

How do students cope with stress during remote learning due to COVID-19 pandemic? Biological and psychological factors- protocol of the study

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Abstract

Introduction: The COVID-19 pandemic negatively affected the mental health of the Polish population. Students are the group with a high risk of developing mood disorders. The scientific data suggest the usefulness of biological substances as potential biomarkers of psychiatric disorders. Saliva is non-invasive, quick to obtain material and a promising fluid for measuring stress and depression severity. In this paper, we present the protocol of our study, which aims to assess the utility of concentrations of biological fluids as potential biomarkers of the stress response and risk of developing depression and anxiety disorders among students of Lublin.

Materials and methods: Total number of 80 students were included in the study. Participants were asked to collect saliva samples simultaneously with responding to the questionnaires concerning their mental state. Afterwards, the samples were examined with an ELISA test to assess the level of fibroblast growth factor (FGF-2), cortisol and chromogranin A (CgA). The relationship between biomarkers and severity of stress, depression, anxiety will be examined.

Results: Cortisol on its own is not a good material to investigate the stress reaction in saliva due to its variable levels among people during stressful situations. Scientific data indicate the usefulness of other biomarkers or their combination to indicate the potential risk of developing depression and/or anxiety.

Conclusions: Introducing objective tests, helpful for mood symptoms recognition, would improve the prevention and diagnosis of psychiatric disorders in vulnerable populations.

Keywords: depression, saliva, biomarkers, cortisol, FGF-2, CgA

Streszczenie

Wstęp: Pandemia COVID-19 wpłynęła negatywnie na zdrowie psychiczne studentów w polskiej populacji. Studenci są grupą w szczególności narażoną na zaburzenia nastroju. Badania naukowe dowodzą użyteczności biologicznych substancji jako potencjalnych biomarkerów chorób psychiatrycznych. Ślina jest nieinwazyjnym, szybkim do uzyskania i obiecującym materiałem biologicznym do badania nasilenia stresu oraz depresji z powodu jej właściwości. W tym artykule, przedstawiamy protokół naszego badania, którego celem jest ocena użyteczności mierzenia stężenia substancji biologicznych w ślinie i ocena ich w kontekście potencjalnych biomarkerów odpowiedzi stresowej, ryzyka rozwoju depresji oraz zaburzeń lękowych wśród studentów Lublina. Będziemy badać następujące biomarkery: czynnik wzrostu fibroblastów-2 (FGF-2), kortyzol oraz

chromograninę A (CgA).

Materiały i metoda: 80 studentów obydwu płci Uniwersytetu Medycznego w Lublinie będzie włączonych do badania. Ochotnicy będą poproszeni o zebranie śliny z jednoczesnym wypełnieniem arkuszy na temat ich samopoczucia psychicznego, warunków socjodemograficznych oraz ewentualnego stałego leczenia. Następnie próbki zostaną poddane analizie metodą ilościową ELISA w kierunku obecności czynnika wzrostu fibroblastów-2 (FGF-2), kortyzolu i chromograniny A (CgA). Na samym końcu, związek pomiędzy biomarkerami i nasileniem objawów stresu, depresji i zaburzeń lękowych będzie poddany analizie statystycznej.

Rezultaty: Kortyzol badany osobno nie jest dobrym biomarkerem do oceny poziomu stresu z powodu zmienności jego poziomu w ślinie w populacji podczas reakcji stresowych. Badania wskazują na użyteczność innych substancji lub kombinacji różnych biomarkerów w celu dokładniejszego zbadania ryzyka wystąpienia zaburzeń nastroju.

Konkluzje: Wprowadzenie laboratoryjnych testów, które byłyby pomocne w obiektywizacji diagnoz z zakresu zaburzeń nastroju, poprawiłoby proces prewencji oraz diagnostyki zaburzeń psychiatrycznych w najbardziej na to narażonych populacjach.

Słowa kluczowe: depresja, ślina, biomarkery, kortyzol, FGF-2, CgA

1. Introduction

The COVID-19 pandemic had a negative impact on the physical as well as on mental health of people around the world, forcing them to accept the new reality of their lives [1,2]. The academic population, as the group most prevalent to stress, had to compromise to stay away from the infection, and the consequence of that was a limitation of social contact and a lack of satisfaction and motivation from studying [3]. E-learning is associated with a higher percentage of depression cases and stress reaction among students [4,5]. Therefore, it is crucial to investigate the correlation between education during the COVID-19 pandemic and the risk of developing mood disorders in this population. Data suggest that people, who were struggling with mental disorders before, could see even more serious mental health problems weeks or months after acute infection [6,7]. Moreover, the literature indicates that remote education and the isolation, which comes with that, can implicate long-term negative effects on students' mental well-being [8,9].

