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CHANGES OF BIOCHEMICAL CHARACTERISTICS OF SALIVA AND THEIR INFLUENCE ON STOMATOLOGICAL STATE OF STUDENTS UNDER STRESS

The dependence of the pH level and enamel resistance of the students under stress is studied in the work. It was determined that stress causes decrease of pH level, increase of cortisol level and decrease of human enamel resistance. The obtained data may be used for a program of prediction and prophylaxis of psychosomatic and stomatological sanitation of students.

Key words: caries, saliva, pH level, cortisol, stress.

Introduction

Stress is an integral part of our life in the modern world. Education by Bologna system in institutions of higher education includes periodical modular control that causes changes of psychosomatic health of students. The degree of such changes depends on emotional stability of young people, their ability to deal with emotional stress and control negative emotions. According to data provided by the authors (Knutsson U., Dahlgren J., Marcus C., 1997), saliva is one of the first to react to changes in the body. Stress activates hypothalamus, which produces CRH hormone or corticoliberin. The hypophysis secretes ACTH hormone or adrenocorticotrophin, and adrenal glands, in their turn, produce cortisol needed to decrease stress. As the result, the level of cortisol increases (Safarzadeh E., Mostafavi F., Haghi Ashtiani M. T., 2005). A cellular membrane of the salivary glands does not let pass biological molecules of molecular mass > 400 Da into the salivary ducts. Molecular mass of all the steroids id > 400 Da and as the result only free forms of steroids penetrate into the salivary ducts (cortisol, testosterone, estradiol, etc.) However, the hormones related to albumin or globulin do not penetrate. The level of cortisol in saliva does not depend on volume and speed of its secretion that is fundamental for results interpreting [1-7].

The **purpose of research** is to determine changes in mouth cavity under emotional stress and dependence of pH level and enamel resistance using physical and chemical methods of analysis.

Main part

As a rule, to evaluate the cariogenic situation in the mouth cavity, chemical methods are used. pH level of the liquid is determined by standard scale test ribbon; the level of oral hygiene by Fedorov-Volodkina index and Oral Hygiene Index (Green and Vermillion); enamel acid resistance by TEP test (by V. R. Okushko modified by L. I. Kosareva 1984). To evaluate the coloring intensity of the stained enamel by 2% methylene blue solution, a 10-level scale of polygraphic colors is used (State Standard 2789-73). The level of cortisol in saliva was determined by immune-enzyme analysis. The normal concentration of free cortisol in saliva follow circadian biorhythm and become maximum in early morning hours. At the daytime, it decreases to 54%, and 89% in the evening. That is why sampling is made between eight and ten o'clock in the morning. For that Sarstedt Salivette cotton swab is used. It is placed to the mouth cavity for 2 – 3 minutes avoiding any contact with hands after mouth wash 10 min before the test.

Today, the mouth liquid can be studied by mass-spectrometric, fluorescent, potentiometric, luminescent, interferometric, and calorimetric measuring devices. These devices can determine chemical and biological composition of liquid. At that they have rather bid dimensions, high price or require a number of reagents and prolonged analysis time [8 – 11].

One of the modern methods of rapid analysis is a gas-discharge visualization (GDV). According to the method, the liquid is introduced into a variable (more than 1 kHz) electromagnetic field of

high strength (20-25 kV/cm), around which a glow appears caused by gas discharge between the object and the electrode. As the test object is a part of the electric circuit, it influences the glow. Thus, the state of the object can be determined by analyzing the discharge image.

GDV method is used in medicine for health screening and monitoring, quantitative determination of stress level and psycho-functional readiness to complex professional activity, to study characteristics of liquids and materials. The research results in most cases are subjective and do not provide quantitative estimation of human's state. However, recent researches allowed finding physical essence of GDV processes and creating fundamentally new class of apparatus for studying both bio objects and liquid-phase bio object, using last achievements of microelectronics, computer image processing methods for analysis of multiparameter data arrays [12].

