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Salivary Acidity as an Alternative Biosensor for Assessing Stress Levels

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ABSTRACT
Background: Stress affects physiological, psychological, and behavioral responses. Noninvasive and low-cost indicators may assist in the assessment of stress levels.
Objectives: This study aimed to determine the relationship between stress levels and salivary pH.
Methods: This analytical observational study used a cross-sectional design involving 81 final-year medical students at University X, selected through simple random sampling. Stress levels were measured using the stress subscale of the Depression, Anxiety and Stress Scale (DASS-42), and salivary pH was measured using a pH meter. Data were analyzed using the Spearman correlation test.
Results: Stress levels were normal in 48 respondents (59.26%), mild in 19 (23.46%), moderate in 11 (13.58%), and severe in 3 (3.70%); no respondents had very severe stress. Mean salivary pH was 7.18 ± 0.34. Mean pH values were 7.27, 7.10, 6.99, and 6.93 in the normal, mild, moderate, and severe stress groups, respectively. Spearman analysis showed a significant association between stress levels and salivary pH ($p < 0.001$).
Conclusion: Higher stress levels were associated with lower salivary pH among the respondents. These findings suggest the potential of salivary pH as an indicator of stress, although further validation is needed.

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INTRODUCTION

American Psychiatric Association (APA) defines stress as the physiological or psychological response to internal or external stressors. Stress involves changes affecting nearly every system of the body, influencing how people feel and behave. It may be manifested by some symptoms: palpitations, sweating, dry mouth, shortness of breath, fidgeting, accelerated speech, augmentation of negative emotions, and longer duration of stress fatigue. Severe stress is manifested by the general adaptation syndrome. By causing these mind - body changes, stress contributes directly to psychological and physiological disorders, affects mental and physical health, and reducing quality of life.[1], 2

Stress is any event or incident, whether in the form of external demands (environmental) or internal demands (physio and psychological), that requires, burdens, and exceeds an individual's capacity.[3] Stress is a condition that can be experienced by anyone. The Ministry of Health states that 17.[4] million Indonesians, or about 11.[4] percent of the Indonesian population experience stress. According to WHO, this number has increased to 6.[26]% of the population in 2020.[4], 5, 6

Stress can arise due to a gap between the demands that must be met by an individual and the biological, psychological, or social resources that the individual possesses.[7] This can affect an individual's cognition, emotions, and behavior, reduce immunity, lower concentration, and even cause biological changes in the body.[8], 9

Stress itself can be explained through psychophysiological mechanisms that occur when an individual experiences stressful conditions. When stressed, the brain activates the Corticotropin Releasing Factor (CRF), particularly in the Hypothalamic - Pituitary - Adrenal axis (HPA). The hypo-thalamus will activate Sympathetic Nervous System (SNS) to release adrenaline and cortisol hormones, leading to an imbalance between SNS and Parasympathetic Nervous System (PNS).[10], 11

Changes in the mechanisms of body organs caused by stress conditions emphasize the importance for individuals to recognize stress indicators in the body, enabling them to manage stress conditions more effectively.[3] Biomarker is an indicator for measuring and detecting pathogenic factors and assessing biological conditions. In contrast, biosensor is a biological device used for various purposes, such as rapid detection and traceability of pathogens.[12]

Some hormonal markers as a biomarker for stress. Cortisol, often called the “stress hormone,” is a primary indicator for assessing stress levels. Other hormones are adrenocorticotropic hormone (ACTH), dehydroepiandrosterone (DHEA), and catecholamines include epinephrine and norepinephrine.[13,14] As an alternative, alpha-amylase, salivary profile like volume, electrolyte content, and salivary acidity or power of Hydrogen (pH) can serve as a biosensor to predict stress levels.[15,16] Compared to other parameters or chemical biosensors, measuring pH from saliva has the advantage of being a more non-invasive method and low-cost, making it a potential preferred solution in the future.

METHOD

Study design

This analytical observational study used a cross-sectional design to examine the relationship between stress levels and salivary pH among final-year students at the Faculty of Medicine, University X, who were about to take the exit examination.

[Author confirmation required: provide the institution’s full name, study location, and study dates.]

Population and sample

The study population comprised final-year medical students at University X. A total of 81 respondents were selected using simple random sampling.