Stress is a natural reaction of organisms that motivates action. However, too intense or prolonged stress may increase the risk of developing anxiety disorders and depression [10]. Diagnostics of the diseases mentioned above are based on medical history, patient observation and diagnostic scales, more often largely subjective [11]. Even so, it is possible to assess symptoms by laboratory tests of substances whose concentrations change during the stress reaction closely related to anxiety and depression [11]. According to the available literature, biological factors associated with depression and stress include: cortisol, lysozyme, chromogranin A (CgA), fibroblast growth factor 2 (FGF-2) and alpha-amylase (sAA) [11-14].

More and more researchers use salivary biomarkers for the diagnosis of many organism disruptions. There are

many advantages of using saliva as a biological material. Collection of this fluid is a non-invasive procedure; therefore, it does not require special conditions or trained personnel to obtain it, which significantly reduces the costs of the initial diagnostic stage. It is also an advantage for patients as the procedure is painless, safe and less stressful. Moreover, saliva is a stable material for 24 hours at room temperature with no risk of coagulation. Hence, the laboratory process can be postponed if necessary, e.g. for technical reasons [11].

The use of biological methods, confirmed in the scientific literature, could be a significant diagnostic aid and, in the future, could be used in the implementation of pharmacological treatment and monitoring its effectiveness or as a screening test for exposure to depression and anxiety disorders.

The aim of the project is to assess the utility of concentrations of the fibroblast growth factor (FGF-2), cortisol, and chromogranin A (CgA) as potential biomarkers of the stress response, risk of developing depression and anxiety disorders among Lublin students.

2. Materials and methods

2.1 Study group

Total number of 80 students of both sexes of the Medical University of Lublin were qualified for the study. Each participant was informed about the objectives, the course of the study and the possibility of withdrawing from the project at every stage. Subjects were asked to sign informed consent.

The exclusion criteria were as follow:

- presence of mental disorders (with the exception of personality disorders, nicotine and / or caffeine addiction);
- taking medications / substances with a sedative, anxiolytic, antidepressant effect.

2.2 Estimating the quantity of the studied population

We determined the number of participants based on the earlier studies. The probability of rejecting the null hypothesis of no differences in the concentrations of the test substances in saliva with the power >0.08 and $\alpha < 0.05$ was established for 80 students.

2.3 Methods

I. Questionnaires

1. Self-constructed sociodemographic questionnaire - containing general questions about age, gender, information about chronic diseases, using drugs and supplements, smoking tobacco, frequency of alcohol consumption, using relaxation techniques.
2. Beck Depression Inventory (BDI) - a self-assessment tool consisting of 21 closed questions to determine the severity of mood symptoms. The answers were from 0 to 3 points, in total 63 points. The higher the score, the higher the intensity of the assessed symptoms. We took 11 points as the cut-off for classification into the group with low (BD) or high severity of depressive features (D) [15].
3. State-Trait Anxiety Inventory (STAI) - a questionnaire consisting of 40 questions to distinguish anxiety as a personality trait from situational anxiety. The STAI is divided into two subscales (X-1, anxiety as a trait; X-2, anxiety as a state). The respondent chooses one out of four categorized responses [16].
4. Perceived Stress Scale (PSS-10) - consisting of 10 questions about subjective feelings related to stressful situations in everyday life, behaviors, and ways of dealing with them. The higher the stress level, the higher the score on the scale. The number of 20 points was used as the cut-off point for qualifying as low (BS) or high (S) stress [17].

II. Saliva testing

Students were asked to collect saliva simultaneously with filling of the questionnaires. It took no less than 15 minutes in order to collect the proper amount of material in test-tubes with a maximum volume of 10 ml. Students were asked not to eat and not to wash teeth at least two hours before the procedure. Moreover, they were asked to come during the morning hours, rested after the night before and without any excessive workout prior to the study. In the collected material, the concentrations of biological substances were determined by the enzyme immunoassay method (ELISA) using commercial kits, following the manufacturer's recommendations.

