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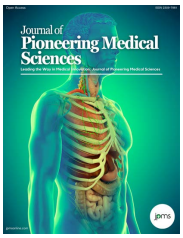

Assessment of Mental Health Status and its Association with Cortisol Levels in Cigarette Smokers and Non-smokers

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Abstract **Objectives:** Mental health status profoundly influences an individual's core being, affecting thought processes, perceptions and consciousness. Increased cortisol secretion under stressful situations can impair brain function and overall health. Smoking, often perceived as a coping mechanism for stress, may exacerbate stress and mental health deterioration. **Aim:** This study aims to assess and compare cortisol levels in smokers and non-smokers and investigate the association between elevated cortisol levels and mental health status. **Methods:** A cross-sectional study was conducted with a sample size of 320 participants, comprising 160 smokers and 160 non-smokers. Saliva samples were collected and analyzed using the enzyme-linked immunosorbent assay (ELISA) to measure cortisol levels. The Mini Mental State Examination (MMSE) was administered to evaluate participants' mental health status. Statistical analyses were performed to examine the relationships between smoking, cortisol levels and mental health outcomes. **Results:** Smokers exhibited significantly higher cortisol levels (59.88 ± 30.84 ng/ml) compared to non-smokers (47.09 ± 19.57 ng/ml). A drowsy mental health status was observed in 24.69% (79) of smokers and 6.56% (21) of non-smokers. Elevated cortisol levels correlated with chronic stress and reduced mental alertness, particularly among smokers. **Conclusion:** Smokers demonstrated heightened cortisol levels, indicative of a chronic stress state and compromised mental health. In contrast, non-smokers showed lower cortisol levels and better mental health status. These findings highlight the role of cortisol as a stress biomarker and underscore the need for targeted smoking cessation interventions to mitigate stress and its mental health consequences. Future research should explore socioeconomic factors, alternative stress-coping mechanisms and longitudinal impacts of smoking on cortisol regulation.

Key Words Smoking, non-smoking, cortisol, stress biomarkers, mental health, nicotine addiction

INTRODUCTION

Mental health is a vital component of overall well-being, encompassing emotional, psychological and social factors. It significantly influences daily life, relationships and physical health [1]. The prevalence of mental health issues among smokers is an important public health concern, given their higher vulnerability to conditions such as depression, anxiety and chronic stress. Smoking is often perceived as a stress-relieving mechanism; however, it paradoxically exacerbates stress and dysregulates mental health over time.

Cortisol, commonly referred to as the "stress hormone," is produced by the adrenal glands in response to stress and plays a key role in the hypothalamic-pituitary-adrenal (HPA) axis [2]. Its release is regulated by circadian rhythms and directly influenced by adrenocorticotropic hormone (ACTH) and corticotropin-releasing hormone (CRH) [3]. Under normal conditions, cortisol levels peak in the morning and decline throughout the day. Chronic stress, anxiety, or depression can disrupt this rhythm, leading to dysregulated cortisol levels and adverse effects on brain function and health [4].

The Mini Mental State Examination (MMSE) is a widely used tool for assessing mental health and cognitive performance. It evaluates five key domains: orientation, registration, attention and calculation, recall and language. The MMSE is valuable for distinguishing cognitive

impairment and monitoring changes over time. However, it relies primarily on verbal and written responses, which may limit its scope in diagnosing specific cognitive dysfunctions [5]. Additionally, the MMSE should be used alongside comprehensive clinical assessments for accurate mental health evaluation.

Smoking, as a coping mechanism for stress, introduces a paradox. While nicotine temporarily reduces stress, withdrawal or the constant need for nicotine intake triggers physiological stress responses, leading to elevated cortisol levels [6,7]. Smokers may display both blunted and heightened cortisol responses due to the combined effects of nicotine dependence and withdrawal stress. This dysregulation of the HPA axis contributes to a heightened state of arousal and stress, worsening mental health conditions over time [8].

Non-smokers, on the other hand, generally experience more stable cortisol levels and mental health status [9]. Their HPA axis demonstrates better balance in response to daily stressors, offering some protection against stress-related mental health disorders. However, other factors, such as lifestyle, environmental stressors and pre-existing conditions, can also influence their cortisol levels [10]. Comparing cortisol regulation in smokers and non-smokers offers valuable insights into how smoking amplifies stress and deteriorates mental health.

