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# INVESTIGATION OF BIOCHEMICAL CHANGES IN BLOOD AND SALIVA OF BASKETBALL PLAYERS BEFORE THE COMPETITION

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**Annotation.** Much research has been conducted to understand how anxiety can affect sports performance, both in practice and in development. It is known that exercise is associated with high levels of stress and threat potential, and the application and application of a number of psychological strategies can be beneficial in managing anxiety. The article is devoted to the evaluation of the level of dihydropyrimidinase-like protein 2 (DBZ2) in platelets and saliva of basketball players before the competition and the level of natural autoantibodies against DBZ2 in blood serum. Instead of control, samples of blood and saliva from those athletes in normal condition were used. The levels of DBZ2 and natural autoantibodies against DBZ2 were determined by indirect immuno-enzymatic assay on polystyrene tablets with a moderate level of adsorption. The results of the research showed a significant decrease in the level of natural autoantibodies against DBZ2 in the serum of the athletes before the competition ($p < 0.01$) and no changes of DBZ2 in their platelets and saliva. Based on the evidence obtained in previous years, our results allow us to conclude that athletes reflect similar changes in the level of DBZ2 in the subcortical regions of the brain and indicate an increase in their level of aggression before competition.

**Keywords:** *athletes, platelets, blood serum, dihydropyrimidinase - like protein 2 (DBZ2), natural autoantibodies against DBZ2, indirect immuno-enzyme analysis, sports competitions.*

At present, in order to prepare athletes in the training process and bring them to a high physical and spiritual level for competitions, the coach must have accurate information about the level of physiological processes in their body. Obtaining such information is possible only with the application of accurate, modern and highly sensitive methods.

Participation in every competition is an event accompanied by excitement for the athletes. At the same time, when people get into stressful situations, based on their life experience and the strength of the nervous system, the effects of negative psychological factors meet them with different marked emotional reactions. Despite the changes in the level of stress hormones, which are an accurate indicator of emotional response, it is a very important problem to determine the adaptation or non-adaptation of the human nervous system to that stressful situation ("fight or flight" reaction).

It is possible to use dihydropyrimidinase-like protein 2 (DBZ 2) [7, 10] instead of the indicator of the adaptation process. In particular, studies conducted on animal models and humans have provided evidence that this protein is actively involved in the development of depression, aggressive behavior, and excitability [1, 8]. Our aim is to determine the level of DBZ2 in platelets and saliva of pre-competition athletes and the level of natural autoantibodies against DBZ2 in serum samples.

**Material and methods.** The studies were conducted on high-level male basketball players (n=8) and blood and oral fluid samples

were taken in their normal condition and before the competition. 5 ml of blood was collected from the vein of the subjects in test tubes containing 5% solution of ethylenediamine tetraacetate (EDTA) instead of anticoagulant, and blood plasma was separated by centrifugation at a speed of 700 RCF for 10 minutes. Platelets were precipitated from the plasma samples in a centrifuge at a speed of 9000 RCF for 16 min, water-soluble proteins were extracted through the extraction buffer and used instead of antigen in 0.1 M tris-HCl buffer (pH 8.6) at a concentration of 20 μg/ml in the indirect immunoenzyme assay method (DIEA). done. The reaction was carried out on medium adsorption polystyrene tablets (Sigma, Germany). Polyclonal rabbit immunoglobulins against DBZ2 in antibody buffer (pH 7.3) were used instead of primary antibodies. Instead of secondary antibodies, ticklish peroxidase - conjugated goat immunoglobulins (Sigma, Germany) against islet rabbit immunoglobulins in antibody buffer (pH 7.3) were used. Instead of the peroxidase substrate, orthophenylenediamine was applied at a concentration of 0.5 mg/ml in 0.05 M citrate-phosphate buffer (pH 4.5). To stop the reaction, 20 min after adding the substrate to the wells of the tablet, 50 μl of 3 M NaOH was added to each well, and the reaction results were read by Molecular Devices Spectra Max 250 (MTX Lab Systems, Inc., USA) at a wavelength of 492 nm (relative wavelength 630 nm) was recorded [3].

Serum above sediments (platelets) in test tubes was used to determine the level of natural autoantibodies against DBZ2. In this form of DIE administration, purified DBZ2 in 0.1 M tris-HCl buffer (pH 8.6) at a concentration of 20 μg/ml was applied instead of antigen. Serum samples were substituted for primary antibodies in DIE at a dilution ratio of 1:75 in antibody buffer (pH 7.3). Mouse immunoglobulins (Sigma, Germany) conjugated with ticklish peroxidase against human immunoglobulins in antibody buffer (pH 7.3) were used instead of secondary antibodies. The subsequent steps of the reaction were carried out as described above.

