„VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND PHARMACY FROM TIMIȘOARA
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PhD THESIS

THE INFLUENCE OF SYSTEMIC DISEASES ON ORAL HEALTH

- ABSTRACT -

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T i m i ș o a r a

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Current models of oral health globally and regionally largely reflect distinct risk profiles among countries, linked to lifestyle, living conditions, and the implementation of preventive measures, as well as preventive oral health systems. The significant role of social-behavioral and environmental factors in oral diseases and oral health has been demonstrated in numerous epidemiological studies. The most important non-communicable diseases (NCDs) - cardiovascular diseases, chronic stress, diabetes, cancer, and chronic obstructive pulmonary disease (COPD) - share common risk factors with oral cavity diseases. The strong correlation between several oral diseases and non-communicable chronic diseases (NCDs) is primarily the result of common risk factors. Severe periodontal disease, for example, is associated with diabetes and has been considered the sixth complication of diabetes. Given the magnitude of health issues, oral diseases represent a major public health problem in all regions of the world. Their impact on individuals and communities through pain and suffering, impairment of functions, and reduction in quality of life is considerable. Globally, the greatest burden of oral diseases falls on disadvantaged and poor population groups. In several industrialized countries, there are positive trends regarding the reduction of oral conditions, such as dental caries among children and tooth loss among adults.

Taking into account the WHO resolution on oral health from 2021, improving oral health as part of the universal health coverage agenda by 2030, the outcomes of this thesis could be useful for identifying the most effective means and methods of promoting oral health within the Romanian target group. Furthermore, they could contribute to the development of a national oral health program.

The results of this doctoral thesis were published in 2022 and 2023 in ISI-indexed international journals: Diagnostics (IF-3.6), Healthcare (IF-2.8), and Materials (IF-3.4), with a **cumulative impact factor of IF-9.8.**

Keywords: *oral health, general health, periodontal disease, salivary biomarkers, total antioxidant capacity, salivary pH, stress.*

Oral health is linked to general health status, mortality, and morbidity. The bidirectional relationship between periodontal disease and type 2 diabetes is well-established through scientific studies. Progressive chronic diseases, such as periodontal disease and other non-communicable diseases, release inflammatory mediators into circulation and exacerbate systemic inflammation. This common inflammatory pathway is considered to be an association between periodontal disease and some systemic diseases, including cardiovascular diseases, rheumatoid arthritis, Alzheimer's disease, and Parkinson's disease. Aspiration of oral bacteria can cause pneumonia, especially in hospitalized patients and elderly adults. If dental caries or periodontal disease is left untreated, oral bacteria can enter the bloodstream, leading to septicemia. Therefore, promoting oral health can improve both oral and systemic health. Additionally, stress is now recognized as a universal premorbid factor associated with many risk factors for various chronic diseases. Acute stress can induce an individual's adaptive response to environmental demands. However, chronic, excessive stress has a cumulative negative impact on health outcomes through "allostatic load." Thus, monitoring quantified levels of long-term stress mediators would provide an opportunity for the prevention or early intervention of stress-related chronic diseases. Stress can lead to both physical and psychological health problems. Chronic stress can lead to negative consequences and can affect the immune, cardiovascular, neuroendocrine, and central nervous systems. In particular, chronic stress can have a serious impact due to sustained high levels of chemicals released in the "fight or flight" response, which involves the release of glucocorticoids by the endocrine system. Cortisol secretion in response to biochemical stress contributes to impairing the hypothalamic-pituitary-adrenal (HPA) axis on health events and cognition. The concentration of salivary cortisol levels correlates with the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Ryan, Booth, Spathis, Spathis, Spathis, Mollart, & Clow, 2016). Dysregulation of the hypothalamic-pituitary-adrenal axis characterizes many childhood predominant diseases. Autism, depression, and post-traumatic stress disorder are examples of pathologies with reported changes in salivary cortisol measurements in pediatric populations (Putnam et al., 2012). In children, salivary cortisol is a significant biomarker for early identification of risk factors related to autoimmune diseases, cardiovascular diseases, and metabolic syndrome (Jessop & Turner-Cobb, 2008) (Cozma et al., 2017; Keil, 2012).