Smokers often find themselves trapped in a vicious cycle where stress leads to smoking and smoking further dysregulates their stress-response system [11]. The disruption of natural cortisol regulation in smokers leads to acute and chronic changes that negatively affect mood and stress levels [12]. Examining the relationship between cortisol levels and mental health status in smokers and non-smokers is essential to understanding the physiological mechanisms through which smoking impacts mental health.

While prior research has largely focused on the general relationship between smoking and mental health, this study aims to address gaps by specifically examining nuanced differences in mental health status and cortisol levels between smokers and non-smokers. Given the complex interplay between smoking, stress and mental health, understanding this relationship can inform more effective treatment approaches, public health strategies and interventions for smoking cessation.

Aim

The study aims to assess cortisol levels in smokers and non-smokers and investigate whether higher cortisol levels are associated with mental health status.

METHODS

A cross-sectional study was conducted among outpatients attending a dental college in Chennai to assess cortisol levels and mental health status in smokers and non-smokers. The study adhered to ethical guidelines and informed consent was obtained from all participants. Ethical approval was granted by the institutional ethics committee, ensuring participant confidentiality and protection throughout the study.

Study Design and Sample Selection

The study included a total of 320 participants, divided equally into two groups: 160 smokers and 160 non-smokers. The sample size was determined based on findings from a pilot study to ensure adequate power for statistical analysis. Participants were selected through stratified random sampling, ensuring representation across various demographic and socioeconomic backgrounds. Inclusion criteria required participants to be 18 years or older, with no history of major psychiatric or neurological disorders. Exclusion criteria included individuals using medications that affect cortisol levels, such as corticosteroids, or with known endocrine disorders.

Data Collection

Data collection included two primary components: mental health assessment and saliva sample analysis.

- **Mental Health Assessment:** The Mini Mental State Examination (MMSE) was administered to each participant to evaluate cognitive and mental health status. This standardized tool assessed five key domains: orientation, registration, attention and calculation, recall and language. Scores were interpreted according to established guidelines to classify participants as either alert or drowsy. Although MMSE is effective for cognitive screening, it was supplemented with additional participant interviews to capture broader mental health insights and minimize reliance on verbal or written responses alone.
- **Saliva Sample Collection:** Saliva samples were collected in sterile containers between 9:00 AM and 11:00 AM to control for diurnal variations in cortisol levels (4). Participants were instructed to avoid eating, drinking (except water), smoking, or engaging in vigorous physical activity for at least one hour prior to sample collection. Samples were immediately stored at -20 °C and later processed in the laboratory.

Assay Procedure

Saliva samples were analyzed using enzyme-linked immunosorbent assay (ELISA) to measure cortisol levels:

- **Reagent Preparation:** All reagents were prepared in accordance with the manufacturer's instructions.
- **Standard and Sample Addition:** Standards and samples were added in duplicate to the microplate. Standard wells received 50 µL of diluted standard solution, while test wells were prepared with 40 µL of sample diluent and 10 µL of saliva sample. Blank wells remained untreated.

- **Incubation and Washing:** The plate was incubated at 37°C for 45 minutes, followed by washing each well five times with 250 µL of wash buffer. Residual buffer was removed by aspiration and blotting.
- **Detection Antibody:** A 50 µL solution of HRP-conjugated detection antibody was added to each well, except blanks and the plate was incubated at 37 °C for 30 minutes.
- **Color Development:** After additional washing, 50 µL of Chromogen Solution A and 50 µL of Chromogen Solution B were added to each well. The plate was incubated in the dark at 37°C for 15 minutes.
- **Stop Solution:** A 50 µL stop solution was added, changing the color from blue to yellow. Plates were gently tapped to mix and optical density (O.D.) was measured at 450 nm using a microplate reader within 15 minutes.

Statistical Analysis

Statistical analyses were conducted using SPSS software. The Shapiro-Wilk Test assessed the normality of data distribution, revealing a non-normal distribution. Mann-Whitney U-Test was used to compare cortisol levels between smokers and non-smokers and examine differences in mental health status. Chi-Square Test assessed associations between smoking status and mental health (alert vs. drowsy).

RESULTS

Mental Health Status

The analysis of mental health status revealed significant differences in alertness between smokers and non-smokers. As shown in the pie chart (Figure 1), the majority of participants (68.75%) were categorized as "Alert," while a smaller but significant proportion (31.25%) were categorized as "Drowsy." The bar graph (Figure 2) further highlights differences in mental health status between smokers and non-smokers. Among smokers, the distribution of alertness was nearly balanced, with 25.31% (81 individuals) classified as "Alert" and 24.69% (79 individuals) as "Drowsy." In contrast, non-smokers exhibited predominantly higher levels of alertness, with 43.44% (139 individuals) categorized as "Alert" and only 6.56% (21 individuals) as "Drowsy."

