Coll. Antropol. **33** (2009) 1: 93–97
Original scientific paper

HIV Infection, as Opposed to Antiretroviral Therapy, Does not Cause Changes in the Concentration Levels of Specific Salivary Electrolytes

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A B S T R A C T

The concentration levels of salivary calcium, magnesium and zinc can vary in different localized oral, as well as systemic diseases and conditions. So far changes in the concentration levels of specific electrolytes in stimulated parotid saliva in HIV-positive/AIDS patients have been proven. The objective of this research was not only to study the concentration levels of calcium in non-stimulated total saliva, but also the concentration levels of magnesium and zinc, which have not been studied so far, and the influence of antiretroviral therapy (HAART). This research was conducted on 60 healthy subjects with an average age of 40.4 years, and 60 HIV-positive patients with an average age of 43.7 years, 45 of whom took HAART therapy. The concentration levels of calcium, magnesium and zinc in saliva were determined by means of an atomic absorption spectrophotometry. No significant differences in the levels of excreted saliva/5 minutes (p=0.116), the concentration levels of salivary calcium (p=0.713), magnesium (p=0.600), nor zinc (p=0.162) were found between HIV-positive patients and the control group. No any correlation was determined between all three types of electrolytes and the number of CD4+ cells, nor the number of HIV-virus copies in peripheral blood of patients. Within the HIV-positive group, with respect to HAART therapy, no differences were found in the concentration levels of salivary magnesium (p=0.588), nor zinc (p=0.096). However, the concentration levels of salivary calcium were significantly higher in HIV-positive patients who underwent HAART treatment (p=0.004). The results of this research show that HIV, as a systematic infection, does not cause changes in the excretion of magnesium, zinc nor calcium in non-stimulated total saliva. Furthermore, it has been proven that HAART treatment does not cause changes in the concentration levels of magnesium or zinc, but can cause an elevation in the concentration level of saliva, which could be related to the calcium mobilization in blood.

Key words: *HIV infections, saliva, calcium, magnesium, zinc*

Introduction

Saliva, as a product of salivary glands, not only has a specific physiological function, but is also an accessible medium for diagnosing of different pathological conditions. Alongside water and organic matter, important elements of saliva are inorganic components whose concentration levels are in correlation with many pathological conditions in human body[1–7]. The concentration levels of salivary calcium can vary in systemic diseases and conditions. However, with certain conditions like asthma, Sjögren's syndrome and familial disautonomy no discrepancy was determined[8,10]. On the other hand, the concentration levels of salivary calcium in patients suffering from diabetes mellitus can be both elevated and decreased[5,11]. The decreased concentration levels of salivary calcium are found in menopausal women[12]. The concentration levels of salivary magnesium can also be related

Received for publication February 12, 2009

to many diseases. In patients suffering from diabetes mellitus[5], GVHD (Graft-versus-host disease; GVHD)[13], and those who have been receiving nourishment via nasogastric probe over a longer period of time[14], the concentration levels of salivary magnesium are elevated. In patients suffering from migraine[15], and those prone to the development of sialoliths[4], the concentration levels of salivary magnesium exhibit a decrease, while with the patients suffering from Sjögren's syndrome no discrepancy in the levels of electrolytes in saliva was found[15]. Certain diseases and conditions exhibit discrepancy in the concentration levels of several electrolytes simultaneously. Accordingly, the concentration levels of salivary calcium and magnesium are elevated in patients suffering from systematic lupus erythematosus[16], and periodontal disease[17], while decreased in patients with hypertesion[18]. Discrepancy in the concentration levels of salivary calcium, magnesium and zinc were not found in patients with juvenile recurrent parotitis,[19] but in patients with periodontitis lower concentration levels of calcium in parotid saliva were found[20]. Zinc is another type of electrolytes in saliva which plays an important role in the prevention of tartar development. Decreased concentration levels of zinc are found in patients suffering from diabetes and taste disorder[5,21].

The concentration levels of salivary electrolytes in HIV-positive/AIDS patients have not been completely recognized. Total saliva contains elevated concentration levels of sodium, with unchanged concentration levels of potassium and phosphate[22]. Stimulated parotid saliva exhibits elevated concentration levels of sodium and chloride, with unvarying concentration levels of potassium, phosphate and calcium[23,24]. On the other hand, some researches have discovered unchanged concentration levels of chloride[25]. So far, there have been no documented data regarding the concentration levels of salivary zinc and magnesium in HIV-positive patients. The objective of this research was to determine the concentration levels of salivary calcium, magnesium and zinc in HIV-positive subjects, as well as the influences of antiretroviral therapy.

