Influence of antioxidant therapy on the salivary components of patients with Fluorosis

Elena Stepco

Chişinau, Republic of Moldova

Summary

The salivary parameters: thiocyanate (SCN), urea, creatinine and protein were examined in 42 patients (19-30 years old) with fluorosis. An imbalance has been determined in the salivary components of the patients with fluorosis. Between the degree of detrimental effects of high-fluoride intake (clinical manifestations in teeth) and the contents of salivary parameters in the patients with fluorosis negative correlation has been shown. Complex antioxidant therapy including calcium gluconate, retinol, alfa-tocopherol and ascorbate during 30 days restored the imbalance partially.

Keywords: fluorosis, urea, creatinine, thiocyanate, antioxidant therapy.

Introduction

Fluorosis is a serious public health problem in many parts of the world, where drinking water contains more than 1 ppm of fluoride (Canada, USA, India, Romania, etc.)[1]. In many regions of Republic of Moldova fluoride concentration in various water sources is about 0.8 mg/l to 11.0 mg/l. Intake of high levels of fluoride increases free radical generation and lipid peroxidation (POL) in tissues, numerous pathological consequences, metabolic dicturbances. Fluorosis, caused by long-term intake of high levels of fluoride, is characterized by clinical manifestations in bones and teeth.

The salivary parameters of the patients with fluorosis may be the reflection of the metabolic disturbances and may constitute clinico-diagnostical means [2,3]. Investigation of salivary indexes is a simple and informative method used in many countries, such as Finland, Japan, USA, Canada, etc.

The **purpose** of this investigation was to search the interrelation between clinical manifestations in teeth and content of salivary parameters in patients with fluorosis. Also, one task of our investigation was the examination of antioxidant therapy influence on the content of patient's salivary components.

Materials and Methods

Forty-two patients (19-30 years old) with fluorosis were examined. Twenty-five (20-30 years old) healthy subjects were examined as the control group (group for comparison). Patients with fluorosis, according to clinical manifestations in teeth (clinical characteristics) were divided in the following groups: 1 - patients with very mild and mild fluorosis (n = 12); 2 - patients with moderate fluorosis (n = 17); 3 - patients with severe fluorosis (n = 13). Patients with fluorosis were treated with complex antioxidant therapy, including calcium gluconate (0.5 g twice a day), vitamins-antioxidants A (retinol palmitate – 100,000 IU/daily), E (alfa-tocopherol acetate - 100 mg/daily), C (ascorbate - 100 mg/daily) during 30 days. The salivary parameters were examined two times during treatment: before the therapeu-

tic course and after the end of treatment, in 30 days.

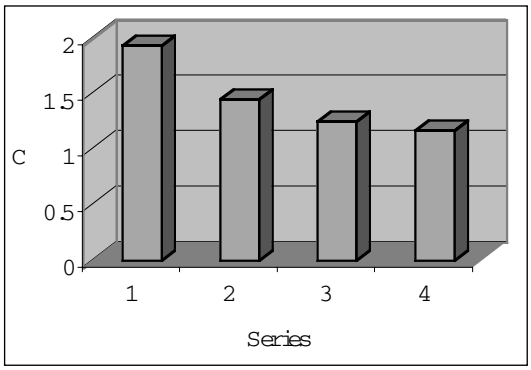
Saliva (mouth liquid) was collected in the morning and centrifuged at 600 g during 10 min. After that saliva was used for examination with using SP "Humalyzer 2000" (Germany). The following parameters were determined in the patient's saliva: thiocyanate (SCN) [4], urea [5], creatinine [6] and protein [7]. We used the "Human" GmbH and "DiaSys International" (Germany) reagents for salivary indexes determination. The results were calculated with the help of statistical Student method [8] and "Microstat": Microsoft Exceel 98 programe.

Results and Discussion

Many components of saliva work synergistically to limit bacterial growth, such as lactoferrin, lysozyme, lactoperoxidase, histatins, etc. Lactoperoxidase is the enzyme for oxidation of thiocyanate (SCN) in presence of hydrogen peroxide to hypothiocyanate (OSCN) with bacteriostatic property [9]. The examination of SCN-ions contents in the saliva of all patients with fluorosis shown a decrease of these ions concentration. The results obtained are presented in *Figure 1*. In patients of the first group, the content of **thiocyanate** in saliva was 1.449 mmol/g of protein (75.0%; $P<0.05$) in comparison with healthy subjects (1.932 mmol/g). In the patients' saliva of the second and third group the contents of thiocyanate were 1.249 mmol/g (64.6%; $P<0.05$) and 1.166 mmol/g (60.4%; $P<0.05$), accordingly.

