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Serum and Oral Fluid Levels of Protein Markers and Hormones in Patients With Alcohol Dependence Syndrome

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Abstract

Background: The development of alcohol dependence syndrome is accompanied by disturbances of neuroplasticity in neural circuits, the imbalance of neurotransmitter metabolism and immune and hormonal statuses in the central nervous system, which are reflected in changes in peripheral markers. Therefore, determining neuropeptide levels in body fluids is a potentially promising strategy for laboratory monitoring of substance use.

Objective: To determine characteristics of changes in oral fluid and serum levels of protein markers and hormones in patients with alcohol dependence syndrome during rehabilitation.

Materials and methods: We formed 2 groups of male participants: a control group of apparently healthy volunteers (n=30) and a group of patients with alcohol dependence syndrome, which was similar in size, age, and gender (20-40 years) to the controls. At the time of admission to the rehabilitation program and 3 months later, serum and oral fluid samples were collected. We used an enzyme-linked immunosorbent assay to determine levels of brain-derived neurotrophic factor (BDNF), glial cell line–derived neurotrophic factor, neuropeptide Y, orexin, pituitary adenylate cyclase-activating peptide, corticotropin, and cortisol in the body fluids.

Results: Laboratory findings revealed that it is possible to determine neuropeptides and hormones in the oral fluid. The wide variability of findings in the oral fluid and no statistically significant correlation with corresponding serum levels were characteristic of the most protein markers. Only the BDNF levels were statistically significantly reduced (3.2-fold decrease) in both the serum and oral fluid. Analysis of the serum and oral fluid BDNF and cortisol levels revealed a moderate correlation ($r=0.51$, $P=.0189$).

Conclusions: For laboratory monitoring of alcohol dependence syndrome, it is possible to determine oral fluid BDNF, which, like cortisol, has demonstrated a statistically significant moderate correlation between the serum and oral fluid levels.

Keywords: dependence syndrome, alcohol, neuropeptides, blood serum, oral fluid, neurotrophins

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Содержание белковых маркеров и гормонов в сыворотке крови и ротовой жидкости у больных с синдромом алкогольной зависимости

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Резюме

Актуальность: Развитие синдрома зависимости сопровождается нарушениями нейропластичности в нейронных цепях, дисбалансом нейромедиаторного обмена, иммунного и гормонального статусов в центральной нервной системе, которые отражаются в изменении периферических маркеров. В связи с этим определение нейропептидов в биологических жидкостях – потенциально перспективная стратегия лабораторного мониторинга наркопатологии.

Цель: Определение характерных особенностей изменений белковых маркеров и гормонов в ротовой жидкости и сыворотке крови больных с синдромом алкогольной зависимости на этапе реабилитации.
Материалы и методы: Были сформированы 2 группы испытуемых лиц мужского пола: контрольная группа – практически здоровые добровольцы (n=30) – и аналогичная по размеру и половозрастным (20–40 лет) характеристикам вторая группа больных с синдромом зависимости от алкоголя. На этапе поступления на реабилитацию, а также спустя 3 мес. осуществляли взятие сыворотки крови и ротовой жидкости. В биожидкостях методом ИФА определяли содержание мозгового (BDNF) и глиального (GDNF) нейротрофинов, нейропептида Y, орексина, пептида, активирующего аденилатциклазу гипофиза (PACAP), АКТГ и кортизола.
Результаты: Анализ результатов лабораторных исследований показал принципиальную возможность определения в ротовой жидкости нейропептидов и гормонов. Особенностью большинства вышеперечисленных белковых маркеров была широкая вариабельность результатов в ротовой жидкости и отсутствие статистически значимой корреляции с сывороточным содержанием соответствующих белков. Только концентрация BDNF была статистически значимо снижена в 3,2 раза и в сыворотке крови, и в смешанной слюне. При этом анализ сывороточного и саливарного содержания BDNF, также, как и кортизола, показал наличие корреляции средней силы (r=0,51, *p*=0,0189).
Заключение: Для лабораторного мониторинга синдрома алкогольной зависимости возможно определение в ротовой жидкости BDNF, который, как и кортизол, показал наличие статистически значимой корреляции средней силы между сывороточным и саливарным содержанием.
***Ключевые слова*:** синдром зависимости, алкоголь, нейропептиды, сыворотка крови, ротовая жидкость, нейротрофины
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Introduction

