INFLUENCE OF PSYCHO-EMOTIONAL STRESS ON BIOCHEMICAL AND MORPHOLOGICAL PARAMETERS OF SALIVARY FLUID OF STUDENTS OF DIFFERENT ETHNIC GROUPS

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Abstract

It is known that the psycho-emotional and mental stress can lead to the disruption of the process of adaptation, which is accompanied by a picture of severe metabolic changes in the body. In this research indirectly defined free radical activity, studied the morphological characteristics of the structure and correlations revealed the biochemical and structural parameters of salivary fluid freshmen three ethnic groups - Russian, Africans, Indians - before and after the impact of psycho-emotional stressor (exam testing).

Key words: *stress, ethnic groups, free-radical activity, pH, saliva, students*

INTRODUCTION

Exam stress is one of the main reasons for mental stress of high school students (Alekseeva 2010, Singh 2012). Emotions are a kind of human body biological adaptation system to various conditions of the social environment. Under the influence of emotional stress on the healthy body, there is a specific integration of information and regulatory power and metabolic processes, which allows the body to maintain homeostasis (Aghajanian 2010, Babar 2015, Madiha Ali 2015).

It is known that oxygen free radicals are involved in metabolic processes regulation as intracellular messengers, which are capable to activate redox reaction (Timochko 1998). According to Dubinina, Kolesnikova (2001, 2010) any stress reaction as a result of the adaptation process deployment is accompanied by oxidative stress development, therefore, to determine the characteristics of free radical oxidation and antioxidant protection is undoubted importance for the opening at the cellular level, the laws of adaptive reactions formation (Birben 2012).

As it is known, the biological fluids structure carries information about the state of biological object formation. Dried bioliquid membrane with fixed shaping elements allows to visualize the molecular level of biological systems organization, thus making it suitable for morphological studies (Shabalin 2002).

Salivary fluid is easily available to obtain, and it can be used to determine the level of stress (Morse, 1981). In addition, there is information about influence of psychoemotional stress certain types on the change of the free radical processes intensity and antioxidant enzyme activities in saliva (Grigoriev, 2004).

In this regard, the aim of this study was to investigate the effect of psychoemotional stressor (exam) on free radical processes, pH and salivary fluid morphological picture of students from different ethnic groups.

MATERIALS AND METHODS

The study involved 129 1st year students of Nizhny Novgorod State Medical Academy at the age of 19 ± 2 years. All volunteers were asked to pass the questionnaire (Table 1).

Table. 1 Questionnaire for research participants

Test tube №
Name/Nickname
Sex (male/female)
Nationality
Date of birth
Place of birth
How deep do you know your pedigree (how many generations)?
How many generations of your family had been living at the place of your birth?
Do you stick to authentic cuisine?
Do you stick to any diet?
What diseases do you suffer from? In what form?
Do you take any medical drugs and which ones?
How regular do you take vitamins?
Do you smoke?
If you do not smoke now, have you smoked earlier?
Do you drink alcohol? If yes, how often.

On the basis of personal data from a number of students were excluded the ones who have diseases or who use drugs or who smoke at the time of the study, and quit smoking at this time. After the screenings volunteers were divided into 3 groups according to ethnicity: Russian (37), Indians (13), Africans (20).

As biological material salivary fluid was collected twice - before and after the exam; primary processing was carried out according to methodical instructions (Buzoverya, 2006). The pH was measured with pH-meter pH-410. Status of prooxidant and antioxidant system indirectly evaluated by chemiluminescent emission intensity (Kuzmina 1983).

The method of wedge dehydration (Shabalin 2002) qualitatively and quantitatively evaluated the overall morphological picture of the salivary fluid. A drop of 15 µl was applied on a glass slide in 3 replicates and then dried at a temperature of 20-25°C, relative humidity of 45%-65% and a minimum mobility of ambient air; the duration of the drying period was 18-24 hours. After finishing of the salivary fluid dehydration we obtained facia - the dried membrane that was examined with microscope (Leica CM E, lens 10, eyepiece 10) in transmitted light; then an electronic photo of facia was made and its elements were investigated (Fig. 1).