One of our tasks was searching the interrelation between clinical manifestations in teeth and salivary parameters content in the patients with fluorosis. The results of our analysis shown negative correlation between clinical characteristics in teeth and thiocyanate content in the saliva of patients with fluorosis. We can see in *Figure 1* that

Figure 1. Thiocyanate concentration in the saliva of patients with fluorosis

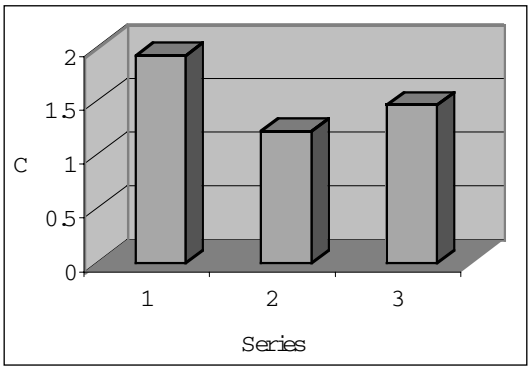


C - concentration, mmol/g of protein;
Series: 1 - healthy subjects; 2 - patients with mild fluorosis; 3 - patients with moderate fluorosis;
4 - patients with severe fluorosis.

with progression of chronic fluoride intoxication the thiocyanate content decreased in the patients' saliva. Very likely, that decrease of thiocyanate in the patients' saliva is a reflection of the host antimicrobial defense deficiency. Oxidation of thiocyanate (SCN) to antimicrobial product hypothiocyanite (OSCN) depends on hydrogen peroxide and Cl-ions concentrations. Cl-ions are used as a donor of myeloperoxidase of saliva [10]. In our early work, the determination of Cl-ions content in the saliva of patients with fluorosis showed a low concentration [11]. Moreover, Hannuksela S. et al. [12] observed the inhibition of lactoperoxidase and myeloperoxidase activities by fluoride. Furthermore, the generation of the actual antimicrobial agent hypothiocyanite of the oral peroxidase system was inhibited by fluor (F).

The course of antioxidant therapy of the patients with fluorosis increased the thiocyanate content in saliva (*Figure 2*). Before the starting of therapy SCN-ions concentration in the patients' saliva was 1.226 mmol/g of protein (63.5%; $P<0.01$) in comparison with healthy individuals (1.932 mmol/g). After the end of treatment the thiocyanate concentration in the saliva of the patients was 1.472 mmol/g (76.2%; $P<0.01$).

Figure 2. Thiocyanate content in the patients' saliva after treatment



C - concentration, mmol/g of protein.
Series: 1-healthy subjects;
2- patients before treatment;
3- patients after treatment.

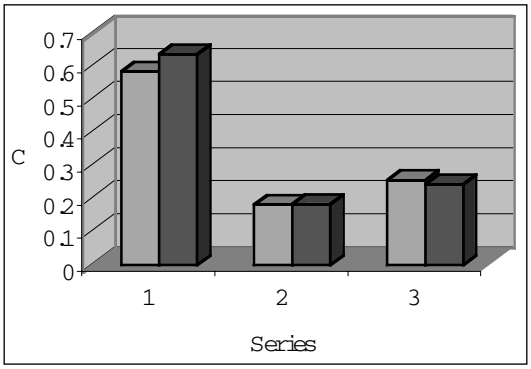
Examination of **protein** content in the saliva of patients with fluorosis showed an increase to 1.402 g/l (152.7%; P<0.01) in comparison with the healthy subjects (0.918 g/l). The antioxidant therapy decreased the protein content in the saliva of patients to 1.273 g/l (138.7%; P<0.05).

Determination of **creatinine** content in the saliva of the patients with fluorosis at the first examination (*Figure 3*) before treatment showed a decrease to 0.182 mmol/l (31.1%; P<0.01) in comparison with the healthy group (0.585 mmol/l) and according to g of protein, 0.184 mmol/g (28.6%; P<0.01) in comparison with the control group (0.637 mmol/g). Treatment with antioxidant therapy increased salivary creatinine content to 0.257 mmol/l (43.9%; P<0.01) and 0.244 mmol/g (38.3%; P<0.01) insignificantly.

Before the therapy, **urea** content in patients' saliva was 42.56 mmol/l (122.4%; P<0.05) and 40.20 mmol/g of protein (106.2%; P>0.05) in comparison with healthy subjects (34.76 mmol/l; 37.86 mmol/g) (*Figure 4*). The therapy course decreased urea content in the saliva of patients according calculation per liter to 40.14 mmol/l (115.5%; P>0.05). Salivary urea content according to g of protein did not change – 36.51 mmol/g (96.4%; P>0.05) and was similar with urea content in the saliva of the control group.

