

## **Exploring Effective Biomarkers as Early Warnings of Stress and Fatigue**

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## **Abstract**

Background: Many college students suffer from mental disorders, affecting academic performance and increasing dropout rates. Hence, assessing students' mental health is crucial. Alpha-amylase (sAA), lysozyme (LZM), and melatonin (MT), which are considered potential biomarkers due to their association with stress, depression, and anxiety, serve as indicators of these conditions. Current study has evaluated the diurnal patterns of sAA, LZM, and MT in saliva from undergraduates, to explore more objective, noninvasive diagnostic methods for mental disorders. Methods: (1) 637 undergraduates completed questionnaires including the Perceived Stress Scale (PSS), Fatigue Severity Scale (FSS), and Pittsburgh Sleep Quality Index (PSQI), along with a demographic survey. (2) 18 participants formed the control group (Con), and 15 formed the experimental group (Exp.), for saliva collection. The standard scores of Con group were PSS \( \frac{2}{2} \)8, PSQI \( \frac{5}{2} \)5, and FSS \( \frac{4}{2} \)0, which indicated the health condition of the Con group students; while for Exp. group were PSS≥57 or PSQI≥5 or FSS≥5. (3) Saliva samples were collected at various times: 9:00, 9:30, 10:00, 11:00, 12:00, 14:00, 16:00, 17:00, 18:00, 20:00, and 23:00. (4) sAA and LZM concentrations were measured using Sandwich-ELISA, and MT activity was assessed with Competitive-ELISA. (5) Data were analyzed using Two-way Repeated-Measures ANOVA and One-way ANOVA. Results: (1) PSS scores were positively correlated with PSQI (r=0.35) and FSS (r=0.41); FSS was also highly correlated with PSQI (r=0.37). (2) Significant differences in sAA activity were noted between Con and Exp groups at awakening time, with higher sAA levels in Exp group at 9:30, 10:00, and 11:00. (3) LZM activity differed significantly between groups, with higher LZM levels in Con group at 17:00, 18:00, 20:00, and 23:00. (4) MT activity differed between groups at awakening time, but no significant differences were found. (5) sAA and LZM activity showed gender-specific differences at certain times. Conclusions: (1) College students face significant stress, necessitating careful mental health monitoring. (2) Diurnal profiles of sAA, LZM, and MT in different stress levels were established. (3) Salivary sAA and LZM at specific times could be potential biomarkers for stress and fatigue. (4) Gender-specific patterns in sAA and LZM activity were observed.

## **Keywords**

Stress, Fatigue, Sleep Quality, A-Amylase, Melatonin, Lysozyme

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