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Salivary Cortisol and Stress Levels in the Malayali Tribe of Thiruvannamalai: A Comparative In Vitro Study with Non-Tribal Groups

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Abstract **Introduction:** Stress is a significant contributor to various health disorders, often linked to elevated cortisol levels mediated by the hypothalamic-pituitary-adrenal (HPA) axis. Salivary cortisol measurement is a reliable, non-invasive biomarker for assessing stress. This study aimed to compare salivary cortisol levels between the Malayali tribal population in Thiruvannamalai, Tamil Nadu and a non-tribal urban control group to evaluate stress differences and explore potential socio-environmental influences on cortisol levels. **Methods:** An in-vitro study was conducted between July and August 2024, involving 44 participants divided into two groups: Malayali tribes (n = 22) and non-tribal individuals (n = 22). Saliva samples were collected using the passive drool technique and analyzed using the Abbkine Human Cortisol ELISA Kit. Environmental and lifestyle factors, including dietary habits and occupational stress, were recorded to assess potential confounders. The Mann-Whitney U test was used for statistical analysis, with significance set at $p<0.05$. **Results:** The Malayali tribal group exhibited significantly higher mean salivary cortisol levels (13.44±2.15 µg/L) compared to the non-tribal group (8.73±1.85 µg/L) ($p<0.05$). Optical density values were also notably higher in the tribal group, reinforcing the elevated cortisol concentration. The ELISA assay demonstrated a non-linear relationship between optical density and cortisol concentration. The findings suggest that socio-economic challenges, environmental hardships and limited healthcare access may contribute to heightened stress levels in the tribal population. **Conclusion:** The study revealed significantly higher stress levels in the Malayali tribal population than in their non-tribal counterparts, likely driven by socio-environmental stressors. These results emphasize the urgent need for targeted interventions such as culturally adapted stress management programs, improved healthcare access and educational initiatives to mitigate chronic stress in tribal communities. Future research should explore broader sample sizes, longitudinal stress assessments and the role of cultural practices in influencing stress responses.

Key Words Stress, salivary cortisol, Malayali tribe, ELISA, biomarkers, socio-environmental stressors, stress management

INTRODUCTION

Background

Stress is a universal biological and psychological response triggered by external stressors. While acute stress can be adaptive by enhancing focus and improving performance, prolonged or chronic stress is linked to serious health risks, including cardiovascular diseases, diabetes, anxiety, depression and immune dysfunction [1].

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in the body's stress response by regulating cortisol secretion. Cortisol, a vital stress hormone produced by the adrenal glands, supports metabolic processes, immune responses and coping mechanisms during challenging situations. However, when cortisol secretion becomes prolonged or dysregulated due to chronic stress, it can disrupt normal physiological processes and contribute to various health disorders [2,3].

Salivary cortisol measurement has gained prominence as a non-invasive and practical method for assessing stress. Unlike blood sampling, salivary cortisol accurately reflects the biologically active portion of cortisol in circulation, offering a more precise indicator of stress-related hormonal

activity. Additionally, salivary cortisol levels are less affected by external factors such as invasive procedures, making this method ideal for field studies and population research [4-6].

The Malayali tribe, predominantly residing in the Thiruvannamalai district of Tamil Nadu, is one of India's many indigenous communities. The tribe maintains a traditional lifestyle centered around agriculture, forest-based work and handicrafts. While this cultural heritage remains rich and resilient, Malayali tribal populations face multiple socio-environmental challenges such as limited healthcare access, economic insecurity and environmental instability [7]. These adversities, coupled with a reliance on natural resources for livelihood, may increase their susceptibility to chronic stress. Despite these concerns, the physiological impact of stress in this population, particularly using objective biomarkers like cortisol, remains largely unexplored [8].

Conversely, non-tribal urban populations experience stress through distinct pathways, including occupational pressures, social demands and fast-paced lifestyles. Factors such as long working hours, financial burdens and health anxieties shape their stress profiles. The influence of these stressors may produce cortisol responses distinct from those seen in tribal populations [9].

Given the stark differences in socio-economic conditions, lifestyle practices and environmental stressors between these two groups, understanding cortisol levels in both populations is crucial to evaluating stress disparities and related health risks.

Rationale and Knowledge Gap

Despite growing awareness of stress-related health risks, the physiological impact of stress in tribal communities remains under-researched. While previous studies have explored social and behavioral aspects of stress in tribal populations, there is limited evidence involving objective biomarkers such as salivary cortisol to assess their physiological stress responses.