Oral saliva samples were also taken from basketball players to determine the level of DBZ2 by the DIEA method. The course of the reaction was performed in a similar manner as the determination of the level of DBZ2 in platelets.

DBZ2 was extracted from cattle brain by partial (0-40%) ammonium sulfate precipitation and gel chromatography on a Sephadex G-150 column (3 X 60 cm). Then the concentration of the protein was concentrated up to 3 times through the ultrafiltration method and dialyzed against distilled water for 6 s at a temperature of +5ºC. After dialysis, the protein was stored overnight in a pre-deionized 40 Mm EDTA solution at a temperature of +5ºC on a vinegar stand to free it from calcium ions. Then, with the application of ampholines in a narrow (pH 4-6) range, the fractions of the protein solution were separated within 5 s of the isoelectric focusing method, and the pH gradient was drawn up after determining the pH values of all samples while eluting from the gel. The fraction corresponding to the isoelectric point of DBZ2 was stored as DBZ2 and frozen. Protein homogeneity was determined by electrophoresis in 4-12% polyacrylamide gel in tris-glycine buffer system (pH 8.3) [6].

The obtained results were collected for each group and statistical analysis was performed based on Student's t-test [13].

**Research results and their discussion**. The results of our research show that the level of natural autoantibodies against DBZ2 in the serum samples of the basketball players before the competition was significantly decreased compared to the normal condition. In particular, the level of natural autoantibodies against DBZ2 in the resting state of basketball players was 0.053±0.006 absorbance optical units (UOV), while their pre-competition level was 0.035±0.002 UOV ($p < 0.01$; Fig. 1). At the same time, the level of DBZ2 in platelets in resting state and before the competition of basketball players was not significantly different (respectively, 0.341±0.002 UOV and 0.299±0.03 UOV, $p > 0.05$). There was also no difference between resting and pre-competition levels of DBZ2 in the saliva of basketball players (0.225±0.016 UOV and 0.22±0.014 UOV, respectively ).

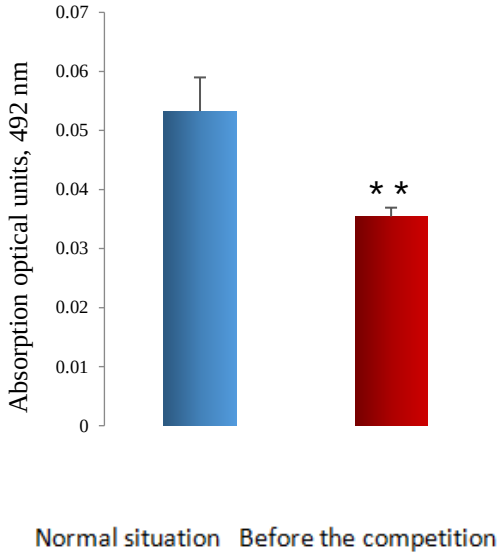


***Fig. 1. Changes in the level of natural autoantibodies against DBZ2 in the serum of athletes during pre-competition. ** - p < 0.01.***

The changes noted in our research in the platelets and serum samples of basketball players are not only related to the processes occurring in the blood, but also allow obtaining accurate information about the processes taking place in the brain regions. Thus, the indicators of the serotoninergic system in platelets fully reflect the changes of the serotoninergic system in the cortex of the human brain [4, 5] and reflect the level of DBZ2 in the cerebral cortex with very high accuracy. On the other hand, it should be emphasized that natural autoantibodies against all proteins (including water-soluble, hydrophobic structural and regulatory proteins) are detected in the human body [12]. Such proteins do not participate in any pathological processes within the body and only perform a regulatory function in protein homeostasis. In previous years, the results of research on animals conducted in the "Molecular basis of integrative activity" laboratory of the Institute of Physiology named after academician Abdulla Garayev (head - bed. A.A. Mekhtiev) testify that the level of natural autoantibodies against DBZ2 in the blood serum is very accurate in the subcortical structures of animals. reflects the level [8].

Thus, the marked decrease in the level of natural autoantibodies against DBZ2 in the pre-competition serum samples of basketball players suggests that the level of DBZ2 in the subcortical structures of their brains is changing in the same degree and direction. In previous years, the results of studies on rats in the dominance model showed that the level of natural autoantibodies against DBZ2 in the serum of aggressive rats and the level of DBZ2 in the amygdala of the brain, which regulates aggression behavior, were similarly and markedly reduced [9]. On the other hand, in patients scheduled for surgery, the level of natural autoantibodies against DBZ2 in their serum is dramatically increased, indicating a high level of excitability [1]. Taking into account the results of the aforementioned studies on animals and humans, our results on basketball players contribute to the conclusion that the level of excitement of high-class basketball players was not observed before the competition, and only an increase in the level of aggression was determined based on the results of our blood serum analysis.