[Author confirmation required: provide the population size, eligibility criteria, sample-size justification, and random-selection procedure.]

Data collection

Stress levels were measured using the Depression, Anxiety and Stress Scale (DASS-42), while salivary pH was measured using a digital pH meter.

[Author confirmation required: specify the DASS-42 language version, stress-subscale scoring and cutoffs, saliva-collection protocol, collection time, precollection restrictions, pH-meter model, calibration procedure, and control of potential confounders.]

Data analysis

Data were analyzed using SPSS. The Kolmogorov–Smirnov test was used to assess normality. Because the data were not normally distributed, the association between stress levels and salivary pH was tested using Spearman correlation.

[Author confirmation required: provide the SPSS version, prespecified significance level, Spearman correlation coefficient, and confidence interval if available.]

Ethical statement

[Author confirmation required: provide the ethics committee name, approval number and date, informed-consent procedure, and confidentiality protections. These details were not reported in the source manuscript.]

RESULTS

The study included 81 respondents. Their characteristics are presented in Table 1.

Table 1. Distribution of sample characteristics

Characteristic	n	Percentage (%)	Mean ± SD
Sex			
Male	32	39.51	
Female	49	60.49	
Age (years)			20.58 ± 0.76
18	2	2.47	
19	1	1.23	
20	32	39.51	
21	40	49.38	
22	6	7.41	

SD: Standard deviation. Percentages were recalculated from the reported counts (N = 81).

The mean stress score was 13.^{06}, within the normal range reported in the source manuscript (0–14). Most respondents had normal stress levels (59.^{26}%). Among respondents with elevated stress, mild stress was the most frequent category (23.^{46}%), followed by moderate and severe stress (Table 2).

Table 2. Distribution of stress levels

Stress level	n	Percentage (%)
Normal	48	59.26
Mild	19	23.46
Moderate	11	13.58
Severe	3	3.70
Very severe	0	0.00
Total	81	100.00

Based on the results of saliva pH measurement using a digital pH meter, the respondents' saliva pH in this study was 7.^{18}, indicating it is still within the normal saliva pH range (6.$^{20\text{-}7}$.^{60}).^{17} From Table 3, it is found that higher stress levels correlate with a decrease in saliva pH.

Table 3. Mean salivary pH by stress level

Stress level	Salivary pH mean ± SD	n
Normal	7.27 ± 0.31	48
Mild	7.10 ± 0.32	19
Moderate	6.99 ± 0.30	11
Severe	6.93 ± 0.55	3
Very severe	—	0
Total	7.18 ± 0.34	81

SD: Standard deviation. No respondents were classified as having very severe stress.

The results of measuring stress levels and salivary pH were subsequently analyzed statistically using SPSS. The normality test of the data with the Kolmogorov-Smirnov test showed that the data were not normally distributed for both stress levels ($p = 0.003$) and salivary pH ($p = 0.001$). The results of the non parametric correlation test using Spearman correlation test were found to be significant ($p < 0.001$).

DISCUSSION

A previous study reported a relationship between reduced saliva volume during stress and a decrease in saliva pH, making it more acidic in normal students induced with stress by exams.[16] In this study, we collected students with varying levels of stress (normal, mild, moderate to severe stress), already determined with the DASS-42 instrument, and found differences in saliva pH among these groups.

In normal individuals or those not under stress, impulses are carried from the salivary nuclei in the medulla oblongata, which regulate saliva secretion, and are then transmitted to the salivary glands by the parasympathetic nervous system, which is more dominant. The acetylcholine, neurotransmitter carried by the parasympathetic nervous system, causes vasodilation of the blood vessels in the salivary glands, allowing the glands to obtain adequate organic and inorganic materials from the blood to increase saliva secretion. This leads to increased saliva flow and higher electrolyte concentration in the saliva secreted by the glands.[18], 19

The release of the neurotransmitter acetylcholine not only widens the blood flow to the salivary glands but also triggers the release of Cyclic Adenosine Monophosphate (cAMP) and increases the mobilization of calcium ions (Ca) into the acinar cells. The rise in Ca2+ within the acinar cells causes the opening of potassium (K) and sodium (Na) ion channels, allowing the movement of Na+, followed by water into the lumen. The result of this acinar cell secretion is called primary saliva, which is hypotonic and nearly identical to extracellular fluid.[18]