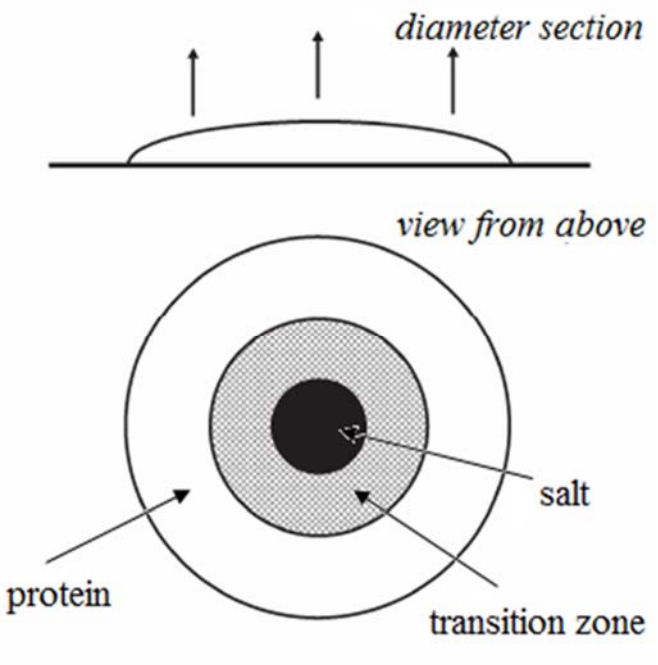


Fig. 1 A drop of a biological fluid on a plane

For the facies we described zoning - the presence of peripheral, central, intermediate zones and the shape, type of branch, size, thickness, and number of crystals in each of these zones (Fig. 2).

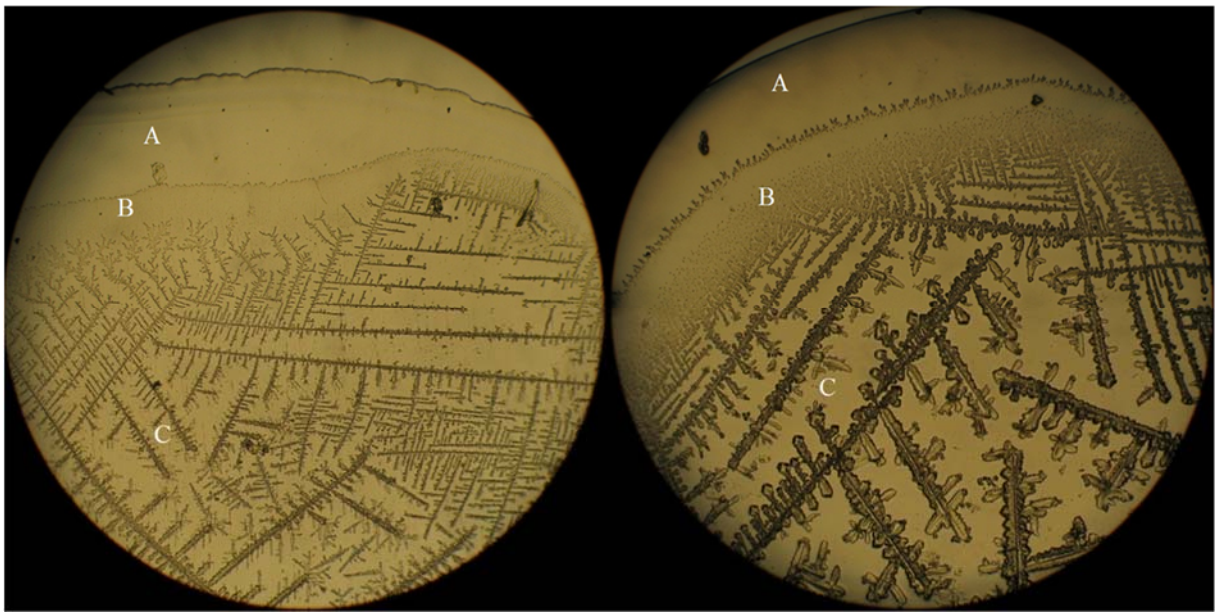


Fig. 2 Samples of salivary fluid facia (A – peripheral zone, B – intermediate zone, C - central zone)

Statistical Analysis

The data were analyzed using STATISTICA 8.0 software package. Statistical processing was performed using the Wilcoxon criterion for dependent samples, data are presented as the median, 25 and 75 percentile. For the study of correlations Spearman rank correlation coefficient was used. Differences were considered to be statistically significant if the significance level was $p<0,05$.

RESULTS

After measuring the pH in salivary fluid in each ethnic group the students were divided into two conditional groups marked pH = 7.00. The salivary fluid pH of Indian and African students under the influence of emotional stress changed randomly, for Russian students with pH level >7,00, we found a statistically significant reduction after exam (0,043).

