Summary
Increased production of reactive oxygen species (ROS) and lipids peroxidation play an important role in the pathogenesis of chronic fluoride toxicity. Our aim was to investigate the salivary parameters: protein, calcium (Ca²⁺), inorganic phosphate, chloride ions (Cl⁻), thiocyanate (SCN⁻ ions), activity of alkaline phosphatase, glutathione reductase and glutathione S-transferase in the monozigota concordant patients with mild dental fluorosis treated antioxidative therapy. An imbalance in the salivary parameters has been determined in patients with fluorosis. The results of our investigation showed difference between salivary parameters of two monozigota concordant sisters with dental fluorosis. Antioxidative therapy was restored imbalance partially.

Key words: fluorosis, glutathione reductase, glutathione S-transferase, alkaline phosphatase, antioxidative therapy.

Introduction
Fluorosis, caused by long-term intake of high levels of fluoride, is characterized by clinical manifestations in bones and teeth. Fluorosis is a serious public health problem in many parts of the world, where drinking water contains more than 1 ppm of fluoride (India, Ethiopia, Canada, USA, Australia, Italy, Romania, Moldova, etc.)[1] For example, in Kenya of fluoride concentration in Nakuru lake water is about 2,800 mg/L, in Tanzania in certain mineral water – 95 mg/L.[2] In mineral waters in Portugal of fluoride concentration is about 14 ppm, in Bulgaria – 5 ppm and in France – 8.5 ppm.[3] In Republic of Moldova the mineral waters in certain localities have fluoride concentration about 1.25 ppm (Riscani) to 8.5 ppm (Camenca). Intake of high levels of fluoride increases free radical generation and lipid peroxidation (POL) in tissues, numerous pathological consequences, metabolic disturbance. Treatment of fluorosis, which affects both young and old alike, has posed a daunting task to the medical fraternity.

Salivary parameters of the patients with fluorosis may be indicative of the metabolic disturbances and have clinico-diagnostical importance.[4,5] Determination of salivary indices is simple and informative method used in many countries.

The aim of our study was the comparative examination the salivary parameters in monozigota concordant patients with dental fluorosis treated with antioxidative therapy.

Materials and methods
Four monozigota concordant patients with mild dental fluorosis were examined. Both pairs of monozigota concordant patients were girls aged 19 or 20 years, lived in locality Anenii Novi. Twenty (20-25 years old) healthy subjects were examined as the control group. Patients with dental fluorosis were treated with the complex antioxidant therapy (AOT), including calcium gluconate (0.5 g twice a day), vitamins-
antioxidants A (retinol palmitate – 100,000 mg/daily), E (alfa-tocopherol acetate – 100 mg/daily), C (ascorbate – 100 mg/daily) during 30 days. The salivary parameters were examined two times: before the therapeutic course and after end of treatment, in 30 days.

Saliva (mouth liquid) was collected in the morning and centrifuged at 600 g for 10 min. After that we used saliva for examination by means of SP “Humalyzer 2000” (Germany).

The following parameters were determined in the patients’ saliva: protein [6], calcium ions (Ca$^{2+}$)[7], inorganic phosphate [8], chloride ions (Cl$^{-}$)[9], thiocyanate (SCN$^{-}$-ions)[10], activity of alkaline phosphatase [11], glutathione reductase [12] making some of our modifications [13] and glutathione S-transferase [14]. Our study we divided into three steps: 1- examination of the salivary parameters in two pairs of homozigota concordant patients (sisters) with dental fluorosis; 2- comparative examination of the salivary components in one pair of homozigota concordant sisters with dental fluorosis; 3- determination of the salivary parameters in two sisters with dental fluorosis after antioxidative therapeutic course (AOT).

Results and discussion

Our results evidenced that all salivary parameters in homozigota patients with dental fluorosis differed from the salivary parameters in control group (healthy).

The next step of our study was comparative examination of salivary parameters in the one sister (first) and another (second) sister of one homozigota concordant pair.

Mineral components of saliva play an important role for teeth, mouth soft tissues and salivary enzymes. Calcium (Ca$^{2+}$) is one of main elements necessary for bones and teeth formation. The calcium ions concentration in the saliva of one (first) of monozigota concordant sisters was 1.583 mmol/L (71.9%) and in another (second) sister was 1.181 mmol/L (53.6%) in comparison with healthy subjects (2.204 mmol/L)(Figure 1). The inorganic phosphate concentration in the saliva of the first sister was 3.618 mmol/L (56.7%) and in the second sister – 3.413 mmol/L (53.4%) in comparison with healthy (6.386 mmol/L) (Figure 1). The chloride ions concentration in the saliva of the first sister was 82.08 mmol/L (86.0%), in second sister was 86.38 mmol/L (90.55%) (Figure 1). The protein content in the saliva of first sister was 1.15 g/L (118.0%), in the saliva of second sister –
1.181 g/L (164.1%) in comparison with healthy subjects (0.975 g/L) (Figure 1). We can see, that the obtained data reflect disturbances of salivary mineral components, protein content and their difference between the first and the second homozigota concordant sisters.