Diagnostic perspectives of the method are restrained by lack of standards for apparatus of discharge parameters creating (signal frequency, form, voltage, current), as well as image analysis. That is why the same experiments by different researchers are not interpreted ambiguously and cannot be used to evaluate the state of the test object with high reliability.

The mouth liquid as bioobject was studied by GDV and other known methods in this work. To confirm the adequacy of the methods, a GDV system was developed. It was used to determine experimentally the spectral intensity distribution of the test object and concentration of components in the mouth liquid. The main feature of the system is the possibility to get images of the test and reference bioobjects under the same environmental conditions and the same parameters of the experimental facility [13].

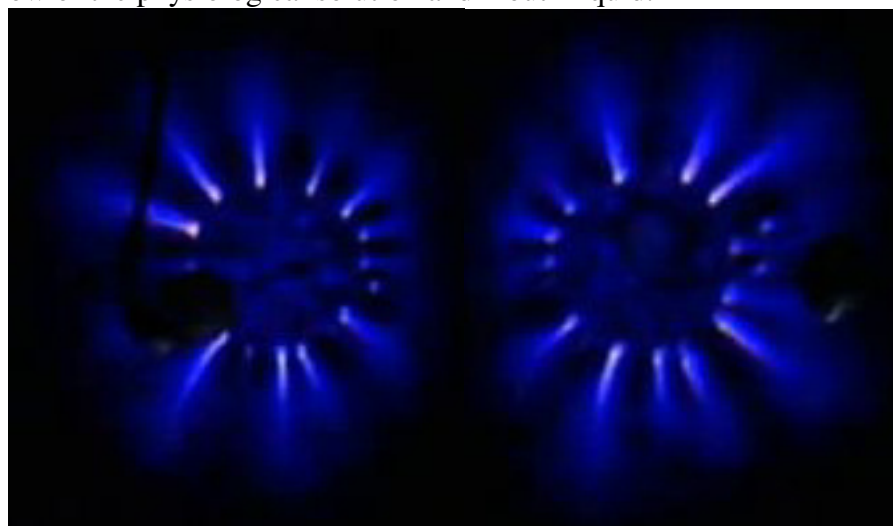Fig. 1 shows glow of the physiological solution and mouth liquid.

Fig. 1. GDV image of the mouth liquid (left) and physiological solution (right)

Different test systems were used for studies. In the first case, the GDV glow image of mouth liquid, and, in the second case, spectral emission intensity was used.

The experimental results show that the state of the mouth liquid can be determined by a complex criterion of its glow image, and the chemical composition – by spectral intensity.

At the time of saliva sampling, a patient was in the state of relative rest, the saliva was secreted without any efforts or stimulations. The patients did not eat or drink anything and did not use a toothbrush or dental floss for one hour before the test. They did not drink alcohol and did not use creams with glucocorticoids for one day before the test. The pregnant patients and patients with acute inflectional diseases, serious somatic diseases or diseases of mucous tunic or gums were not eligible for testing. The level of free cortisol in saliva was determined by immune-enzyme analysis using HART – UV spectrophotometer (BioTek Instruments, USA). Statistical processing of the experimental results were done using traditional methods of variation statistics [14 – 15].

The experimental results showed coincidence within 10 – 15% of the parameters of state of mouth liquid received by GDV method and other known methods.

For experimental researches, 36 somatically healthy students of the Dentistry Faculty were selected before and during module control. There were 19 young men and 17 girls in the age of from 17 to 19 years. The stomatological state was determined in addition to the student's card (Form №025-3/o approved by the order of MOZ of Ukraine №302 of 27.12.99).