The main purpose of the thesis is to evaluate the influence and connection of oral health with systemic conditions of patients. In this regard, the thesis has two converging research directions:

- the first research direction aims to explore the subtle connections of the periodontal patient profile in Romania (age, demographic aspects, associated risk factors, and present systemic conditions).

- the second research direction focuses on identifying changes in salivary markers influenced by stress in a group of students during exam periods (evaluation of salivary pH and Total Antioxidant Capacity (TAC) under stress conditions).

The thesis is divided into two sections: the general part and the special part:

The general part presents the current state of knowledge, WHO recommendations, and the resolution regarding the integration of oral health within the context of general health and non-communicable diseases (NCDs). The multidisciplinary approach to oral diseases and their early detection, through the use of salivary markers, facilitates the early identification of common risk factors for both oral and general conditions. Conducting thorough measurements of evidence regarding the link between oral and non-communicable diseases (NCDs) is of significant relevance in guiding and influencing decisions in the field of public health. Therefore, this thesis endeavors to undertake a comprehensive analysis to evaluate the robustness of thematic-analytical estimations establishing links between oral and systemic diseases, highlighting their bidirectional relationship. Additionally, our objective is to investigate whether future research has the potential to reshape health prevention programs, recognizing the importance of comorbidities and environmental factors within the population. In this context, the central theme of the thesis seeks to determine whether the results regarding the Romanian population align with a recent study (Botelho J et al. - 2022) that highlighted strong associations between 28 non-communicable diseases (NCDs), including five types of cancer.

The special section of the thesis will investigate the two research directions through specific approaches and analyses, structured into 4 studies, aiming to correlate the specific results of the Romanian population with international research outcomes.

The special part of the thesis is structured into 4 studies:

-Study 1 - Exploring the relationship between oral health, periodontal disease, smoking, and systemic diseases in the Romanian population.

-Study 2 - Identifying the Total Antioxidant Capacity (TAC) of saliva under stress conditions.

-Study 3 - Evaluating changes in salivary pH under stress conditions.

-Study 4 - Correlating salivary pH and TAC among dental students under various stress conditions.

Study 1 is a missing epidemiological study for Romania. The aim of the study is to understand the symptomatology of periodontal diseases within the context of the characteristics of the Romanian population and to establish the degree of correlation with various systemic diseases, chronic diseases classified as non-communicable diseases (NCDs). There are estimates that periodontal disease affects 10% of the global population, thus the impact on the healthcare system and quality of life is significant. However, with the increase in life expectancy, there is a growing geriatric population that will require oral treatments, especially for periodontal diseases. Smoking has been consistently linked to periodontal disease and tooth loss. Numerous studies have demonstrated that cigarette smoking, which exposes individuals to a wide range of toxins, is a significant risk factor for various health problems, including cardiovascular diseases, cancer, and associated chronic diseases.

Study 1 is a correlational, comparative, and cross-sectional study, and the hypotheses are that: there are differences between the frequency of smoking and the severity of periodontal disease, and there is a correlation between comorbidities (understood as systemic conditions) and the severity level of periodontal diagnoses. Validity: For the measured items from the Berna form, a global total was created. It has a Cronbach's alpha coefficient of 0.75, indicating reliability of the scale from sub to acceptable levels. A group of 242 patients diagnosed with various forms of periodontal disease (according to the new periodontology guidelines) were clinically evaluated according to the Berna online form and a general health and risk factor questionnaire (smoking).

The results showed that regarding patients' age/periodontal disease, Spearman's Rho correlation demonstrates that:

-there is a significant positive relationship between the mean age and the mean value of probing depth (an item from the periodontal chart) Rho (238) = 0.182, p <.01. The same is true for the total diagnosis, with a significant positive association between age and the total diagnosis Rho (238) = 0.360, p <.001,

-with a significant positive association between age and the degree of diagnosis Rho (238) = 0.183, p <.01, and

-a significant positive association between age and the stage of diagnosis Rho (238) = 0.467, p < 0.001.

