RESEARCH STUDIES

State of antioxidant system glutathione – glutathione S-transferase in deep fluoridation of tooth enamel in children with high risk of dental caries

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Abstract

Background: The high prevalence of dental caries in children determines the relevance of the search of ways to prevent this disease. The goal was a comparative study of the impact of fluor-prevention on the activity of glutathione S-transferase (GST) and content of glutathione of saliva, which play a protective antioxidant and antitoxic role, in children with high risk for dental caries.

Material and methods: The study observed 100 children from 7 to 12 years old, 50 children had severe pathology of central nervous system (CNS). Within three years, all children underwent the preventive measures for dental caries, including oral hygiene, complex of vitamins, minerals and antioxidants. Additionally for 60 children was conducted deep fluoridation (DF), a combination of laser therapy with DF, and DF with photodynamic therapy (PDT).

Results: The activity of GST, the contents of glutathione and total protein in the saliva were determined by spectrophotometric methods. DF of tooth enamel in healthy children reduced the activity of GST in the saliva in the process of observation, in children with disorders of the nervous system activity of the enzyme increased. Combination of DF with laser therapy decreased the activity of GST in the saliva of children with pathology of CNS, and DF with PDT – it increased.

Conclusions: Completing of fluor-preventive complex for caries was effective for both healthy children and children with pathology of CNS.

Key words: glutathione, glutathione transferase, fluoridation, tooth caries.

Introduction

The high prevalence of dental caries in children determines the urgency of finding ways for prevention of this disease. Dental caries is a complex pathology characterized by demineralization of tooth structure [1]. Etiological factors of caries process are diverse: the accumulation of plaque as a result of poor oral hygiene, excessive consumption of refined sugars and low resistance of tooth enamel. According to the majority of authors of the scientific and medical literature, in childhood the leading etiological-pathogenetic factor in caries is the low resistance of enamel of both temporary and permanent teeth as the result of insufficient concentration of fluoride in drinking water [2]. The results of numerous research materials of WHO and International Dental Federation (IDF) confirm the effectiveness and safety of fluoride in optimal quantities for the prevention of dental caries. Fluoride promotes more intensive incorporation of calcium in the tissues of the teeth. In response to hydroxyapatite crystals of enamel, fluor (F^-) forms compounds more resistant to acids, reducing the permeability of the tooth enamel, strengthening its microcrystalline lattice [3]. Fluor also has a bactericidal action to decrease the enzymatic activity of acid-producing bacteria inhibiting their proliferation in the oral cavity. Deficiency of the fluor/fluoride in the diet contributes to the development of tooth caries [4]. Children from unfavorable social environment, inmates of orphanages and boarding schools are at particular risk because their health is influenced by the combined action of adverse social, psychological, biological and genetic factors, which reduce the adaptive capabilities of their organisms [5, 6]. The group of the particular risk includes children with pathology of the central nervous system and mental development [7, 8]. In the scientific and medical literature the state of the antioxidant system of saliva in children with caries and intellectual disabilities is known insufficiently.

One of the defense systems of saliva is a system of glutathione – glutathione S-transferase. Glutathione S-transferase using a water-soluble antioxidant, reduced glutathione, as a coenzyme catalyzes reaction of conjugation of glutathione with many toxic substances protecting the human organism. Glutathione, performing the function of an antioxidant, inactivates harmful free radicals, and as detoxicant, it promotes the excretion of various toxins from the organism.

The goal was a comparative study of the state of glutathione – glutathione S-transferase system performing protective antioxidant and antitoxic role in caries-active healthy children and children with pathology of central nervous system during fluor-preventing action.

