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**Stress and Anxiety in High School: Examination of the
Relationship between Physiological and Psychological Indicators
among Adolescents.**

Ph.D. thesis

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Stress and Anxiety in High School: Examination of the Relationship between Physiological and Psychological Indicators among Adolescents.

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Introduction

In recent decades, significant social and economic changes have transformed people's lifestyles. It's a paradox that while our lives have become much more comfortable due to various devices assisting us both at home and in the workplace, we spend significantly more time engaged in work-related activities. For adults, this entails job-related tasks, while for children, it involves tasks related to learning. The ongoing commitments, increasing expectations, and the pressure to meet these expectations often serve as significant sources of stress, affecting everyone regardless of age or gender, albeit varying in intensity and duration.

Stress exerts its effects on the body through the neuroendocrine system, leading to behaviors that facilitate adaptation in individuals. While stress is a natural part of life, when it becomes chronic or improperly managed, it contributes to the emergence of various psychological and consequently physiological problems. The undeniable relationship between stress and mental illness poses severe issues for both adults and children to the extent that mental health disorders are recognized as significant public health concerns worldwide.

Stressors that trigger stress processes can be external or internal factors, primarily environmental, biological, and mental factors, leading to the production of free radicals. These reactive oxygen species can damage cells when the internal balance, homeostasis, is disrupted, and the level of antioxidants in the body decreases. In this case, free radicals outnumber antioxidants, resulting in oxidative stress, which can damage lipids, proteins, and DNA, leading to molecular changes and the development of various diseases in the long term.

Research on oxidative stress is currently a significant topic, as health damage due to mental stress has been proven. Psychological stress significantly contributes to the development of cardiovascular diseases since acute mental stress releases catecholamines, resulting in high heart rate and increased blood pressure. Prolonged exposure to stress in the body leads to various physiological changes in the neuroendocrine, immune, and cardiovascular systems, resulting in health problems. Chronic psychological stress exposure can lead to the development of diseases such as metabolic and cancerous diseases.

Furthermore, it has been demonstrated that persistent oxidative stress results in the development of cardiovascular diseases, which are the leading cause of death in developed countries. If stress is present both mentally and physically, it can exacerbate cardiovascular

reactions by altering the body's redox balance, thereby collectively contributing to the higher mortality rate caused by cardiovascular diseases.

In recent years, numerous studies have focused on anxiety related to mental stress, directing attention towards the adolescent age group due to the frequent occurrence of anxiety and depression among them. These mental stressors develop as a result of intensive physiological and psychological changes inherent in adolescence, identity crisis, and the construction of a new identity. Stressors can also originate from the environment, with both family circumstances and factors related to learning and the school environment exerting a strong influence on adolescents' mental well-being. The examination of stress and anxiety among adolescents and young people is of paramount importance because anxiety limits their academic abilities, affects their social relationships, family life, and can lead to serious problems such as eating disorders, depression, or even suicide.

Therefore, it is essential not only to uncover the causes but also to understand the relationships between stress and anxiety during adolescence. This understanding will enable targeted interventions, pedagogical, and psychological interventions, to help this age group so that the stress and anxiety they experience do not result in academic failures, deteriorating relationships, and ultimately, but not least, psychosomatic symptoms and chronic illnesses in their lives.

Objective

The aim of our research was to assess the acute and chronic stress exposure, as well as anxiety levels among high school students attending various types of secondary education institutions (vocational schools, technical high schools, and grammar schools, according to the former classification: vocational training schools, technical secondary schools, and grammar schools) at two different times: at the end of the school year and during the first week of the educational period following the summer break. The first data collection took place in May at the end of the school year, while the second data collection occurred in the first week of September.

The aim of the study was to determine whether the perceived stress, obtained through standardized questionnaires, could be objectively validated through laboratory measurements, specifically measuring the quantity of cortisol in hair samples. Additionally, our goal was to

explore the factors influencing the levels of stress and anxiety, as well as certain lifestyle factors associated with stress and anxiety (such as alcohol consumption and smoking).

Materials and methods

Our study was conducted in May 2019 and September 2019 in secondary educational institutions in a county-level city (including vocational schools, vocational high schools, and gymnasiums). Data collection was carried out using questionnaires and biological sampling. The questionnaires were completed at two time points, concurrently with the biological sampling. The first data collection took place at the end of the 2018/19 academic year, while the second data collection occurred on the first day of the 2019/20 academic year. The biological samples were processed at the laboratory of the Faculty of Health Sciences, University of Pécs, following the completion of the questionnaires. The entire quantity of samples was utilized during the laboratory procedure.