Subjects and Methods

This research was conducted on 60 voluntary HIV-positive subjects, 47 male and 13 female, with an average age of 44.7 years (ranging from 26 to 71 years of age). The number of CD4+ lymphocytes varied from 25–744/μL, with the number of HIV virus copies in blood ranging from 0–468,000/mL. Out of 60 HIV-positive subjects, 45 took antiretroviral therapy, according to current treatment guidelines, HAART (Highly Active Antiretroviral Therapy; HAART).

The control group consisted of 60 healthy subjects, 37 male and 23 female, with an average age of 40.4 years (ranging from 21 to 79 years of age). None of them had any clinically visible pathological changes in the mucous membrane of the oral cavity.

Non-stimulated total saliva was collected according to Wu-Wang et al.[26], i.e. in calibrated test tubes during a period of 5 minutes, between 8 am and 11 am, was being examined. After the syalometric assessment of amount, the samples were frozen at –70 °C until the start of the analysis. The concentration levels of calcium, magnesium and zinc were determined by atomic absorption spectrophotometry according to aforementioned methods[27–30].

The results were presented descriptively, as the mean value ± standard deviation. Due to the exponential increase in the number of HIV virus copies in peripheral blood it was presented as a decimal logarithm. Depending on the type of variables and the principal of data distribution, the differences between the two groups were examined using χ^2 test, t-test and Pearson's exact correlation test.

Results

There was no significant difference between the HIV-positive group and the control group according to age ($t=1.486$; $p=0.140$), nor sex ($\chi^2=3.214$; $p=0.072$). By means of syalometric assessment, the average amount of 1.14 ± 0.31 ml saliva/5 minutes was collected in HIV-positive subjects, and 1.26 ± 0.49 ml saliva/5 minutes in the control group, with no significant difference between the two groups ($t=–1.585$; $p=0.116$) (Table 1).

Average concentration levels of salivary magnesium in HIV-positive subjects was 0.276 ± 0.149 mmol/L, and 0.262 ± 0.152 mmol/L in the control group, with no significant difference between the two groups ($t=0{,}526$; $p=0.600$). The concentration levels of salivary calcium in HIV-positive subjects was 0.756 ± 0.442 mmol/L, and 0.787 ± 0.453 mmol/L in the control group, once again with no significant difference between the two groups ($t=–0.369$; $p=0.713$). The concentration levels of salivary zinc in HIV-positive subjects averaged 1.831 ± 1.195 mmol/L, in addition to 1.566 ± 837 mmol/L in the control group, with no significant difference between the two groups ($t=1.408$; $p=0.162$) (Table 1).

The concentration levels of salivary electrolytes were compared between the HIV-positive subjects, in reference to antiretroviral therapy (Table 2). The concentra-

TABLE 1
THE AMOUNT OF COLLECTED SALIVA AND THE CONCENTRATION LEVELS OF SPECIFIC SALIVARY ELECTROLYTES IN HIV-POSITIVE AND CONTROL SUBJECTS

	HIV-positive (N=60)[1]	Control group (N=60)[1]	p*
Amount of saliva (mL/5 minutes)	1.14 ± 0.31	1.26 ± 2.34	0.116
Magnesium (mmol/L)	0.276 ± 0.149	0.262 ± 0.153	0.600
Calcium (mmol/L)	0.756 ± 0.442	0.787 ± 0.453	0.713
Zinc (μmol/L)	1.831 ± 1.195	1.566 ± 0.837	0.162

[1] mean ± standard deviation
* t-test

TABLE 2
THE CONCENTRATION LEVEL OF SALIVARY ELECTROLYTES IN HIV-POSITIVE PATIENTS WHO TOOK ANTIRETROVIAL THERAPY (HIGHLY ACTIVE ANTIRETROVIRAL THERAPY; HAART) AND THOSE WHO DID NOT

	With HAART (N=45)[1]	No HAART (N=15)[1]	p*
Magnesium (mmol/L)	0.282 ± 0.151	0.258 ± 0.149	0.588
Calcium (mmol/L)	0.850 ± 0.464	0.477 ± 0.190	0.004
Zinc (μmol/L)	1.980 ± 1.209	1.387 ± 1.067	0.096