Psychoactive substance use is a serious, multifaceted, and recurring public health issue worldwide, which has significant social and economic consequences.[1,2] One of the fundamental problems in psychiatry and narcology is the fallibility of the diagnostic process due to a large share of subjective methods. Practicing narcologists come across such problem less frequently because the very fact of psychoactive substance use is revealed by chemical and toxicological analysis of body fluids and in some cases hair or nails. However, direct or indirect markers of psychoactive substance use do not allow to assess patients' psychophysical state, normalization of which is stated as the only criterion for successful rehabilitation in modern clinical guidelines.[3] It is known that the development of alcohol dependence syndrome is accompanied by disturbances of neuroplasticity in neural circuits, the imbalance of neurotransmitter metabolism and immune and hormonal statuses in the central nervous system.[4] In addition to data, indicating brain tissue changes in the expression and concentration of such biomarkers as brain-derived neurotrophic factor (BDNF), glial cell line–derived neurotrophic factor (GDNF), neuropeptide Y (NPY), orexin, and pituitary adenylate cyclase-activating peptide (PACAP), there are some data on the possibility and prospects of determining their serum levels for laboratory monitoring of chemical addictions. Thus, measuring serum BDNF levels is a valuable diagnostic strategy for predicting relapse, determining withdrawal severity at different stages, and assessing treatment adherence and rehabilitation in people with substance use disorders.[5-7]

An even more complex and less studied issue is a diagnostic value of determining changes in oral fluid levels of the above-mentioned peptide markers. Nevertheless, oral fluid garners significant interest, owing to both the noninvasive nature of its collection and analysis and available positive data regarding the correlation of some laboratory analytes between blood and saliva, including cortisol levels. The disadvantages of oral fluid include the low concentration of the metabolites in question and wide variability of findings due to the influence of both external factors and oral cavity tissues.[8-9] Unifying biomaterial sampling and using highly sensitive analytical methods can partially overcome these obstacles.

This study analyzed changes in serum and oral fluid levels of protein markers (BDNF, GDNF, NPY, orexin, and PACAP) and hormones (cortisol and corticotropin [ACTH]) in patients with alcohol dependence syndrome during rehabilitation. We specifically selected cortisol, blood and salivary levels of which correlate well, and BDNF, serum level of which correlates well with the use of psychoactive substances, including ethanol.[10] Thus, we selected a set of markers, which included standard well-known markers with well-predictable changes and new promising markers that may be potentially useful for developing informative algorithms for laboratory monitoring of substance use.

The study aimed to determine characteristics of changes in oral fluid and serum levels of protein markers and hormones in patients with alcohol dependence syndrome during rehabilitation.

Methods

The study participants were divided into 2 groups. The control group (group 1, n=20) included apparently healthy men aged 20-40 years. The absence of somatic and mental disorders was confirmed during health screening and medical examinations at the Narcological Dispensary of the Ministry of Health of the Krasnodar Region (Krasnodar, Russian Federation). Group 2 comprised male patients aged 20-40 years (n=30) with F10.252 diagnosis "Dependence syndrome due to use of

alcohol, stage 2, regular consumption" who underwent outpatient treatment and rehabilitation at the Narcological Dispensary.

This observational study was approved by the independent ethics committee of Kuban State Medical University (Krasnodar, Russian Federation) (protocol No. 96 dated January 29, 2021) and conducted in accordance with the World Medical Association Declaration of Helsinki (2013) and the Federal Law of the Russian Federation No. 323-ФЗ dated November 21, 2011 "On the Fundamentals of Health Protection of Citizens in the Russian Federation."

At the time of admission to the rehabilitation program and 3 months later, venous blood samples (7-8 mL) were collected from the cubital vein into test tubes with clot activator to obtain serum. At the same time, oral fluid samples were collected by the spitting method (unstimulated saliva). Saliva was collected in clean dry containers in the morning after preliminary preparation of the patients (brushing teeth and thoroughly rinsing the mouth with boiled water one hour before the procedure). The patients were instructed to refrain from eating, drinking, and smoking during that time. Sampled oral fluid was centrifuged at 1000*g* for 20 minutes, and the supernatant was collected for further studies. The body fluid samples were frozen immediately after collection and stored at −800 °C for no more than 2 months before laboratory studies.

We determined the following markers (Cloud-Clone Corp, China; product numbers of corresponding enzyme-linked immunosorbent assay [ELISA] kits are provided in parentheses) in serum and oral fluid by enzyme immunoassay: BDNF (SEA011Hu), GDNF (SEA043Hu), NPY (CEA879Hu), orexin (CEA346Hu), PACAP (CEB347Hu), cortisol (CEA462Ge), and ACTH (CEA836Hu). The optical density analysis of wells in the microplate format was performed using the FLUOstar Omega multimode microplate reader (BMG Labtech, Germany).