-concerning age and somatic conditions, again a significant positive association between the two variables can be observed Rho (238) = 0.443, p<0.001.

In other words, we observe that as patients get older, the probing depth increases, the severity of the diagnosis both globally and in terms of degree and stage increases, and the frequency of somatic conditions increases (see table 9).

There is also a significant positive association between the frequency of systemic diseases and the severity of the diagnosis taken as a whole, Rho (242) = 0.151, p < 0.05, and taken as a stage, Rho (242) = 0.199, p < 0.01. Thus, we can observe that as the severity of the diagnosis increases, the patient presents more comorbidities (fig. 5). As shown in figure 5, there is a correlation between the presence of systemic conditions, especially cardiovascular diseases, and the presence of local factors, namely dental plaque, tartar, dental crowding, and inadequate dental restorations.

Regarding smoking as a risk factor associated with periodontal disease, the post-hoc analysis Hochberg GT2 indicates that the non-smoker group has a statistically significantly lower severity of diagnosis (Mdif = -,81, p= 0.01), with a strong effect size (Cohen's d = 0.73). The group that smokes less than 10 cigarettes per day also has a significantly lower severity of diagnosis than the group that smokes 10-20 cigarettes per day (Mdif = -1. 13, p<.01), with a strong effect size (Cohen's d = .79). The group that smokes less than 10 cigarettes per day also has a statistically significantly lower severity level of diagnosis than the group that smokes more than 20 cigarettes per day (Mdif = -1.5, p <.001), with a very strong effect size (Cohen's d = 1.1). For the rest of the comparisons, no statistical significance was recorded, or statistically significant differences were observed. Therefore, based on a simple

ANOVA analysis, we can consider that non-smokers have a lower severity in terms of degree and stage of diagnosis than those who smoke more than 20 cigarettes per day.

The conclusions of this epidemiological study indicate that age, smoking, and systemic diseases are correlated with periodontal disease in Romanian patients, emphasizing the need for a strong interdisciplinary approach. By addressing these factors and encouraging cooperation among healthcare professionals, we can improve the management and prevention of periodontal disease, ultimately enhancing both oral and systemic health of patients.

Emerging evidence suggests that oral infections may be linked to various systemic diseases, although the exact mechanisms underlying these connections are not yet fully understood. Larger and longer-term randomized intervention studies are needed to shed more light on this matter. These findings carry important implications for the assessment, planning, and treatment of periodontal conditions as well as for the evaluation of the effectiveness of periodontal care.

Study 2 of the thesis aims to identify the Total Antioxidant Capacity (TAC) in saliva in a group of students undergoing stress during academic exams. Oxidative stress refers to the imbalance between the generation of reactive oxygen species (ROS) and the body's ability to counteract them with antioxidants. This imbalance within the human body represents a notable risk factor contributing significantly to the development of non-communicable diseases (169). Total Antioxidant Capacity (TAC), defined as the moles of oxidants neutralized per liter of solution, is a biomarker that measures the potential antioxidant capacity of fluids in the body (170*). In recent years, there has been a shift in focus among clinicians and researchers towards recognizing the importance of salivary antioxidant capacity as a crucial line of defense against chronic degenerative diseases*. Saliva, with its unique composition and properties, has gained recognition for its potential role in protecting against the harmful effects of oxidative stress. Understanding and harnessing the antioxidant capacity of saliva could provide promising avenues for preventing and treating chronic degenerative diseases (171). To analyze this important aspect for oral health, we monitored salivary TAC in a group of students at the Faculty of Dental Medicine, who are constantly subjected to periods of stress and who, in terms of age and living conditions, are a homogeneous working group. Test-related anxiety encompasses specific emotional and physiological emotions and disturbances evoked by the testing stimulus and