The study results on alkaline phosphatase, glutathione reductase and glutathione S-transferase activity are shown in Figure 2. It is a known fact that fluorine (F-ion) acts as inhibitor for alkaline phosphatase. The activity of alkaline phosphatase in the saliva of first sister was 8.1 IU/L while in the saliva of second sister - 3.6 IU/L. (Figure 2) Our results are in accordance with the results of Gao Y. et al.[15], who showed the low level of alkaline phosphatase activity in blood and urine of children (8-15 years) with fluorosis.

Intake of high levels of fluorine/flouride causes chronic oxidative stress and increase free radical generation and lipid peroxidation (POL). In this case, we can look forward an induction of antioxidative defense system in patients with fluorosis. The results of examination the activity of two glutathione-dependent enzymes (glutathione reductase and glutathione S-transferase) in the saliva of two sisters with fluorosis are shown in Figure 2. We can see, that activity of both enzymes were increased. The salivary glutathione S-transferase activity in first sister increased to 3756 IU/L (128.3%) and in second sister to 3727 IU/L (127.3%) in comparison with healthy subjects (2927 IU/L). The activity of glutathione reductase in first sister was 184.7 IU/L (110.5%) and in second – 196.5 IU/L (117.6%) in comparison with healthy (167.1 IU/L).

Glutathione-associated metabolism is a major mechanism for cellular protection against agents, which generate oxidative stress and POL. Genetic and biochemical evidence has demonstrated, that glutathione and glutathione-dependent enzymes play a central role in the cellular defense against toxic environmental agents. Glutathione participates in detoxication at several different levels through the antioxidant responsive element, which is found in the promoter of many of the genes that are inducible by oxidative and chemical stress.

Glutathione reductase regulates the low content of oxidized glutathione (GSSG) and the high level of its reduced form (GSH) into the cell.[16] GSH is the main component of redox-buffer of the intracellular medium. Glutathione S-transferase, which participates on the second line of defense against mediators of oxidative stress, plays a very important protective role. Enzyme catalyzes the conjugation of GSH with different toxic and mutagenic compounds, which are generated during lipid peroxidation processes. Examination of the salivary glutathione reductase activity in two sisters.
with fluorosis was shown small increasing of its activity. More considerable increase of the glutathione S-transferase activity estimated in the saliva of both sisters with fluorosis. On the basis of our results we can state that an imbalance between the salivary enzymes of the antioxidant defense system is the result of chronic intoxication with fluoride contained in drinking water.

Antioxidative therapy (AOT) increased the concentration of thiocyanate, calcium and decreased the content of protein in the saliva of both sisters with dental fluorosis. (Figure 3) The salivary calcium concentration in first sister increased from 71.9% to 100.5% and in second sister – from 53.6% to 100.4%. The salivary protein content in first sister decreased from 117.6% to 97.4% and in second sister – from 164.1% to 143.6%. Also, AOT increased the alkaline phosphatase activity in the saliva of both sisters with fluorosis only partially. (Figure 4)

Our results demonstrated different tolerance to fluoride in the homozigota concordant sisters of one family with similar clinical manifestations on teeth. Well known about physiological and biochemical variations between the human organisms. [17] Also, we know about different tolerance to fluoride in the living organisms.[18] Fluoride content in the human organism is dependent on it’s intake into organism with food, drinking water, and absorption into intestine. Between fluoride absorption into

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**Figure 3.** Salivary components in two homozygotic concordant sisters with dental fluorosis after antioxidative therapy (AOT) (%)

1,3,5-first sister; 2,4,6-second sister. 1,2-thiocyanate; 3,4-calcium; 5,6-protein. Lower column-before AOT; upper column-after AOT. Concentration in the saliva of healthy-100%.

**Figure 4.** Activity of alkaline phosphatase in saliva of two homozygotic concordant sisters with dental fluorosis after AOT

1-after AOT; 2-before AOT. External cycle-first sister; inner cycle-second sister.

Activity of alkaline phosphatase in saliva before and after AOT therapy
intestine and acidic medium of gastric juice there is positive direct correlation. [19] In our previous study we found the difference between content of protein, creatinine and urea in the saliva of the homozigota concordant sisters. [20]

Conclusion

The results of our investigation showed an imbalance between salivary parameters in the homozigota concordant patients with dental fluorosis, as a result of chronic intoxication with fluoride contained in drinking water. Moreover, between the salivary described parameters in first and second sisters was considerable difference.

Due to our results, we can confirm that these homozigota concordant sisters live in one family, have similar clinical manifestations on teeth (mild dental fluorosis) and different tolerance to fluoride and metabolic disturbances.

Complex antioxidant therapy (AOT) restored salivary parameters imbalance in both sisters with dental fluorosis only partially.

References
