

Initially, the epidemiological data were collected from the medical record: personal data, reason for hospitalization, comorbidities, time of treatment with antiretroviral therapy, medications in use (antiretroviral drugs and others) and their history of smoking, alcohol and illicit drug use.

Saliva collection
Samples of total saliva were collected at the same time of day (9:00 a.m. - 11:00 a.m.). The stimulated total saliva was collected using a mechanical stimulus. These samples of saliva had previously been used in a study that investigated at the flow and levels of amylase and total proteins. Each saliva sample was centrifuged and frozen at -20^{o}C until analysis by ICP-OES.

Salivary flow analysis
Salivary flow analysis was performed using gravimetric technique[18] and hyposalivation was considered when the salivary flow was $\leq$ 0.6 mL / min.

Chemical analysis of saliva
The levels of the salivary electrolytes sodium, potassium, magnesium, calcium and phosphorus were evaluated by ICP-OES in the laboratory of environmental analyzes of the Department of Chemistry of the University. The saliva samples were initially transferred to falcon tubes with a capacity of 15 mL. One milliliter of the sample was diluted in 9 mL of deionized water obtained from the Milli-Q system totaling a final volume of 10 mL. In this way, a dilution factor of 10 was obtained. Subsequently, this factor was multiplied by the analytical response provided by the equipment in mg / L. The final result was given in mg / L. Dilution of the samples was necessary due to some factors such as high viscosity and high content of organic matter present in the saliva, besides the little amount of sample available. Dilutions were performed using a micropipettor and deionized water with resistivity of 18 MΩ cm obtained using the Milli-Q system (Millipore, Bedford, MA, EUA).

A spectrometer Thermo Scientific modelo ICAP 6500 (Massachusetts – USA, Ano 2007) was used with axial vision. The instrumental operating conditions of ICP-OES are shown in Table 1. The argon gas was used with a purity of 99.996% (White Martins/Praxair, São Paulo). ICP-OES is an analytical technique based on the optical emission spectra of the elements. The equipment has a wide analytical application and is often used in routine analysis laboratories. It consists basically of argon plasma, an optical system and a detector.[18]

The multielement analytical curve is necessary in many instrumental techniques in chemistry, since it is a kind of equipment calibration. It was prepared in 1% (v / v) nitric acid medium, including blank, from successive dilutions of a standard solution containing the analytes Ca, K, Mg, Na and P in the range of 0.1 to 2.0 mg L^{-1}. The linear correlation coefficients obtained were higher than 0.99 for all elements analyzed. Thus, the linearity criteria were reached. The ability of the analytical method to establish a direct relationship in a given concentration range was verified by linearity. Thus, the correlation coefficient was used to indicate how straight the line can be considered as a mathematical model for the case study.

Table 1. Instrumental parameters of ICP-OES.

Instrumental parameters	Value
Radiofrequency	40 MHz
Radiofrequency power	1150 W
Plasma gas flow	12,0 L min^{-1}
Auxiliary gas flow	1,0 L min^{-1}
Gas flow in the nebulizer	0,7 L min^{-1}
Torch	Quartz with axial view
Nebulizer	Concentric
Replicates	3
Analytical lines	Ca: 422.673 nm K: 769.897 nm Mg: 280.270 nm Na: 589.592 nm P: 213.618 nm

Statistical analysis

The data collected were tabulated in an Excel for Windows software spreadsheet and analyzed using the SPSS program – Statistical Package for the Social Sciences (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, version 19.0 Armonk, NY: IBM Corporation, USA. Initially, the data were assessed for normality using the Levene Equality Test of Variances. Subsequently, the groups were compared using Student's t-test. The level of significance was 5%. Data on the oral condition were tabulated in Excel for Windows software (Microsoft Corporation, Brazil) and statistically described by means of percentages and averages.

RESULTS

One hundred and two participants were included in this research (51 individuals diagnosed with AIDS and 51 controls). Sociodemographic characteristics of participants regarding age, gender, skin color, marital status and origin are presented in the Table 2. Most of the HIV-infected patients were white males, aged 39 to 48 years, single and coming from the city of Curitiba. Patients in the control group had the similar characteristics.

The main cause of hospitalization of patients was caused by pneumocystosis (39.2%). However, other diseases and conditions were able to cause hospitalization of patients: pulmonary tuberculosis (9.8%), CMV infection (7.8%), gastrointestinal diseases (7.8%), syphilis (5.8%), HSV infection (5.8%), fungal infection (5.8%), herpes zoster (3.9%), toxoplasmosis (1.9%), cryptococcosis (1.9%), and severe anemia (1.9%).