These findings indicate that non-smokers are more likely to maintain mental alertness, while smokers demonstrate a higher likelihood of experiencing drowsiness or reduced alertness. The chi-square test confirmed a statistically significant association between smoking status and mental health status (Chi-square value = 48.931, $p<0.001$). This suggests that smoking may negatively impact mental alertness, contributing to higher rates of drowsiness.

Cortisol Levels in Smokers and Non-Smokers

The comparison of cortisol levels between smokers and non-smokers revealed substantial differences. As detailed in Table 1, the mean cortisol level among smokers was

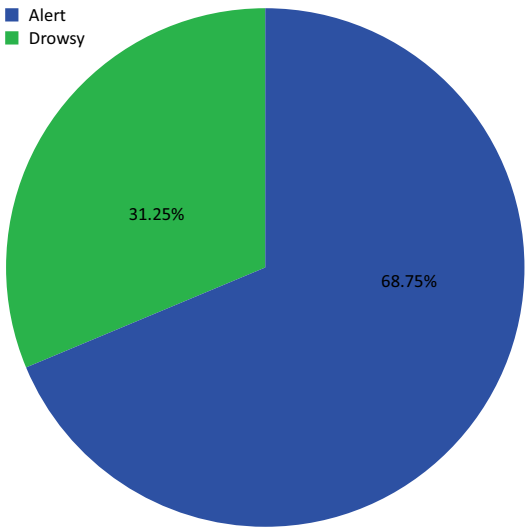

Figure 1: Mental health status of study participants

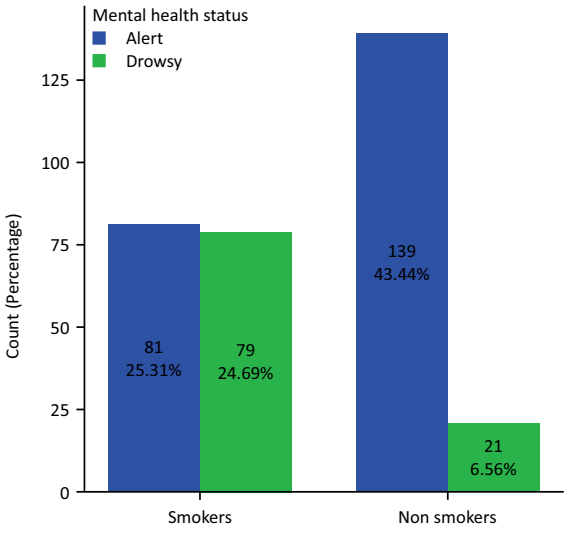

Figure 2: Bar chart showing mental health status of smokers and non smokers

significantly higher (M = 59.88, SD = 30.84) compared to non-smokers (M = 47.09, SD = 19.57). These findings suggest that smoking is associated with elevated cortisol levels, reflecting a heightened stress response. Additionally, the standard deviation for smokers was notably larger than that for non-smokers, indicating greater variability in cortisol levels within the smoking group. This variability may be influenced by additional factors, such as smoking frequency, duration, or withdrawal effects.

The standard error of the mean (SEM) was higher for smokers (SEM = 2.44) than for non-smokers (SEM = 1.55), suggesting that the cortisol level estimates were more precise in the non-smoking group. These results highlight the role of smoking in contributing to dysregulated stress responses as measured by cortisol.

Table 1: Cortisol levels among smokers and non smokers

	Cortisol levels (ng/mL)			
	N	Mean	Standard Deviation	Standard Error Mean
Smokers	160	59.8771	30.84336	2.43838
Non smokers	160	47.0912	19.56951	1.54711

Table 2: Mann Whitney U Test Results Regarding the cortisol levels among smokers and nonsmokers

	Cortisol levels				
	N	Mean Rank	U	Z	P
Smokers	160	183.10	16416		0.000
Non smokers	160	137.90			

Table 3: Mann Whitney U Test Results regarding the cortisol levels and mental health status

	Cortisol levels				
	N	Mean Rank	U	Z	P
Alert	220	171.68	13590	-3.209	0.001
Drowsy	100	135.9			

Statistical Analysis of Cortisol Levels

Statistical tests confirmed the significant differences in cortisol levels between smokers and non-smokers. The Mann-Whitney U test (Table 2) indicated a statistically significant difference in cortisol levels between the two groups ($U = 16416$, $Z = -4.00$, $p<0.001$). Smokers had a higher mean rank (Mean Rank = 183.10) compared to non-smokers (Mean Rank = 137.90), reinforcing the link between smoking and elevated cortisol levels.