III. Examined biomarkers

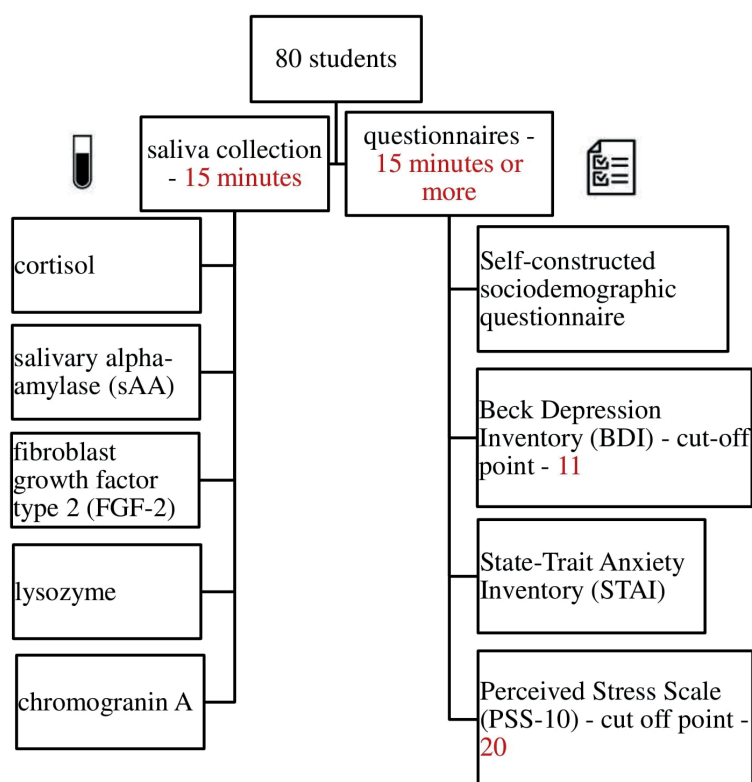
The levels of the following markers are going to be measured:

1. Cortisol - a hormone secreted by the adrenal cortex. Exposure to stressors activates the hypothalamic-pituitary-adrenal (HPA) axis, and the end product resulting from these pathways is cortisol. It intensifies the effects of other "stress hormones" - adrenaline and norepinephrine, causing a sharp increase in the number of substances used to cope with situational stress [11]. A meta-analysis by Miller et al. confirmed that the substance determined in saliva could be considered a biomarker of acute stress [18,14].
2. Chromogranin A - glycoprotein released from the adrenal medulla and sympathetic nerve endings. Its concentration has been shown to increase as a result of isolation stress or immobilization, and numerous studies indicate that its amount in the saliva is a promising, sensitive biomarker of psychosomatic stress [11,14]. The rapid and specific increase in salivary chromogranin occurs in response to various stress factors in the body, such as during student exams [19].
3. Lysozyme - a protein synthesized and released from monocytes and macrophages. It gives saliva antibacterial properties, plays a role in the response against viruses, and induces the lysis of neoplastic cells. Studies conducted with the group of students showed significantly lower values of lysozyme in saliva during the examination session than in the non-examination period. Currently, it is used and recognized as a biomarker of chronic stress [11,14].
4. Fibroblast growth factor type 2 (FGF-2) - A mitogen for cells found in the salivary glands and saliva involved in the physiological response related to neurodegeneration and stress regulation. It has been considered an endogenous regulator of fear expression, and its concentration is inversely proportional to the severity of anxiety and stress [11].
5. Salivary alpha-amylase (sAA) - is the main digestive enzyme in the mouth. It performs immune functions, and its concentration increases sharply and faster than cortisol as a result of exposure to stress factors [11,14].

3. Statistical analysis

Statistica 13 software TIBCO Software Inc., CA, USA was used to evaluate:

- distributions of analyzed variables;
- sociodemographic and health characteristic of examined population;
- differences in the levels of biological markers concentration in saliva of participants with and without anxiety and / or depression;
- the relationship between the subjective assessment of mental well-being and the concentration of the tested biomarkers;
- sensitivity and specificity of the tested substances as



markers of psychological well-being.