Table 2. Sociodemographic characteristics of individuals with HIV infection and controls.

Variables	Patients (%)	Controls (%)	p
Age			
18 – 28 old	6 (11.7)	8 (15.7)	
29 – 38 old	12 (23.5)	13 (25.5)	0.999
39 – 48 old	21 (41.2)	18 (35.3)	
≥ 49 old	12 (23.5)	12 (23.5)	
Gender			
Male	29 (56.9)	29 (56.9)	1.000
Female	22 (43.1)	22 (43.1)	
Skin color			
White	29 (56.9)	44 (86.3)	0.001
Not White	22 (43.1)	7 (13.7)	
Marital status			
Single / Divorced	28 (54.9)	18 (35.3)	<0.001
Married	23 (45.1)	33 (64.7)	
Total	51 (100)	51 (100)	

*Chi-square test ($p < 0.05$).

The mean salivary flow of subjects with HIV infection was significantly higher when compared to controls (Case group = 1.0 mL / min ± 0.63 / Control group = 0.7 mL / min ± 0.46; p = 0.012). However, when the hyposalivation criterion was used, 22 (43%) individuals with HIV infection had this condition. Hyposalivation was observed in 19 (37%) individuals of the control group.

Table 3 shows the values of average, mode, minimum and maximum for sodium, potassium, magnesium, calcium, and phosphorus ions. Sodium, potassium and calcium levels in the saliva of individuals with HIV infection were significantly higher than in controls. On the other hand, no significant difference was observed in salivary levels of magnesium and phosphorus ions according to the groups.

The oral health of the patients and controls is presented in Table 4. In this study, oral condition of the HIV-infected individuals was worse than the controls, since they had a higher percentage of lost teeth, dental caries, periodontal disease, residual roots, and oral candidiasis.

The use of drugs, addictions, and other comorbidities is shown in Table 5. The results revealed that not all patients with HIV infection were being treated with antiretroviral drugs. Of the total sample, 76% were already using antiretroviral therapy and the most used drugs were: Atazanavir, Lamivudine, Tenofovir and Ritonavir. During the anamnesis, it was recorded that 58.8% of the individuals in the case group and 9.8% of the controls were smokers. On the other hand, alcoholic individuals represented 15.6% in both groups.

Table 3. Levels of salivary calcium, potassium, magnesium, sodium and phosphorus of patients and controls.

Variables	Patients				Controls				p
	Mean±SD	Mode	Max	Min	Mean±SD	Mode	Max	Min	
Calcium (mg/L)	49.9±22.4	NV	138.3	0.4	47.9±24.8	NV	151.2	18.6	0.000*
Potassium (mg/L)	791.5±274.9	NV	1,845.2	0.008	678.9±191.9	NV	1,162.8	144.8	0.000*
Magnesium (mg/L)	2.5 ±2.1	1.2	13.1	-0.004	2.0±1.2	1.9	7.38	0.508	0.240
Sodium (mg/L)	314.0±252.3	252.6	1,564	-0.29	177.1±138.2	279.4	858.7	41.6	0.001*
Phosphorus (mg/L)	117.7±38.7	NV	194.5	110.42	122.3±48.5	N/A	231	41.9	0.600

*Student's t test ($p < 0.05$); NV = No value.

Oral health	Case group (N = 51)	Control group (N =51)	p
Lost teeth	35 (68.6%)	15 (29.4%)	< 0.001*
Coated tongue	32 (62.7%)	16 (31.3%)	0.001*
Dental caries	30 (58.8%)	19 (37.2%)	0.029*
Periodontal disease	23 (45.0%)	9 (17.6%)	< 0.001*
Residual root	15 (29.4%)	0 (0%)	< 0.001**
Oral candidiasis	12 (23.5%)	1 (1.9%)	0.018*
Melanotic maculae	10 (19.6%)	0 (0%)	< 0.001**
Dry mucosa	9 (17.6%)	4 (7.8%)	0.234
Atrophic glossitis	8 (15.6%)	0 (0%)	0.005**
Oral ulcer	7 (13.7%)	0 (0%)	0.005**

*Fisher's exact test ($p < 0.05$); **Chi-square test ($p < 0.05$).

DISCUSSION

Acquired immunodeficiency syndrome was recognized around 1981 in the USA. The number of infected and sick people has increased dramatically in that short period of time. It is estimated that about 21.7 million people Worldwide had access to antiretroviral treatment.[19] HIV destroys the cells of the immune system, altering its functioning and reducing its count. In this way, the ability to avoid disease is gradually compromised. In addition to the immune system, HIV infection often compromises the functioning of the salivary glands. Some patients may develop diffuse infiltrative lymphocytosis syndrome and others only manifest hyposalivation. To date, there is still much discussion about salivary changes in patients with HIV infection.