includes cognitive component responses (e.g., worry) and emotional and physiological arousal components (e.g., emotional excitement) (177). The Spielberger State-Trait Anxiety Inventory (STAI) was created by Spielberger et al. in 1970 to assess anxiety levels based on both temporary states and enduring traits. State measurement evaluates individuals' present moment anxiety levels, asking them to rate the intensity of their feelings on a four-point scale from "not at all" to "very much." On the other hand, trait anxiety measurement explores how individuals generally experience anxiety, assessing them on a four-point scale from "almost never" to "almost always" (178). The results of the study demonstrated correlations between individuals' stress conditions (STAI questionnaire), comorbidities (systemic diseases), and associated risk factors (smoking). A total of 80 fifth-year students from the Faculty of Dental Medicine at UMFTVB who agreed to participate in the study completed the STAI Questionnaire to assess stress during exams, the systemic health questionnaire, and smoking as a risk factor. They collected 3ml of saliva in tubes during the exams (in 2023). The students ranged in age from 20 to 53 years, with a mean age of M = 23.62 (±4.19). Pearson correlation results show a statistically significant negative result between the STAI test and TAC during the stress-free period (-0.02**, N=80, $p<0.01$). Additionally, a significant correlation of 0.02 (N=80, $p<0.05$) is observed between acute/chronic illness and STAI during the exam period. The results of this study show a significant positive correlation between the amount of tobacco consumed and the total STAI score during the stress-free period (0.03**, N=80), as shown in table 15. Furthermore, there is a statistically significant negative correlation between stress-free TAC and stress-free total STAI (-0.02*, N=80), as well as stress-free STAI total (-0.02*, N=80).

In conclusion, this study based on the STAI test and TAC recordings suggests the occurrence of changes in saliva antioxidant capacity under different stress conditions. Dental medicine students exhibited higher stress levels before academic evaluations compared to the stress-free period during the study. Since stress remains a concern even after graduation, these findings can help mitigate the impact of stress during the transition to professional life. Further research on larger samples of students is warranted to obtain more specific data regarding stress-related salivary biomarkers. Such studies would provide a deeper understanding of the practical utility of interpreting these biomarkers (195).

Study 3 of the thesis aims to investigate the modification of salivary pH according to stress conditions. Since salivary pH is regulated by the sympathetic and parasympathetic nervous systems, it can serve as a stress biomarker (177). Stress is an inherent part of human life and permeates nearly every human experience. Stressful encounters, as well as a person's psychological state and reactions to them, activate the hypothalamic-pituitary-adrenocortical (HPA) axis of the sympathetic nervous system, leading to the secretion of excessive levels of stress hormones, especially cortisol and catecholamines. Recently, salivary pH levels have been suggested as a potentially useful and inexpensive biomarker indicating levels of psychological stress (206). Salivary pH, or its degree of salivary acidity, maintains oral morphological elements in a state of equilibrium, with a pH of 7 being necessary for optimal functioning of various components (207). A decrease in oral pH may be responsible for disrupting other salivary stress biomarkers, such as cortisol (208), IgA (209), and salivary alpha-amylase. Therefore, pH may precede stress-induced disruption of salivary component levels measured previously as stress biomarkers. Additionally, salivary pH has been found to reliably reflect skin or urinary pH. Therefore, the use of salivary pH as a stress biomarker may be a response to the need to establish non-invasive and feasible methods for measuring physiological stress indicators.

The aim of this study was to determine whether salivary pH could be a stress biomarker, using stressful exams during academic activity. Specifically, based on the cognitive model of stress determination, the study attempted to determine:

1. The relationships between challenge and threat appraisals, experienced stress and test anxiety, and pH levels;
2. The predictive value of pH according to stress levels.