4. Discussion, clinical implications

The university students, as an exceptional stress-prone and exposed population, are the target group of the study. The meta-analysis published in 2022 with 25,246 participants indicated that the prevalence of anxiety and depression is significantly higher among students compared to the general population (32% and 31% vs. 22% and 20%, respectively). This data supported the urgent need to identify practical, effective solutions and interventions to support students' mental health. In a study from Medical University of Poznań, the cortisol level indicated in the student's saliva increased most dynamically in the morning. Its concentration was the highest in the evening hours at the beginning of the academic year compared to later [20].

The exams required to complete education could be a potential stressor for young individuals. A study conducted at the School of Nursing at Osaka Prefecture University with the participation of 15 women aged 21-26 showed that acute stress caused by the examination increased the concentrations of CgA and IgA in saliva and did not increase the cortisol [19]. In two independent experiments to assess the relationship between examined stress and cortisol, 35 students examined in writing and 37 students giving an oral presentation were tested. Increased cortisol concentration in saliva was detected before the exam, while the values decreased afterwards. In the case of oral presentation, the level of cortisol was

elevated both before and after the exam [21]. The results of a study published by Tayamman et al. indicated that the students who gave the oral presentation in front of the audience had increased cortisol, alpha-amylase and chromogranin A levels than the group watching their statements [22]. Based on previous reports, saliva is not a good predictor of acute stress reaction. At the same time, some studies show that students immediately before exams are characterized by elevated levels of cortisol and a decrease in its concentrations after the examination period [20, 21]. The inconclusive findings of studies may result from the circadian rhythm of cortisol secretion and changes in its concentrations during the day depending on interpersonal variability. Typically, exams are carried out in the morning when the A-amylase and chromogranin-A concentrations in the body are different from cortisol. In the morning hours, cortisol levels are high, and A-amylase and chromogranin-A levels drop down. In assessing biological markers of stress, the time of saliva uptake may significantly impact on the interpretation of the results [22]. Despite the studies of the correlation between exposure to stress and biological substances in saliva, there is still a deficit of information about the interaction of biological markers with the presence of stress and depression. According to the results of the Polish study from 2022, every third student had depressive symptoms [23]. The high risk of mood disorders in this population is the result of many factors: more responsibilities, a new environment, a large amount of learning, exams, and lifestyle changes caused by self-empowerment [23].

Early detection of depression programs could prevent the economic burden on the state, e. g. due to the inability to work of patients with depression.

The number of studies assessing the relationship between the symptoms of mood disorders and the concentration of biological markers of stress, depression and anxiety is insufficient to type the most promising diagnostic marker. The main challenge in revealing biomarkers of mood disorders is the complexity of basic human physiological systems, their interplay and intra variability.

The data from 2020 did not confirm the relationship between salivary cortisol levels and symptoms of depression among students [24]. These results are consistent with two Japanese reports, where no differences in the cortisol concentration in saliva were observed [24,25]. However, a study carried out on a group of young individuals suffering from mood disorders, indicated a significantly higher ratio of morning to evening salivary cortisol levels compared to the healthy controls [25]. This suggests that single-point measurements of biological markers may not be sufficient to diagnose mental health problems. Hence, there is a requirement not only to find reliable markers of stress, anxiety and depression, but also to determine time of the day when examination should be performed. Patients with mood disorders were also characterized by higher values of salivary amylase concentration compared to people suffering from other psychiatric disorders [26]. On the other hand, young depressed patients were characterized by lower amylase values in saliva compared to healthy individuals, but only at noon [27]. Similar results were obtained by Tanaka et al. and authors concluded salivary amylase may be a useful biological marker of depression [28]. Researchers observed lower values of salivary amylase concentration among women suffering from depression compared to the healthy population [28]. It should be noted that physiological factors, such as pregnancy, menstrual cycle, may affect the saliva concentration of many biological substances. For example, symptoms of depression during pregnancy were associated with higher levels of alpha-amylase in saliva [29].

The unclear results of the previous research and the lack of publications about the other potential salivary biomarkers of stress, depression and anxiety indicate the requirement to explore this issue. The results of the clinical trials focused on identifying easy-to-test biomarkers of well-being could be used to create an objective tool for mental health assessment. Extending the current medical diagnostic with laboratory tests could accelerate and improve the diagnosis of stress, anxiety and depression. Biological markers can complete an interview with the patient. Moreover, some biomarkers could

be practical screening tests for detecting a vulnerable population's stress, anxiety and depression symptoms. This would enable quick assistance and supervision of at-risk patients, a positive factor in effective treatment and faster recovery.

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Conflict of interest

The authors have declared no conflict of interest.

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