[1] mean ± standard deviation.
* t-test

tion levels of magnesium in saliva of those patients who took antiretroviral therapy was 0.282 ± 0.151 mmol/L, and in those who did not take the therapy 0.258 ± 0.149 mmol/L, with no significant difference between the two groups (t=–0.545; p=0.588). The concentration levels of calcium in saliva of patients who took antiretroviral therapy ranged between 0.850 ± 0.464 mmol/L, as opposed to those who did not take the therapy, 0.477 ± 0.190 mmol/L, with a statistically significant difference (t=–3.019; p=0.004). The concentration level of zinc in saliva of patients who took antiretroviral therapy, was 1.980 ± 1.209 μmol/L, i.e. 1.387 ± 1.067 μmol/L in those who did not, once again with no significant difference between the two groups (t=–1.691; p=0.096) (Table 2).

Pearson's exact correlation test was use to examine the concentration levels of salivary magnesium, calcium and zinc in relation to the number of CD4+ lymphocytes in peripheral blood of the HIV-positive subjects. No significant correlation between the number of CD4+ of lymphocytes in peripheral blood of HIV-positive patients and the concentration levels of magnesium (r=–0.09; p=0.495), calcium (r=–0.06; p=0.651) and zinc in saliva (r=–0.18; p=0.889) was determined. In the end, Pearson's exact correlation test was use to examine concentration levels of magnesium, calcium and zinc in relations to the number of HIV virus copies in peripheral blood in HIV-positive subjects. Similarly, no significant correlation between the number of HIV virus copies and the concen- tration levels of magnesium (r=–0.272; p=0.188), calcium (r=0.101; p=0.631) nor zinc in saliva of HIV-positive subjects (r=0.032; p=0.880) was determined (Table 3).

TABLE 3
PEARSON'S CORRELATION OF THE NUMBER OF CD4+ LYMPHOCYTES AND THE NUMBER OF HIV VIRUS COPIES IN HIV-POSITIVE SUBJECTS WITH THE CONCENTRATION LEVELS OF SPECIFIC SALIVARY ELECTROLYTES (N=60)

	Magnesium (mmol/L)	Calcium (mmol/L)	Zinc (μmol/L))
CD4+ lymphocytes	0.495*	0.651*	0.889*
Number of HIV virus copies (log)	0.188*	0.631*	0.880*

* p value of Pearson's correlation

Discussion

No significant difference in the concentration levels of magnesium, calcium nor zinc between HIV-positive patients and the healthy subjects were determined in this research. Other researches so far have shown that the concentration levels of specific electrolytes in saliva can be a result of local, as well as systematic factors[4,5,9,10,12,15,17,18,20,21]. Certain, mainly localized, diseases, like in children suffering from juvenile recurrent parotitis[19], do not exhibit changes in concentration levels of salivary calcium, magnesium nor zinc. On the other hand, Ben-Aryeh et al. believe that elevated concentration levels of salivary calcium, magnesium and sodium in patients suffering from systematic lupus erythematosus could be an indicator of the development of subclinical disease of salivary glands, within the original disease[16]. Nager et al. have identified elevated concentration levels of salivary potassium, calcium and phosphate in patients suffering from GVHD[13]. After two weeks of pilocarpine treatment, these values normalized, which made them reach a conclusion that changes in the concentration levels of salivary electrolytes are an indicator of subclinical disease of salivary glands, within the original disease, GVHD. HIV-disease/AIDS is a systematic infective disease which causes changes in organic, but also inorganic composition of saliva. However, most researches so far have been directed towards salivary immunoglobulin, while only a few researches have studied salivary electrolytes in HIV-positive/AIDS patients[31,32]. Most researches have shown elevated concentration levels of salivary chloride[23,24]. Apart from that, elevated concentration levels of sodium, with no simultaneous changes in the concentration levels of potassium and phosphate have also been discovered in the stimulated total saliva of HIV-positive subjects[22]. In stimulated parotid saliva elevated concentration levels of sodium, and, at the same time, decreased concentration levels of potassium were found. In another research, Yeh et al. discovered the elevated concentration levels of chloride in both non-stimulated and stimulated parotid, as well as in submandibular saliva of HIV-positive patients[24]. Mandel et al. discovered elevated concentration levels of sodium and chloride in stimulated parotid saliva, while the concentration levels of potassium and phosphate retained their values[23]. They also discovered an unchanged concentration level of calcium in stimulated parotid saliva, similar to our findings for non-stimulated total saliva. Lin et al. showed neither changes in the concentration levels of chloride and potassium in stimulated parotid saliva of HIV-positive patients, nor changes in the concentration level of calcium, similar to our findings for non-stimulated total saliva[25].