After the primary saliva reaches the acinar duct and salivary duct, two main active transport processes occur that further modify the ion composition of the saliva. In the first process, all Na+ is actively reabsorbed, and K+ is actively secreted to replace the sodium lost in the lumen, resulting in a significant decrease in the sodium concentration in saliva while potassium increases. However, sodium reabsorption in the duct lumen is much greater than potassium secretion, causing a negativity inside the duct lumen. To balance this negativity, a cotransport process occurs that leads to chloride (Cl) ions being reabsorbed while a large amount of HCO3- is secreted by the duct epithelium into the duct lumen. Therefore, when reaching the oral cavity under normal conditions, secondary or final saliva is more isotonic, having high concentrations of K+ and HCO3-, while containing low levels of Na+ and Cl-.[18]

In stressful conditions, there is an increased influence of impulses from the sympathetic nervous system, which causes the main concentration of salivary gland secretion to consist of proteins, while the total saliva volume is lower compared to normal conditions, where impulses from the parasympathetic nervous system are more dominant. This is in line with other research on saliva volume, which found that the higher the stress level, the less saliva volume is produced.[18]

In addition to playing a role in protein secretion and the volume of saliva produced, an increase in the neurotransmitter norepinephrine can also affect the concentration of electrolytes present in saliva. Norepinephrine causes vasoconstriction of the blood vessels supplying the salivary glands. As a result, there is

a decrease in the amount of water and Na+ transported to the lumen of the acinar and salivary ducts. The decrease in water and Na+ concentration in primary saliva causes a reduction in the cotransport process of Na+ with K+ and HCO3-, resulting in a final saliva that is low in K+ and HCO3-. This leads to changes in the buffering capacity of saliva, primarily due to a decrease in HCO3- as the main electrolyte. The reduction of HCO3- will eventually cause the pH of saliva to decrease.[18], 20

The electrolyte composition of saliva is also related to the rate of saliva secretion. At low flow rates, saliva tends to have low concentrations of Na+ and Cl-, while the concentrations of K+ and HCO3- are high. In contrast, at high saliva flow rates, the secreted saliva contains high concentrations of Na+ and Cl- and low concentrations of HCO3- and K+. This variation in saliva composition ultimately also affects the pH of saliva. Although impulses carried by the sympathetic nerves produce a smaller saliva flow compared to impulses carried by the parasympathetic nerves, sympathetic nerve impulses can also cause an increase in saliva flow. The increase in saliva flow rate causes the process of electrolyte reabsorption and secretion along the acinar and salivary ducts to become imperfect, resulting in a decrease in the concentration of HCO3- secreted into the final saliva.[18], 20

In previous experimental research conducted by measuring saliva pH during the induction of stressors and after the stressor induction ended, the results supported this study. The treatment group had a saliva pH of 6.[95] when the stressor was induced, whereas when the stressor induction ended, the treatment group's saliva pH gradually increased to 7.[41].[16], 21 This is also consistent with Table 3, where research respondents with lower saliva pH were found to have higher stress levels, while respondents with higher saliva pH were found to have lower stress levels and tend toward normal.

[Author confirmation required: review the physiological account of primary and final saliva tonicity and the relationship between flow rate, bicarbonate concentration, and salivary pH. Clarify the distinction between a biomarker and a biosensor, verify the prevalence figures in the Introduction, and add study limitations and relevant Sustainable Development Goal implications.]

CONCLUSION

Higher stress levels were significantly associated with lower salivary pH among the final-year medical students studied. Salivary pH may have potential as a noninvasive indicator of stress; further studies are needed to establish its validity and diagnostic performance before routine use.

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LIST OF ABBREVIATIONS

ACTH: Adrenocorticotropic hormone; APA: American Psychological Association; cAMP: Cyclic adenosine monophosphate; CRF: Corticotropin-releasing factor; DASS-42: Depression, Anxiety and Stress Scale, 42-item version; DHEA: Dehydroepiandrosterone; HPA: Hypothalamic–pituitary–adrenal; pH: Potential of hydrogen; PNS: Parasympathetic nervous system; SD: Standard deviation; SNS: Sympathetic nervous system; SPSS: Statistical Package for the Social Sciences; WHO: World Health Organization.

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