The results of the study demonstrate that by comparing the results obtained between the two pH measurements (stress/non-stress pH), it is observed that, overall, the mean values did not undergo significant changes. Specifically, during the stress-free period, the mean pH value recorded was 6.73, while during the stress period, the mean pH value was 6.58, as shown in Table 16. However, Pearson correlation results show a statistically significant correlation of 0.02 ($N=80$, $p<0.05$) observed between acute/chronic illness and STAI during the exam period. There is also a statistically

significant positive correlation between the presence of acute or chronic illness and salivary pH level 0.03** (N=80), p<0.01 during the exam period, as well as with the administration of any form of treatment 0.04** (N=80), p<0.01 (Table 17). The research results also show a statistically significant positive correlation between the amount of tobacco consumed and the total STAI score during the stress-free period (0.03**, N=80), as shown in Table 18. For all analyses, $p < 0.05$ was used to assess overall differences.

The results of this research are consistent with findings from other studies highlighting the dose-response relationship between perceived stress levels and salivary pH: as participants' stress levels increased, pH levels decreased. Furthermore, stress levels decreased and pH levels increased during the relaxation period (outside of exams) (195,222).

Study 4 of the thesis aims to investigate the correlation between salivary pH and Total Antioxidant Capacity (TAC) among dental students under various stress conditions. This study is based on the following hypotheses:

1. There are significant differences between TAC and pH levels before exposure to a stressor.
2. We hypothesize that both pH and TAC levels decrease following exposure to a stressor.
3. The physiological response of TAC and pH is positively correlated with an individual's self-perception of anxiety in anticipation of a potentially stressful situation or event.

Total Antioxidant Capacity (TAC), quantifying the number of moles of oxidants neutralized per liter of solution, serves as a biomarker assessing the antioxidant potential of body fluids (223). Numerous analyses detailing various tests commonly used to measure Total Antioxidant Capacity (TAC) have consistently shown a robust correlation between results obtained through different methods.

For the first hypothesis, paired-sample t-tests were used to test if there were significant mean differences between self-perceived anxiety scores, TAC, and pH results obtained before and after exposure to an experimental manipulation (administration of an exam). The results indicate significant mean differences only

before and after the administration of the exam regarding TAC measurements ($t(27) = 4.356$, $p < 0.001$). On average, individuals had a TAC score of $M = 3.62$ before the stressor application, while after the administration of the exam, the average TAC score was $M = 2.19$. Regarding other tests, there seems to be a mean difference between the pretest pH ($M = 6.62$) and posttest pH ($M = 6.73$); however, this difference is not statistically significant. Similarly, concerning the self-reported STAI, where the pretest STAI (considered as the sum of the two subscales constituting it) has an average of $M = 86.17$, while the posttest STAI has an average of $M = 79.35$. (Table 19).

Pearson correlation results show a statistically significant negative result in the relationship between STAI and TAC during the stress-free period (-0.02**, $N = 80$, $p < 0.01$). Additionally, a significant correlation of 0.02 ($N = 80$, $p < 0.05$) is observed between acute/chronic illness status and STAI during the examination period. There is also a statistically significant positive correlation between the presence of acute or chronic illness and pH level (0.03**, $N = 80$, $p < 0.01$) during the examination period, as well as with treatment administration (0.04**, $N = 80$, $p < 0.01$).

In conclusion, our study, based on STAI, TAC, and pH measurements, suggests the occurrence of changes in salivary antioxidant capacity under different stress conditions. Dental students exhibited higher stress levels before academic evaluations compared to the stress-free period during the course. Such studies would offer a deeper understanding of the practical utility of interpreting salivary biomarkers. Salivary biomarker studies are increasingly recognized as a practical and reliable tool for identifying oral indicators of systemic diseases and exposure to risk factors. Salivary constituents serve as a reflection of the overall body, earning them the reputation of being a "mirror of health." The widespread dissemination and increasing acceptance of saliva as a diagnostic tool have proven invaluable in supporting individuals, researchers, healthcare professionals, and community health programs in the more accurate detection and monitoring of diseases, ultimately contributing to improving the overall health of the population (195,222).