Statistical data were processed using StatPlus, version 7 (AnalystSoft Inc, USA). The distribution of the data was assessed using the Shapiro-Wilk test. The nonparametric Mann-Whitney test was used to check the equality of the medians between the 2 study groups. The Spearman correlation coefficient was used to assess the relationship between the serum and oral fluid levels. Differences between the groups were considered significant at $P < .05$. The data in the tables are presented as medians and interquartile ranges.

Results and Discussion

Determination of the baseline levels at the rehabilitation start reasonably coincided with the discharge from the hospital where the patients underwent detoxification and stabilization. This resulted in relatively mild laboratory changes compared with the levels of the corresponding analytes in the controls. The patients with alcohol dependence syndrome were observed to have low serum BDNF and ACTH levels but increased GDNF concentrations (Table 1). Thus, compared with those in the controls, serum BDNF and ACTH levels in group 2 were 3.2- and 4.1-fold lower, respectively. Under the same conditions, at the initial stage of the rehabilitation, serum GDNF levels in the patients with alcohol dependence syndrome were increased by 36% compared with the controls. The rest of the analytes in the serum did not significantly differ from the values of the similar markers in group 1.

Table 1

Serum and oral fluid levels of some markers in patients with alcohol dependence syndrome at the rehabilitation start (Median [IQR])

Таблица 1

Содержание некоторых маркеров в сыворотке крови и ротовой жидкости больных с синдромом алкогольной зависимости в начале реабилитации (Q1-Q3)

Analytes	Study groups / body fluids		
	Group 1 (control)	Group 2 (alcohol dependence syndrome)	
	Serum	Serum	Oral fluid
BDNF, pg/mL	3600.9 (2420.4-5050.4)	1108.5* (712.5-2133.9)	68.4 (58.2-81.6)
GDNF, ng/mL	0.84 (0.71-0.94)	1.14* (1.07-1.26)	0.16 (0.16-0.22)
NPY, pg/mL	88.8 (76.4-92.5)	72.8 (61.0-88.6)	39.6 (2.1-111.5)
Orexin, pg/mL	297.5 (219.4-727.5)	296.3 (181.0-495.2)	482.3 (121.4-1157.6)
PACAP, pg/mL	91.4 (77.5-124.3)	102.5 (81.4-119.5)	132.6 (103.1-186.6)
Cortisol, ng/mL	117.4 (106.4-126.5)	119.1 (110.5-128.7)	12.0 (9.7-15.4)
ACTH, pg/mL	37.0 (16.9-45.3)	9.0* (5.5-13.3)	720.8 (446.6-818.8)

Note: *, statistically significant differences when compared with a similar marker in the control group

Прим.: * – статистически значимые различия при сравнении со значением аналогичного маркера контрольной группы

Table 2

Serum and oral fluid levels of some markers in patients with alcohol dependence syndrome 3 months after the rehabilitation start (Median [IQR])

Таблица 2

Содержание некоторых маркеров в сыворотке крови и ротовой жидкости больных с синдромом алкогольной зависимости через 3 мес. после начала реабилитации (Q1-Q3)

Analytes	Study groups		
	Group 1 (control)	Group 2 (alcohol dependence syndrome)	
	Serum	Serum	Oral fluid
BDNF, pg/mL	3600.9 (2420.4-5050.4)	1656.0*^ (1026.0-3606.0)	60.0 (58.3-92.7)
GDNF, ng/mL	0.84 (0.71-0.94)	1.17* (1.07-1.28)	0.17 (0.16-0.19)
NPY, pg/mL	88.8 (76.4-92.5)	74.3 (63.9-84.8)	18.6 (2.53-66.3)
Orexin, pg/mL	297.5 (219.4-727.5)	79.4*^ (64.5-181.0)	937.2^ (717.8-1013.0)
PACAP, pg/mL	91.4 (77.5-124.3)	277.8*^ (187.9-310.5)	28.5^ (19.8-38.3)
Cortisol, ng/mL	117.4 (106.4-126.5)	115.6 (108.3-122.3)	10.7 (8.6-13.8)
ACTH, pg/mL	37.0 (16.9-45.3)	12.0* (8.2-13.3)	639.8 (610.5-669.1)

Notes *, statistically significant differences when compared with a similar marker in the control group; ^, statistically significant differences when compared with data obtained at the rehabilitation start