A second Mann-Whitney U test examined the relationship between cortisol levels and mental health status (Table 3). Results demonstrated a significant difference in cortisol levels between individuals classified as "Alert" and "Drowsy" ($U = 13590$, $Z = -3.209$, $p = 0.001$). The mean rank of cortisol levels was higher in the "Alert" group (Mean Rank = 171.68) compared to the "Drowsy" group (Mean Rank = 135.90). These findings suggest that individuals in an alert mental state tend to have higher cortisol levels, potentially reflecting better stress regulation or heightened physiological responses to stress.

DISCUSSION

The assessment of mental health status and its association with cortisol in smokers and non-smokers offers significant insights into the physiological and psychological impact of smoking. Smoking's complex interaction with mental health and stress regulation, mediated through cortisol, underscores the paradoxical effects of nicotine. While nicotine is often perceived as a stress-relieving agent, chronic smoking disrupts the hypothalamic-pituitary-adrenal (HPA) axis, leading to dysregulated cortisol levels and heightened physiological stress responses [13,14].

The findings of this study align with prior research showing that smokers exhibit significantly higher cortisol levels than non-smokers, reflecting heightened stress responses [15]. As observed in Parriot *et al.*, elevated baseline cortisol levels among smokers suggest that smoking acts as a chronic stressor, creating a cycle where stress induces smoking and smoking exacerbates stress [16-18]. This physiological dysregulation contributes to mental health challenges such as reduced alertness and increased drowsiness, as evidenced by this study.

Non-smokers, in contrast, displayed more stable cortisol regulation and fewer mental health issues, consistent with findings from Audrain *et al.* [19]. This suggests that their HPA axis functions more effectively, contributing to better stress resilience and mental well-being. Furthermore, Hahad *et al.* [20] and Diaz-Martinez *et al.* [21] findings on cortisol awakening response (CAR) highlight the exaggerated stress reactivity in smokers, which further exacerbates their mental health issues throughout the day.

CONCLUSION

This study reveals a significant association between smoking and elevated cortisol levels, underscoring the role of smoking in exacerbating stress and mental health deterioration. Smokers exhibited higher cortisol levels and increased rates of mental health challenges, such as reduced alertness and heightened drowsiness, compared to non-smokers. These findings emphasize that smoking, rather than alleviating stress, perpetuates a cycle of nicotine dependence and chronic stress. Non-smokers, by contrast, demonstrated better-regulated cortisol levels and superior mental health status, likely due to more effective HPA axis functioning. This underscores the physiological benefits of avoiding smoking and maintaining healthier stress-regulation mechanisms. Future research should focus on longitudinal designs, incorporating external factors like socioeconomic status and genetic predispositions, to better understand the relationship between cortisol, smoking and mental health. Additionally, public health policies should prioritize smoking cessation programs that include targeted stress management strategies to improve mental health outcomes in smokers.

Limitations and Future Directions

Several limitations need to be addressed. Cortisol levels vary significantly due to daily fluctuations and external factors such as diet, sleep and environmental stressors. While sample collection times were standardized, these external variables were not fully controlled, potentially influencing the findings. The study's cross-sectional design limits causal inferences. Longitudinal studies could provide a more comprehensive understanding of the dynamic changes in cortisol and mental health among smokers and non-smokers over time.

The study also did not account for factors such as socioeconomic status, smoking frequency, genetic predisposition and comorbid conditions, which may influence the results. Future research should explore these variables and their interaction with cortisol regulation. Additionally, cultural and gender differences in stress responses among smokers warrant further investigation. Exploring alternative stress-coping mechanisms and their efficacy could also inform smoking cessation interventions.

These findings highlight the critical need for integrated public health programs addressing both the physiological and psychological components of smoking. Smoking cessation initiatives should incorporate stress management techniques and leverage biomarkers like cortisol to monitor stress responses and recovery post-cessation.

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Conflict of Interest

The authors declare no conflicts of interest related to this study. All efforts were made to ensure unbiased analysis and interpretation of the findings.

Financial Consideration

This research was conducted without any external financial support or funding. All resources were provided by the affiliated institutions. The absence of external funding ensures the study's independence and objectivity.

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