Material and methods

The study involved 100 children from 7 to 12 years old: 50 healthy children and 50 children with pathology of the central nervous system (CNS). Clinical and biochemical studies have been conducted in accordance with ethical and

legal standards. All children were observed during three years. For the prevention of dental caries four times a year caries-active children underwent deep fluoridation (nano-fluoride) method Knappwost C. (1999). 60 caries-active children were divided into 3 groups: 1- deep fluoridation (DF); 2- DF + laser therapy (LT); 3- DF + photodynamic therapy (PDT) [9]. During the initial examination and 3 years later children's saliva (oral fluid) was taken, centrifuged at 5000 rpm / min for 10 minutes. After that the activity of glutathione S-transferase (GST, Habig W., Jacoby W., 1974)[10], the content of reduced glutathione (GSH, Sedlak J., 1968)[11] and total protein (Lowry O.H, 1951) were determined spectrophotometrically (Diasys Diagnostics, DE). Composition of saliva varies throughout the day, so it is important to calculate the obtained results as to the protein content of saliva. The obtained results were statistically processed using the Excel and Microsoft: Microstat 2007 program.

Results and discussion

Reduced glutathione (GSH). Results of the GSH determination are shown in Table 1. Content of GSH in saliva of caries-active healthy children varied insufficiently during 3 years of observations. In the saliva of caries-active children with pathology of the CNS GSH content after three years of observations significantly increased to 164% (P_t <0,001) in the calculation per gram of protein as compared to the primary supervision (the first visit). This «relative» increasing is the result of the protein content decreasing in saliva of children as a result of preventive carried out measures.

Table 1

Dynamics of glutathione reduction in the saliva of children

Units	Healthy children		Children with pathology CNS	
	1st visit	in 3 years	1st visit	in 3 years
Caries-active	Comparison group/control			
mcmol/l	35,20 ± 2,40	33,52 ± 2,00	35,52 ± 1,50	39,60 ±1,12
mcmol/g	11,95 ± 0,61	13,15 ± 0,75	10,91 ± 1,30	17,89 ± 1,15*
Caries-active	Deep Fluoridation of tooth enamel (DF)			
mcmol/l	41,36 ± 1,90	41,68 ± 1,07	35,20 ± 1,28	36,64 ± 0,96
mcmol/g	18,93 ± 2,28	12,24 ± 0,52	15,38 ± 1,10	13,01 ±1,37
Caries-active	DF + laser irradiation therapy			
mcmol/l	33,76 ± 1,63	36,56 ± 1,14	37,04 ± 2,21	34,00 ± 1,12
mcmol/g	14,72 ± 1,17	14,91 ± 1,23	15,00 ± 1,25	12,29 ± 0,93
Caries-active	DF + photodynamic therapy			
mcmol/l	35,76 ± 2,37	37,76 ± 0,83	38,48 ± 1,04	34,80 ± 1,36
mcmol/g	16,66 ±1,30	13,77 ± 1,52	15,91 ± 0,69	14,42 ± 1,29

*Note. Symbol * - reliability, P_t<0,001.*

Antioxidant content of saliva depends on the presence of cariogenic microorganisms and activity of dental caries. A significant increase of GSH content in the saliva of children with caries (6-14 years old) was also observed by Han D. et al. [12], who wrote that the content of GSH may be biomarker for the presence of cariogenic bacteria in the oral cavity and activity of dental caries [12]. Combination of the DF with laser therapy did not affect the content of GSH in the saliva of healthy children, slightly lowering its content in the children with disorders of the nervous system (82%, P_t>0,05). The combined using of the DF and photodynamic therapy also had no significant effect on the content of GSH in the saliva of children of both groups. In the saliva of children in both groups after DF the levels of GSH were almost identical (161.6 mcmol/g and 158,8 mcmol/g), that is an interesting fact in our view. Fluor is the most powerful oxidizing agent among the halogens. It is well-known that fluor as a strong oxidizing agent causes the formation of free radicals and initiation of oxidative stress. Well-known are the literature data about the toxic effects of fluor/fluorides on the biosynthesis of proteins, lipids, carbohydrates, and nucleic acids. Taking this into account, it is necessary carefully to analyze the expected effects of fluor-preventive methods and their combination with other ones used in dentistry. This is especially true for children with pathology of the CNS.