In the questionnaire survey, 125 participants took part, and 100 individuals provided hair samples at the first time point. At the second measurement time point, 87 participants completed the questionnaire, and 84 individuals provided hair samples.

Measurement tools used in the questionnaire survey:

The measurement instrument used consisted of a self-designed questionnaire containing items tailored to socio-demographic characteristics, as well as standardized measures such as the Spielberger State-Trait Anxiety Inventory, the Family Dynamics Measure-II questionnaire Clear Communication subscale, the Multiple Social Perceived Support Scale, and relevant question groups from the HBSC and ESPAD questionnaires to assess health behavior and psychosomatic symptoms.

The Spielberger State-Trait Anxiety Inventory consists of 20 items measuring the intensity of anxiety on a four-point scale. While state anxiety assesses the momentary, currently experienced anxiety, trait anxiety provides information about the level of general anxiety characteristic of the individual's personality.

The Clear Communication subscale of the Family Dynamics Measure-II consists of four items assessing family communication using a 6-point Likert scale.

We measured the level of support received from the family using the family-specific four-item subscale of the Multiple Social Perceived Support Scale (MSPSS), which, similar to the Clear Communication Scale, assesses the perceived level of family support.

The HBSC (Health Behaviour in School-aged Children) study, involving participation from over 50 countries, is an international research program that examines the health, well-being, health behavior, and social environment of adolescents aged 11, 13, 15, and 17 years old. This research program, conducted every four years, is supported by the World Health Organization (WHO).

Biological sampling

During hair sample collection, the inclusion criterion required the student providing the sample to have hair at least 3 cm long. Prior to sampling, students were informed that only the amount of cortisol would be determined from their hair sample. Students who had received steroid treatment in the month preceding the sampling or were still undergoing treatment at the time of sampling were excluded.

We collected approximately 20 mg of hair strands with scissors from participants who consented to the study. This quantity amounted to about 30-40 hair strands. According to literature recommendations, hair should be collected from the posterior vertex of the head, as this area exhibits the highest hair growth rate (1 cm/month) and the least individual variability in cortisol levels. This means that cortisol concentration measurement is based on the average hair growth of 1 cm/month during the three-month period prior to sampling. The 3 cm segment of hair closest to the scalp was placed in a pre-labeled Eppendorf tube. Cortisol concentrations were determined from this 3 cm hair segment. The samples were processed within one week but can be stored at room temperature for up to 12 months.

We accurately weighed the samples on an analytical balance and then finely chopped them with surgical scissors. The chopped samples were washed twice for 3 minutes each in 1 ml of isopropanol at room temperature, and then the supernatant was aspirated because it was not used. Subsequently, the samples were dried.

The next step was extraction. The samples were incubated in 1 ml of methanol for 16 hours (200 rpm, 52°C). The supernatant was aspirated into an Eppendorf tube, then incubated with 1 ml of acetone at room temperature and 200 rpm for 5 minutes. The supernatant was aspirated again. This process was repeated once. Subsequently, evaporation with nitrogen followed,

then measurement, where the samples were taken up in PBS buffer: 10 mg of sample + 75 microliters of PBS. The measurement was performed using a modified cortisol saliva ELISA kit according to the instructions provided in the cortisol kit (Cortisol Saliva Kit REF: DSNOV20, LOT: S-CORT-5240A NovaTec Immundiagnostica GMBH). The ELISA Microplate reader used was Type: 357, REF: 51119100, SN: 357-906094T (ThermoFisher).

Results and conclusions

Stress is a part of life. It's an impact that affects everyone, motivating but also potentially causing harm. Investigating stress-related anxiety and the appearance of psychosomatic symptoms, along with exploring their underlying causes, is of paramount importance from health, economic, and social perspectives. Our study focused on 16-17-year-old adolescents who, amidst characteristic physiological, hormonal, and psychological changes, also struggle with the expectations set by parents and school. It has been proven that these circumstances significantly contribute to the anxiety of young individuals. Our goals included differentiating among factors that trigger anxiety, identifying the most prevalent ones, uncovering the root causes, and establishing an objective measurement method alongside the commonly used questionnaire-based research approaches.

The differentiating factors of anxiety

Among the differentiating factors, gender and type of school, as well as the mother's highest level of education, clearly stood out. The analysis of the results showed significant differences in both state and trait anxiety between boys and girls (May STAI-S: $t=-4.00$; $p<0.01$; May STAI-T: $t=-7.58$; $p<0.01$; September STAI-S: $t=-5.89$; $p<0.01$; September STAI-T: $t=-7.82$; $p<0.01$), as well as among different types of schools (May STAI-S: $F=3.44$; $p<0.001$; May STAI-T: $F=8.60$; $p<0.001$; September STAI-S: $F=8.60$; $p<0.001$; September STAI-T: $F=11.76$; $p<0.001$). The statistical tests draw attention to higher levels of stress among girls and students attending gymnasiums.