Прим.: * – статистически значимые различия при сравнении со значением аналогичного маркера контрольной группы, ^ – статистически значимые различия при сравнении с данными, полученными на этапе начала реабилитации

The oral fluid levels of the analytes in the control group were not determined by laboratory methods, so they cannot be correlated with any reference or normal values. Nevertheless, we conducted a correlation analysis between the serum and oral fluid analytes and were able to compare some markers with literature data. Salivary cortisol is widely used in clinical laboratory practice. Its concentration is usually no more than 10% of the serum level and is 15-18 nmol/L (5.4-6.5 ng/mL) in the morning.[11] According to our findings, the oral fluid cortisol level in the patients with alcohol dependence syndrome averaged 10% of the serum level (Table 1) and was 2- or 3-fold higher than the well-known reference level of this analyte.[12] Such findings may indicate a greater prospect of using the salivary cortisol for laboratory monitoring during treatment and rehabilitation in patients with dependence syndrome.

Literature also has data on the oral fluid BDNF level in different groups of participants, which was 255.97 (standard deviation [SD]±83.90) pg/mL in people without chronic diseases.[13-15] We compared our findings in patients with alcohol dependence syndrome with the literature data and can assume that salivary BDNF levels significantly decrease similar to the changes in its serum levels. Salivary levels of other proteins analyzed in this study were not determined by other researchers; therefore, we can draw a conclusion regarding the diagnostic value of their salivary levels based on the statistical analysis of correlations between the serum and salivary levels. What we thought was an interesting discovery were high oral fluid levels of NPY, orexin, and ACTH, which might be linked to cross-reactions of salivary peptides with antibodies in the ELISA kits, as well as to the fact that in some cases high levels of some peptides are observed: substance P levels in oral fluid were shown to be much higher than those in plasma.[16,17] Nevertheless, due to the recorded wide range of fluctuations the diagnostic potential of oral fluid peptide levels will most likely not be realized.

The second stage of the study was conducted 3 months after the rehabilitation start and characterized by a number of specific changes in the analytes (Table 2).

We observed a slight increase in the serum BDNF levels (49% increase from baseline). The orexin levels were 3.7-fold lower than those at the rehabilitation start. This being said, the baseline value was within the reference range, so such change after 3 months of abstinence from alcohol can reflect specific events associated with metabolic changes in the nervous tissue during recovery. On the contrary, the serum PACAP level has a 2.7-fold increase from baseline (before the beginning of the study). The oral fluid orexin increased from baseline (1.9-fold increase in the median value), while the PACAP level had a 4.6-fold decrease. These findings contradicted the data regarding serum. Serum or plasma are regarded as the most reliable body fluids for laboratory diagnostics, as they are the most informative in terms of internal characteristics of the body, whereas other body fluids are highly dependent on various external and internal factors. Although even air contact can have an effect on saliva, the state of the oral tissues has the strongest impact on the composition and properties of this body fluid. Therefore,

the correlation analysis between serum and salivary peptide levels plays a decisive role in assessing prospects of saliva diagnostics. At the same time, a reliable correlation of cortisol levels, the only steroid in our study, has been known for a long time, and salivary cortisol is used in clinical laboratory practice.[18] In our study, the Spearman correlation coefficient determined when comparing the serum and oral fluid cortisol levels was 0.71 ($P=.0108$), which can be characterized as a high strength of the relationship between the laboratory analytes. We found a statistically significant correlation when analyzing the serum and salivary BDNF levels ($r=0.51$, $P=.0189$). The oral fluid levels of the rest analytes did not correlate with the corresponding serum levels of the biomarkers.

Conclusions

Analysis of laboratory findings revealed the fundamental possibility of determining oral fluid levels of such neuropeptides and hormones as BDNF, GDNF, NPY, orexin, PACAP, cortisol, and ACTH using the ELISA method. While salivary cortisol has been used in clinical laboratory practice for a long time, and literature has some information on determining the BDNF level in certain groups of people, other biomarkers were determined in saliva for the first time. One of the characteristics of the above-mentioned protein markers was a wide variability of findings in oral fluid and the absence of statistically significant correlation with the serum levels of the corresponding proteins. Apart from cortisol, only BDNF level in body fluids of patients with alcohol dependence syndrome proved its high diagnostic significance. We found the decreased BDNF level at the local and systemic levels and a moderate correlation between the serum and salivary BDNF levels.

Author contributions

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Acquisition, analysis, or interpretation of data: Popov, Popova, Zavgorodnyaya
Manuscript drafting and revising: Bykov, Kurzanov, Esaulenko
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