Salivary flow, buffer capacity, pH, calcium, phosphate and fluoride ions concentrations are essential factors in the determination of caries disease and periodontal diseases. This study analyzed samples of total saliva collected from a group of patients hospitalized at Hospital Oswaldo Cruz (Curitiba - Brazil). This hospital is a reference center for the treatment of AIDS in the state of Paraná. The study revealed that individuals with HIV infection had a reduced salivary flow and an increase in the concentration of some inorganic components when compared with values of healthy individuals.

Salivary flow is an important parameter because it reflects the functioning of the salivary glands. In healthy conditions, adult subjects produce 500 to 1,500 mL of saliva per day or between 0 and 6 mL / min.[20] The mean salivary flow in subjects with HIV infection was 0.7 mL / min and considered lower when compared to controls. These findings corroborate the results of Lin *et al.*[10] and Liberali *et al.*[13] These authors also observed a significant reduction in the salivary flow of individuals with HIV. In addition to the disease caused by HIV, we believe that other factors may also have contributed to reduce the salivary flow. One of these factors was smoking, since 55% of the sample was smoker and 43% presented hyposalivation. These results corroborate the findings of Dyasanoor and Saddu[21] which showed that 43% of smokers develop hyposalivation. Long-term smoking significantly reduces salivary flow and increases oral and dental disorders associated with dry mouth, such as: cervical caries, gingivitis, dental mobility, calculus and halitosis.[22]

Antiretroviral therapy, especially protease inhibitors, may interfere with the functioning of the salivary glands and cause hyposalivation. According to Navazesh *et al.*[23], HAART based on protease inhibitors represents a significant risk factor for the development of reduced non-stimulated and stimulated salivary flow as well as increased salivary glands. In our study, 76% of subjects with HIV infection used antiretroviral therapy. Of these, the following protease inhibitors were used: Ritonavir (31%), Atazanavir (20%), Lopinavir (12%), Fosamprenavir (4%) and Tipranavir (2%).

Water is the main component of saliva, accounting for 99% of its composition. Solid components, organic

Use of medication and addicts	Patients infected by HIV/AIDS (N = 51)	Controls (N = 51)
Antiretroviral therapy	35 (68.6%)	0 (0%)
Non-protease inhibitors	17 (33.3%)	0 (0%)
Protease inhibitors + Non-protease inhibitors	16 (31.4%)	0 (0%)
Protease inhibitors	2 (3.9%)	0 (0%)
No use of antiretroviral drugs	16 (31.4%)	0 (0%)
Drugs		
Antibiotics	40 (78.4%)	0 (0%)
Analgesics	29 (56.8%)	0 (0%)
Antiemetics	27 (52.9%)	0 (0%)
Vitamins and minerals	24 (47.0%)	0 (0%)
Anti-ulcer	22 (43.1%)	0 (0%)
Antifungals	20 (39.2%)	0 (0%)
Antiviral	17 (33.3%)	0 (0%)
Anxiolytics*	12 (23.5%)	0 (0%)
Antidepressants *	9 (17.6%)	0 (0%)
Corticosteroids	7 (13.7%)	0 (0%)
Antimalarials	6 (11.7%)	0 (0%)
Anticonvulsants*	4 (7.8%)	0 (0%)
Antipsychotics*	4 (7.8%)	0 (0%)
Centrally acting analgesics*	4 (7.8%)	0 (0%)
Drugs used for tuberculosis treatment (rifampicin + isoniazid + pyrazinamide + ethambutol)*	4 (7.8%)	0 (0%)
Hormones for the thyroid	4 (7.8%)	0 (0%)
Antihypertensive drugs*	3 (5.9%)	0 (0%)
Expectorant syrups	3 (5.8%)	0 (0%)
Intestinal flora replenisher	3 (5.8%)	0 (0%)
Vermicides*	3 (5.8%)	0 (0%)
Antiallergic*	2 (3.9%)	0 (0%)
Anticoagulants	2 (3.9%)	0 (0%)
Antidiabetics*	2 (3.9%)	0 (0%)
Anti-inflammatories	2 (3.9%)	0 (0%)
Laxative regulators	2 (3.9%)	0 (0%)
Antidiarrheal*	1 (1.9%)	0 (0%)
Antiflactulence	1 (1.9%)	0 (0%)
Bronchodilators*	1 (1.9%)	0 (0%)
Diuretics	1 (1.9%)	0 (0%)
Muscle relaxants	1 (1.9%)	0 (0%)
Addictions		
Alcoholism	8 (15.6%)	8 (15.6%)
Smoking	30 (58.8%)	5 (9.8%)

*Drug with the potential to induce dry mouth/hyposalivation.

and inorganic molecules are found dissolved in the aqueous component and vary widely from individual to individual; as well as several times during the day in the same person. The inorganic part is composed of strong and weak ions, being the most important: sodium, potassium, chlorine, calcium, bicarbonate, magnesium and ammonia.[24,25] In our study, most of these ions were analyzed in the saliva of patients with HIV/AIDS. However, only calcium, sodium and potassium presented higher levels when compared to the control group.