The mother's level of education shows a correlation with the child's stress exposure, as daughters of mothers with higher education attending gymnasiums exhibit the highest levels of anxiety. Their significantly higher level of anxiety compared to students in other types of schools is striking. The examination of differentiating factors clearly directed attention to girls and adolescents attending gymnasiums, among whom we observed a pronounced presence of psychosomatic symptoms, typically manifested as headaches, gastrointestinal symptoms,

apathy, and insomnia. Furthermore, we found a correlation between the level of anxiety and the intensity of symptoms. Higher levels of anxiety result in more frequent and more intense psychosomatic symptoms.

Results of measuring the cortisol level

Due to the psychological and physiological characteristics of the examined age group, there is no positive correlation between stress-induced anxiety and cortisol levels measured from hair. Cortisol levels were significantly higher in boys ($Z=-4.07$; $p<0.001$) and students attending vocational schools during the first measurement ($\chi^2=26.17$; $p<0.001$), while the differences between boys and girls disappeared during the second measurement ($Z=-0.46$; $p=0.64$), with only the differentiating effect of school type remaining ($\chi^2=11.02$; $p=0.004$). When examining the highest level of parental education, the father's highest level of education did not prove to be a differentiating factor, while the mother's highest level of education showed a significant correlation with the child's trait anxiety during the May data collection ($F=3.10$; $p=0.04$). Both state and trait anxiety were highest among mothers with tertiary education in both measurement periods. The cortisol level was influenced by the mother's highest level of education during the first, May measurement occasion, but in this case, children of mothers with higher education had lower cortisol levels. The increase in anxiety level is accompanied by a decrease in stress hormone concentration, which is observable in both genders. Adolescents tend to react more sensitively and intensely to stress, indicating characteristic functioning of the HPA axis in adolescents. The results obtained complement previous studies by showing that this negative correlation is observable not only in the 10-12 age group but also in 16-17-year-olds. It can be concluded that in this age group, anxiety is a better indicator for subjective testing, which more accurately indicates the cause of psychosomatic symptoms associated with anxiety than determining the objective biological marker.

Given the results, there is a need for further research aimed at identifying an appropriate biological marker to reliably indicate stress exposure. This would help in finding a reliable, objective indicator of stress exposure.

Summary

Our research primarily aimed to uncover anxiety among high school students, identify the differentiating factors of anxiety, and explore the relationships between various data

collection and measurement methods used to examine anxiety. Based on our findings, our recommendations target both the high school student population and their surrounding environment. Additionally, we propose suggestions for further research directions, particularly focusing on the measurement methods for investigating chronic stress.

The examination of anxiety among high school students is not a new area of research. According to detailed descriptions within the theoretical framework, this age group is particularly vulnerable due to the physical and psychological changes of adolescence, regardless of external circumstances, coupled with the stress and pressure from school tasks. Our findings particularly highlight the stronger anxiety among girls and those attending gymnasiums, which also delineates the directions for intervention. Reducing the high level of anxiety among high school students can be achieved through various interventions, which are especially crucial for high school students, particularly girls, based on our findings.

It is important to note, however, that when planning school interventions for this age group, we cannot overlook the institutional environment in which students spend most of their days. In our view, interventions aimed at the mental well-being of adolescents within the school environment can only be effective if educators are also involved in the process. We see this as necessary because only mentally healthy educators can educate and nurture mentally healthy adolescents.

The examination of factors differentiating anxiety has highlighted that certain family characteristics, such as family communication and support, significantly influence the level of anxiety in students. Therefore, we cannot exclude parents when considering the institutional environment surrounding adolescents. Early childhood institutional education places great emphasis on maintaining contact with parents and supporting parental competence. However, we observe a loosening of the relationship between parents and educators in primary school, which becomes even more pronounced in high school. We believe that fundamental changes in thinking are needed in this regard. It is essential to recognize that today's parents do not possess adaptive parenting patterns, and the generational gaps are rapidly increasing. Therefore, previously successful parenting practices and strategies are not suitable for Generation Z. Parents and educators are faced with the same questions every day, emphasizing the importance of collaboration for the benefit of adolescents.

For the examination of chronic stress, we consider further analysis in larger samples across different age groups to be of paramount importance. This will allow for a refinement of

the method's results and the definition of a population where cortisol measured from hair serves as a reliable biological marker of stress exposure.

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