Based on the pre-exam chemiluminescent emission intensity values of salivary fluid 3 categories have been established for each ethnic group: with low (0-0,35), medium (0.35-0.5) and high (0.5<) level of free radical oxidation (FRO). For students with low and middle levels of the original salivary fluid FRO, regardless of ethnic affiliation, stress caused an increase of chemiluminescence emission intensity. Under the influence of a stressor Russian students showed improvement of FRO, while Africans and Indians demonstrated its decline (Table 2). However, ethnic dependence, namely, was established for students with a high pre-exam CL glow.

Table. 2 Changing the chemiluminescent emission intensity of salivary fluid for students under the influence of exam stress (* p<0,05)

Period of study	Ethnic group	The intensity of the chemiluminescence emission (conventional units)		
		0-0,35	0,35-0,5	0,5<
before exam	Russian	0,259 [0,215;0,295]	0,395 [0,370;0,413]	0,769 [0,606;0,992]
	Africans	0,313 [0,263;0,323]	0,426 [0,386;0,446]	0,649 [0,596;0,676]
	Indians	0,331 [0,311;0,341]	0,414 [0,402;0,424]	0,601 [0,546;0,685]
after exam	Russian	0,370 [0,262;0,463]	0,550 [0,459;0,703]*	0,945 [0,603;1,201]
	Africans	0,361 [0,353;0,369]*	0,498 [0,448;0,540]*	0,548 [0,537;0,660]
	Indians	0,380 [0,191;0,461]	0,453 [0,319;0,537]	0,446 [0,355;0,539]*

It is known that the method of wedge dehydration allows you to capture specific elements that characterize all the features of the metabolic processes inherent in the body, so it is necessary to search for correlation to determine the specific structural elements, reflecting the change of certain biochemical parameters.

During the correlation analysis we found statistically significant correlation between chemiluminescent emission intensity and some elements of the facia structure (for all students) (Table 3).

Table. 3 The correlation of free radical activity level in salivary fluid and the parameters of facia structure

The structural parameters of facies	The correlation coefficient (R)	The correlation coefficient (R)
The amount of the central zone crystals	-0,488	0,034
The size of the central zone crystals	-0,645	0,017
The thickness of the central zone crystals	0,661	0,002
Inclusions in central zone	-0,392	0,016
Elements of the intermediate zone	0,344	0,043

Moreover, we found a significant correlation of the sign of emotion, received after the exam, and such parameters of facia structure as:

- the width of the peripheral zone - for African (-0,604) and Indian (0,069) students;
- intermediate zone elements - for Russian students (-0,349).

DISCUSSION

According to the studies of processes features acetylation of drugs (xenobiotics detoxification II phase) from the representatives of different ethnic groups Piruzyan (2004) found that the incidence of slow acetylators has no difference in the populations of Russian and Nigerians (representatives of sub-Saharan Africa). Furthermore, Spitsyn's study (2008) shows that variation of the allele rapid acetylation frequency in European populations, the inhabitants of India and the African sub-Saharan Africa is in the same range - 20-40%.

It is known that, on the one hand, the oxidative stress affects the activity of enzymes that metabolize xenobiotics (antioxidant enzymes). On the other hand, change in the xenobiotic acetylation activity (N-acetyl) leads to increased oxidative stress.

Thus, the interrelation of the pro-antioxidant status and activity of enzymes metabolizing xenobiotics and their ability to vary depending on the effects of environmental factors (Dubinskaya 2013) shows why the chemiluminescent emission intensity indicators, indirect reflecting prooxidant status of the body, have the same distribution limits for different ethnic groups representatives, that found in our study. In Smelysheva's study (2005) have been studied the effects of stress during the exam on the pH and composition of microelements (Na, K, Ca, chlorides) in the salivary fluid and gastric juice of healthy students. The author has registered decrease in pH of salivary fluid after exam passing, which confirms our findings with respect to Russian students.

According to the research of Grigorieva (2004) and co-workers, mixed saliva composition in Russian population representatives is enriched with protein because of enhanced positive psychological and emotional activity. This is the reason for the increase of peripheral facia zone.

Therefore, we can talk about similar reaction to the impact of emotional stress for Russian and Indian students and inverse reaction among sub-Saharan Africa students on the basis of correlation analysis.

So, we can assume a weak ability to adapt to the conditions of emotional stress for Russian and Indian students on the basis of the data obtained. At the same time African students demonstrated stimulation of adaptive response capabilities under the influence of exam stress. These data may be useful for the development of a participatory approach for adaptation of students in different ethnic groups.

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