The concentration levels of salivary magnesium, which have not been studied in HIV-positive patients so far, exhibited no change regarding the illness, similar to certain autoimmune diseases, such as Sjögren's syndrome[15], in contrast to other autoimmune diseases, such as lupus erythematosus, where concentration levels of salivary magnesium were elevated[16]. Similarly, the concentration levels of salivary zinc, which has neither been studied in

HIV-positive patients so far, exhibited no changes in relations to the disease observed in this research, as well as other, mostly localized, oral diseases, such as juvenile recurrent parotitis[19], and as opposed to certain systemic diseases like diabetes, which exhibit decreased concentration levels of salivary zinc[5].

Though patients were not classified according to the extent of HIV infection, the progress of the disease was evident from the concentration levels of CD4+ cells and the number of HIV virus copies in peripheral blood[33]. According to the our research, these variables were in no correlation with the concentration levels of either electrolyte studied, so we believe that their concentration level is not related to the progress of the disease as well.

The results of this research show a significant elevation in the concentration levels of salivary calcium in HIV-positive patients who took antiretroviral therapy. HIV-positive patients are known to be prone to osteopenia. Low Body Mass Index, weight loss, smoking, alcoholism and drug abuse, as well as menstrual cycle disorder are listed as possible reasons. Apart from that, chronic inflammations are known to be linked to progressive bone resorption, malabsorption of calcium, lipodystrophia and lactic acidemia which additionally contribute to osteopenia.[34,35] Progressive osteopenia in HIV-positive patients also can be caused by antiretroviral medications, which have a negative effect on bone metabolism and can lead to osteoporosis, especially in patients who take proteases inhibitors.[36,37] The cause of antiretroviral medications effect on bone structure is still unclear. There is a possibility that it affects the remodeling of bones directly, and/or the metabolism of vitamin D, indirectly. Whether all these effects are related to the calcium mobilization in blood, to the concentration levels of bounded and ionized calcium in blood, and finally to the elevation of concentration level of this electrolyte in saliva remains to be seen.

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HIV INFEKCIJA, ZA RAZLIKU OD ANTIRETROVIRALNE TERAPIJE, NE UZROKUJE PROMJENE KONCENTRACIJE NEKIH SALIVARNIH ELEKTROLITA

S A Ž E T A K

Koncentracija salivarnog kalcija, magnezija i cinka može biti promijenjena u raznim lokalnim oralnim kao i sustavnim bolestima i stanjima. Poznato je da se u stimuliranoj parotidnoj slini kod HIV-oboljelih/AIDSA nalaze promjene koncentracije nekih elektrolita. Svrha ovog istraživanja bila je istražiti koncentracije kalcija u nestimuliranoj ukupnoj slini kao i do sada neistraživanih koncentracija magnezija i cinka te utjecaj antiretroviralne terapije (HAART). U studiju je uključeno 60 zdravih ispitanika prosječne dobi 40.4 godina i 60 HIV-pozitivnih bolesnika prosječne dobi 43,7 godina, od kojih su 45 primali HAART. Koncentracija kalcija, magnezija i cinka u slini određena je atomskom apsorpcijskom spektrofotometrijom. Nije nađeno značajnih razlika između HIV-pozitivnih bolesnika i kontrolnih ispitanika u u količini izlučene sline/5 minuta ($p=0,116$), kao niti u koncentraciji salivarnog kalcija ($p=0,713$), magnezija ($p=0,600$) i cinka ($p=0,162$). Unutar skupine HIV-pozitivnih s obzirom na terapiju s HAART nije bilo razlika u koncentraciji salivarnog magnezija ($p=0,588$) i cinka ($p=0,096$), no koncentracija salivarnog kalcija bila je značajno viša kod HIV-pozitivnih bolesnika koji su primali HAART ($p=0,004$). S druge strane, nije ustanovljena korelacija između sva tri ispitivana elektrolita i broja CD4+ stanica kao niti broja kopija virusa HIV-a u perifernoj krvi. Rezultati istraživanja pokazuju da HIV kao sustavna infekcija ne izaziva promjene u lučenju magnezija, cinka i kalcija u nestimuliranoj ukupnoj slini kao. Također, pokazano je da HAART ne izaziva promjene koncentrcije magnezija i cinka ali može uzrokovati povišenu koncentraciju kalcija u slini, što bi moglo biti povezano s osteopenijom i mobilizacijom kalcija u krvi osteopenijom.