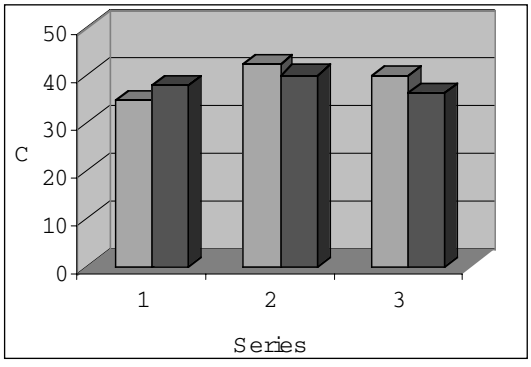
Syntheses of creatinine and urea are interrelated processes in the human organism. Urea production and synthesis of creatinine have the common intermediates, arginine and ornitine. Ornitine is one of the intermediates of creatine synthesis, and it is needed as a substrate for the first reaction of urea production (ornitine cycle). The received results showed an imbalance between these salivary parameters in the patients with fluorosis. Antioxidant therapy restored the imbalance partially.

Figure 3. Creatinine content in the saliva of patients with fluorosis



C - concentration: mmol/l (first column), mmol/g of protein (second column).
Series 1-3 also as in Figure 2.

Figure 4. Urea content in the saliva of patients with fluorosis



C - concentration: mmol/l (first column), mmol/g of protein (second column).
Series 1-3 also as in Figure 2.

Conclusion

The results of our investigation showed an imbalance between salivary parameters of the patients with fluorosis, as a result of chronic intoxication with fluoride contained in drinking water. We can confirm that between the degree of detrimental effects of high-fluoride intake (clinical manifestation in teeth) and the contents of salivary parameters in the patients with fluorosis negative correlation has been shown. Complex antioxidant therapy restored the salivary parameters imbalance partially.

References

1. Fluorides. Environmental Health. Criteria 227. *WHO*, Geneva, 2002.
2. Hofman L.F. Human saliva as a diagnostic specimen. *J Nutr.* 2001; **131**(5):1621-1625.
3. Streckfus C.F., Bigler L.R. Saliva as a diagnostic fluid. *Oral Dis.* 2002; **8**(2): 69-76.
4. Degiampietro P., Peheim E., Drew D. Determination of thiocyanate in plasma and saliva without deproteinisation and its validation as a smoking parameter. *J Clin Chem Clin Biochem.* 1987; **25**(10): 711-717.
5. Tobacco A., Meiattini F., Moda E. Enzymatic colorimetric test. *Clin Chem.* 1979; **25**(2): 336.
6. Bartels H., Bohmer M. Photometric colorimetric test for endpoint measurements. Method with deproteinisation. *Clin Chem Acta* 1971; **32**(1): 81-85.
7. Watanabe N., Kamei S., Ohkuto O. Urinary protein as measured with a pyrogallol red-molybdate complex: Manually and in a Hitachi 726 automated analyzer. *Clin Chem.* 1986; **32**(8): 1551-1554.
8. Gubler E.V. Mathematical methods for analysis and recognition of pathological processes. Ed. Medicine: Leningrad, 1978: 48-51.
9. Pruit K.M., Mansson-Rahemtulla B., Tenovuo J. Detection of the hypothiocyanite (OSCN) ion in human parotid saliva and the effect of pH on OSCN-generation in the salivary peroxidase antimicrobial system. *Arch Oral Biology* 1983; **28**(6): 517-525.
10. Pruitt K.M., Kamau D.N., Miller K. Quantitative, standartized assays for determining the concentrations of bovine lactoperoxidase, human salivary peroxidase and human myeloperoxidase. *Anal Biochemistry* 1990; **191**(2): 278-286.
11. Gavriliuc L.A., Stepco E.A., Hornet V.I. Imbalance of the biochemical parameters in saliva of patients with fluorosis (Disbalans nekotorih biohimicheskih parametrov sliuni u bolinih fluorosom). *Anale stiintifice USMF*, Chisinau 2004; **5**(3): 607-611.
12. Hannuksela S., Tenovuo J., Roder V. Fluoride inhibits the antimicrobial peroxidase systems in human whole saliva. *Caries Research.* 1994; **28**(6): 429-434.

Correspondence to: Dr Elena Stepco, Assistant Professor,Children Dentistry Department, “Nicolae Testemitanu” State University of Medicine and Pharmacy, Bul. Stefan cel Mare 165, Chisinau, Moldova, MD 2004.