Potassium level was significantly higher in the saliva of patients with HIV infection. Apparently, the potassium level changes as HIV infection progresses without treatment. This same finding was observed in the study developed by Mahajan *et al*.[26] The potassium ion is the major cation in the intracellular space. Its content in the body of a healthy adult is about 3,700 mmol and muscle tissue represents its primary reserve in the body. Potassium has two significant balances: a) the external balance between the organism and b) the environment and the internal balance between the intracellular compartment and the extracellular compartment within the organism.[27] In saliva, potassium has higher levels than plasma and functions as an osmoregulator and in the active transport of components through the cell membrane.

The sodium ion is a component that depends directly on the salivary flow. It participates as an osmoregulator and in the active transport of components through the cell membrane. In our study, the level of this electrolyte increased significantly in the saliva of patients when compared to controls. The increase in the level of salivary sodium was much more expressive than the results observed by other authors.[26] This fact may be due to the different methods of analysis performed, because ICP-OES is regarded as one of the "gold standard" techniques for trace element analysis.[28]

Calcium is also dependent on the salivary flow and acts as activator of certain enzymes and in the remineralization of the enamel.[29,30] Our results corroborate the findings of Biocina-Lukenda *et al*.[16] These authors observed elevated calcium levels in the saliva of patients with HIV infection when treated by HAART.

Phosphate also participates in the remineralization process of dental enamel in the buffer system and is an osmoregulator.[31] It reaches a higher concentration than plasma without depending on the salivary flow. No significant difference was observed in the level of salivary phosphate between the groups studied. A similar result was observed by Mandel *et al*.[32] when they longitudinally studied phosphate levels in the parotid saliva of individuals with and without HIV infection. Meanwhile, there is still no consensus that dental caries progression is associated with HIV infection.[33]

Magnesium ion may also play an important role in the prevention of periodontal disease. According to Aun,[34] magnesium has the ability to reduce inflammation caused by bacterial toxins. In addition, it is already

established that reduced concentrations of magnesium are associated with an increased inflammatory response to bacterial challenge.[35] No significant difference in the level of magnesium was observed in the saliva of patients and controls. This same result was also found by Biocina-Lukenda *et al*.[16]

Afridi *et al*.[36] observed significantly lower levels of calcium, potassium and magnesium in all biological samples analyzed (blood, serum and scalp) of AIDS patients compared to healthy controls. In the present study, the inorganic analysis of saliva was performed by ICP-OES. This analytical technique quantifies elements (metals, semimetals and rare earths) in several types of samples. It is based on the detection of electromagnetic radiation emitted by neutral atoms or excited ions in the regions of the visible and ultraviolet electromagnetic spectrum. In addition to its high sensitivity, one of the great advantages of ICP-OES is its ability to simultaneously analyze several chemical elements. Additionally, it is capable of determining a wide variation in the concentration of chemical elements in the same sample. Previous studies have already used the ICP-OES successfully in the analysis of saliva samples.[37,38]

HIV-infected patients, particularly those with AIDS, are predisposed to a number of acid-base disorders involving water and electrolytes, (often with opposite effects), since they are exposed to infections, inflammatory, oncological, and pharmacological agents whose combination compromises their homeostatic capacity.[39] Patients with HIV need multidisciplinary care involving the dentist because the state of immunosuppression caused by the virus leads to the risk of opportunistic infections and neoplasms that may manifest in the mouth. In addition, through an anamnesis and physical examination, the professional may encounter suggestive signs and symptoms, which contribute to the early diagnosis of the disease.

CONCLUSION

Previously, we identified significant changes in the levels of amylase and total proteins in the saliva of hospitalized patients due to complications associated with AIDS.[13] In conclusion, individuals with HIV infection had the lowest salivary flow and higher levels of sodium, calcium, and potassium ions in saliva when compared to healthy individuals.

CONFLICT OF INTEREST

No potential conflict of interest was reported by any of the authors in this study.

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