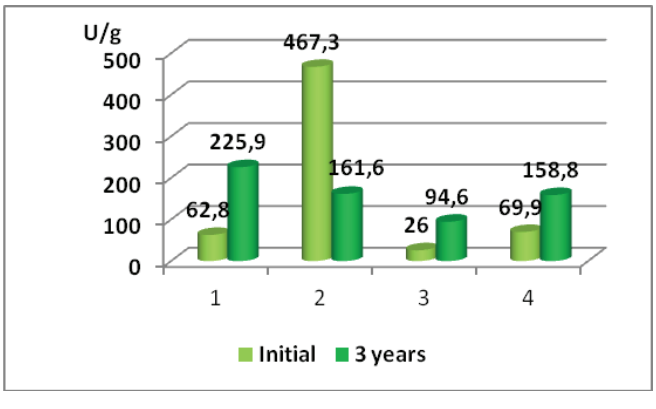

Fig. 1. The activity of glutathione S-transferase in the saliva of caries-active children.

1 – Healthy caries-active children; 2 – children with pathology of CNS. 3 – Healthy children after the DF; 4 – children with pathology of CNS after the DF.

Glutathione S-transferase (GST) plays an important role in antioxidant and antitoxic defense system in the human organism and controls the redox-system of saliva. Results of the GST activity in the saliva of caries-active children after the DF are shown in *figure 1*. Activity of GST in both caries-active groups, healthy children and children with pathology of the CNS, during the three years of supervision was significantly increased by 3,6 times. The DF significantly reduced the activity of the enzyme in the saliva of healthy children, increasing its activity in the saliva of children with disorders of CNS. Fluor, being the most powerful oxidizing halogen, probably induced the increase of the antioxidant activity of GST in the children with pathology of the CNS.

Figure 2 shows the results of the combined effects of laser therapy (LT) with the DF and DF with photodynamic therapy (PDT) on the activity of GST in the saliva of children. Laser

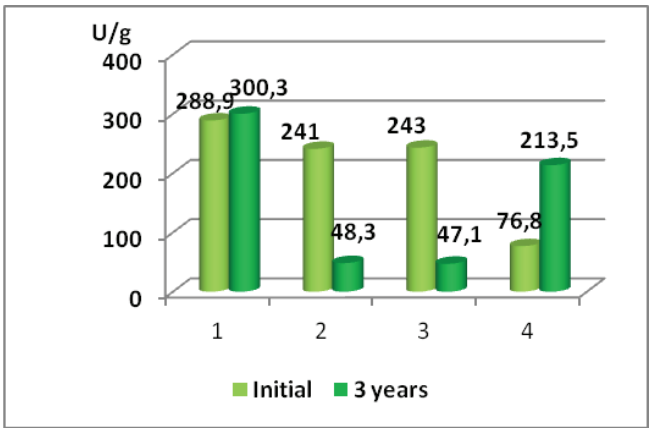

Fig. 2. The activity of glutathione S-transferase after the combined methods of prevention.

1 – Healthy caries-active children (DF + LT); 2 – children with pathology of CNS (DF + LT). 3 – Healthy children (DF + PDT); 4 – children with pathology of CNS (DF + PDT).

irradiation did not affect the level of the GST activity in the saliva of healthy children, and decreased the activity of enzyme by 5 times (Pt <0.05) in the saliva of children with disorders of the CNS. The combination of DF and photodynamic therapy reduced the GST activity in the saliva of healthy children by 5 times (Pt <0.05), increasing its activity in the saliva of children with pathology of the CNS by 2,8 times.

Thus, on the basis of the received results of the DF influence and its combination with other methods of caries prevention (LT and PDT) may be made the following conclusions:

All methods of the fluor-prevention applied to children with pathology of CNS had the positive effect on the antioxidant system glutathione – glutathione S-transferase of saliva without any toxic effects on the metabolism of children.

Glutathione and glutathione S-transferase may be used as biomarkers in the estimation of the state of oral tissues, including tooth caries.

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