The experimental study of the somatological state showed that 85,7% of students had caries with 14,6 process intensity that is very high index. DMFT structure was the following: D – 297, M – 12, F – 193. At the repeated examination DMFT structure did not change. Fedorov-Volodkina Hygiene Index was good in 37,1 %. 28,5% of the examined students had satisfactory level of hygiene and 31,4% had unsatisfactory level of oral hygiene. By Oral Hygiene Index (Green and Vermillion) 48,5% of students showed good hygiene, at the same time 28,5% of the examined group had satisfactory hygiene index. 22,9% of students showed unsatisfactory hygiene. The students were divided into two groups depending on changes of cortisol level in saliva under stress (Table 1). The first group included 68,4% of the examined students (26 students, including 15 girls and 11 young men), whose Cortisol Saliva Level (CSL) was increased. The second group included 31,6% of students (10 students, 2 girls and 8 young men), whose CSL under stress increased relative to the initial level but still was normal.

Table 1

Students distribution by CSL in rest state and under stress

Cortisol saliva level 8-10 o'clock in the morning	Rest			Stress		
	Young men	Girls	%	Young men	Girls	%
Normal up to <6,65 ng/ml	19	17	100	8	2	31,6
Increased level > 6,65 ng /ml	-	-	-	11	15	68,4

The first response to stress was saliva pH change (table 2). The experimental results in state of rest show that normal saliva pH level (6,8 – 7,4) was in 76,9%, 6,0 – 6,7 level was in 23,1% of the students in the first group. Level under 6,0 was not detected. Under the stress pH level in the first group significantly decreased and was the following: normal level was in 11,5%, 6,0 – 6,7 was in 57,7% and under 6,0 was in 30,8%.

pH level of the second group, which was stress tolerant in the state of rest, varied as follows: normal level was in 80%, 6,0 – 6,7 was in 20%. Under the stress there was the following distribution: normal level was in 60%, 6,0 – 6,7 was in 30%, and less than 6,0 was in 10%.

Table 2

Saliva pH results

Saliva pH level	Group 1 (stress sensitive)						Group 2 (stress tolerant)					
	State of rest			Under stress			State of rest			Under stress		
	Young men	girls	%	Young men	girls	%	Young men	girls	%	Young men	girls	%
Normal (6,8-7,4)	8	12	76,9	2	1	11,5	6	2	80	5	1	60
6,0-6,7	3	3	23,1	6	9	57,7	2	-	20	2	1	30
< 6,0	-	-		3	5	30,8	-	-		1	-	10

Caries resistance test (CRT) in state of rest showed that in the first group 38,5% of students have high enamel resistance to acids. 46,2% of students have average enamel resistance and only 15,3% of the examined students had significantly low resistance. In the second group of stress tolerant students the results were the following: 30% of students had high resistance of enamel, 50% had average resistance and 20 % had low enamel resistance. To determine the caries resistance at modular control, another CRT was done. However, no significant changes in CRT results depending on stress situation were discovered. The difference of resistance levels was within 10% (Table 3). One can suppose that changes of stomatological state occur after some time in result of prolonged stress.

Table 3

CRT results

CRT	Group 1 (stress sensitive)						Group 2 (stress tolerant)					
	State of rest			Under stress			State of rest			Under stress		
	Young men	girls	Young men	girls	Young men	girls	Young men	girls	Young men	girls	Young men	girls
1-3	6	4	38,5	4	3	26,9	1	2	30	-	2	20
4-6	3	9	46,2	4	10	53,9	1	4	50	2	4	60
7-9	2	2	15,3	3	2	19,2	-	2	20	-	2	20

Conclusions

In this work, the mouth liquid under emotional stress was researched using GDV and compared to known methods of chemical analysis. The experimental results showed the dependence of the pH level and caries enamel resistance. It was determined that deadaptation of psycho-emotional state, lowering of stress resistance and stress itself influence negatively on health of students and cause saliva pH decrease, increase of CSL and lowering of caries resistance of enamel. The examined students with low level of stress resistance have reduced body reactivity and require regular medical check-up. Those with high CSL need oral cavity sanation and sanitary informing.

The obtained data may be used for a program of prediction and prophylaxis of psychosomatic and stomatological sanitation of students. At that, the GDV method can be used for rapid analysis of mouth liquid state.

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