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## BASKETBOLÇULARDA YARIŞDAN QABAQ QANDA VƏ AĞIZ SUYUNDA BİOKİMYƏVİ DƏYİŞİKLİKLƏRİN TƏDQİQİ

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**Annotasiya.** Həm təcrübədə, həm də rəqabət şəraitində həyəcanlılığın idman performansına necə təsir edə biləcəyini anlamaq üçün çoxlu araşdırmalar aparılmışdır. Məlumdur ki, idman yüksək səviyyədə stress və narahatlıq potensialına malikdir və bir sıra psixoloji strategiyaların tətbiqi narahatlığın idarə olunmasında faydalı ola bilər. Məqalə yarışdan

qabaq yüksəkdərəcəli idmançıların trombositlərində və ağız suyunda dihidropirimidinazaya bənzər zülal 2-nin (DBZ2) səviyyəsinin və qan zərdabında DBZ2-yə qarşı təbii autoanticisimlərin səviyyəsinin qiymətləndirilməsinə həsr olunub. Kontrol yerinə həmin idmançılardan sakit vəziyyətdə qan və ağız suyunun nümunələri istifadə olunub. DBZ2-nin və DBZ2-yə qarşı təbii autoanticisimlərin səviyyələri adsorbsiyanın orta səviyyəsi olan polisteren planşetlərində dolayı immuno-enzim analizi üsulu ilə yerinə yetirilib. Tədqiqatların nəticələrində yarışdan qabaq idmançıların zərdabında gözə çarpan DBZ2-yə qarşı təbii autoanticisimlərin səviyyəsinin azalması ($p < 0.01$) və onların trombositlərində və ağız suyunda DBZ2-nin heç bir dəyişikliklərinin olmaması qeyd olunub. Əvvəlki illərdə aldığımız dəlilləri əsas tutaraq, əldə etdiyimiz nəticələr idmançıların beynin qabıqaltı nahiyələrində DBZ2-nin səviyyəsinin eynixarakterli dəyişikliklərinin əks etdirdiyini və yarışdan qabaq onların aqressiya səviyyəsinin artmasına dəlalət etdiyi barədə qənaətə gəlməyə bizə imkan verir.

**Açar sözlər:** *idmançılar, trombositlər, qan zərdabı, dihidropirimidinazaya bənzər zülal 2 (DBZ2), DBZ2-yə qarşı təbii autoanticisimlər, dolayı immuno-enzim analizi, idman yarışları.*

## ИССЛЕДОВАНИЕ БИОХИМИЧЕСКИХ ИЗМЕНЕНИЙ В КРОВИ И СЛЮНЕ БАСКЕТБОЛИСТОВ ПЕРЕД СОРЕВНОВАНИЯМИ

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**Аннотация.** Много исследований было посвящено пониманию того, как тревога может повлиять на спортивные результаты как на практике, так и в соревновательных условиях. Хорошо известно, что спорт потенциально может вызывать высокий уровень стресса и тревоги, и что в практике и применение ряда психологических стратегий могут быть полезны для управления тревогой. Статья посвящена определению уровня дигидропипимидиназа - подобного белка 2 (ДПП2) в тромбоцитах и слюне и естественных аутоантител к ДПП2 в сыворотке у высококлассных спортсменов перед началом соревнований. Пробы крови и слюны, взятые у этих же спортсменов в спокойном и расслабленном состоянии, служили в качестве контроля. Определение уровня ДПП2 и естественных аутоантител к нему были выполнены с помощью непрямого иммуноферментного анализа на полистироловых планшетах с умеренным уровнем сорбции. Полученные результаты указывают на значительное снижение уровня естественных аутоантител к ДПП2 в сыворотке ($p < 0.01$) и на отсутствие изменений уровня ДПП2 в тромбоцитах и слюне у этих спортсменов перед началом соревнований. Основываясь на полученных ранее нами данных, можно придти к заключению о том, что результаты отражают аналогичные изменения уровня ДПП2 в подкорковых структурах головного мозга спортсменов и указывают на повышение уровня их агрессивности перед началом соревнований.

**Ключевые слова:** *спортсмены, тромбоциты, сыворотка крови, дигидропипимидиназа-подобный белок 2 (ДПП2), естественные аутоантитела к ДПП2, непрямой иммуноферментный анализ, спортивные соревнования.*