Existing research on stress physiology has predominantly focused on urban populations, often overlooking the environmental, economic and cultural factors unique to tribal groups [10,11]. Consequently, there is a significant gap in understanding how socio-environmental stressors impact cortisol secretion patterns in indigenous populations like the Malayali tribe.

Chronic stress is known to dysregulate cortisol secretion patterns, often manifesting as a flattened Cortisol Awakening Response (CAR) and an altered diurnal cortisol rhythm. Such disruptions are well-documented in urban populations but remain underexplored in tribal communities [12-14]. Exploring these cortisol patterns in tribal populations may provide critical insights into stress-related health disparities and highlight the need for targeted interventions.

Additionally, few comparative studies have evaluated cortisol levels between tribal and non-tribal groups. Since environmental conditions, socio-economic challenges and cultural practices may vary greatly between these populations, a comparative analysis can enhance understanding of stress physiology across different contexts. By investigating these differences, this study aims to bridge the existing knowledge gap and contribute to the development of tailored strategies to mitigate stress-related health risks in vulnerable populations. Such insights are vital for informing healthcare policies and promoting culturally appropriate stress management interventions [15].

Aim and Objectives

The primary aim of this study was to assess and compare salivary cortisol levels as a biomarker of stress in Malayali tribal individuals and a non-tribal control group in Thiruvannamalai, Tamil Nadu.

The study tested the following hypotheses:

- **Null Hypothesis (H):** There is no significant difference in salivary cortisol levels between the Malayali tribal population and the non-tribal control group.
- **Alternative Hypothesis (H):** There is a significant difference in salivary cortisol levels between the Malayali tribal population and the non-tribal control group.

By conducting this comparative analysis, the study aimed to provide valuable insights into the impact of socio-environmental stressors on stress physiology in these populations, potentially guiding targeted interventions to improve community health outcomes.

METHODS

An *in-vitro* study was conducted among Malayali tribal individuals residing in the Javvadhu Hills of Thiruvannamalai district, Tamil Nadu, between July and August 2024. The study included a total sample size of 44 participants (n = 22 per group), calculated using G*Power software version 3.1.9.7 with a 95% power level, based on data from a previous study by Chellappa *et al.* [16].

Study Population and Sampling

The participants were divided into two groups:

- **Group I:** Malayali tribe members from Thiruvannamalai
- **Group II:** Non-tribal individuals selected from the outpatient department of a private dental college

The control group was matched with the intervention group by age and gender to minimize confounding variables.

Inclusion and Exclusion Criteria

Inclusion criteria consisted of individuals aged 18 years and above from both genders who were systemically healthy, willing to participate and had not previously taken part in similar studies. Individuals who refused participation, were bedridden, or had systemic illnesses were excluded from the study.

To achieve the required sample size:

- Snowball sampling was utilized to recruit participants from the Malayali tribe
- Systematic sampling was applied to select non-tribal participants from the outpatient dental clinic

Saliva Collection Procedure

To minimize the impact of cortisol diurnal variation, all saliva collection sessions were scheduled in the afternoon. Participants were given clear instructions 30 minutes before sample collection to refrain from:

- Oral hygiene practices (brushing or rinsing)
- Consumption of alcohol, tobacco, caffeine, or dairy products

This ensured the elimination of potential dietary or behavioral factors that could interfere with the results.

Saliva samples were collected using the passive drool technique, a non-invasive method involving unstimulated saliva collection. This method was chosen to ensure consistency in sample quality and minimize variability.

Sample Handling and Storage

To reduce bias, pre-trained researchers responsible for analyzing the samples were blinded to their source. Saliva samples were stored at -70°C within one hour of collection to ensure biomarker stability until analysis.

Before analysis, the samples were centrifuged at 3000 rpm for 15 minutes and only the clear supernatant was used for evaluation to ensure optimal sample clarity.

Biochemical Analysis

Salivary cortisol levels were measured using the Abbkine Human Cortisol (COR) ELISA Kit (Abbkine Scientific Co., Ltd, USA), with a calibration range of 12.5-200 µg/L and a detection limit of 1.0 µg/L. The ELISA procedure followed a two-site sandwich technique, ensuring accurate cortisol detection.

In the assay procedure:

- Standards and saliva samples were added to microplate wells pre-coated with cortisol-specific antibodies
- Cortisol present in the samples bound to the immobilized antibodies
- A horseradish peroxidase (HRP)-conjugated cortisol detection antibody was added
- Any unbound substances were thoroughly washed away
- A chromogen solution was introduced, producing a color change proportional to cortisol concentration
- The reaction was halted and the color intensity was measured using an ELISA reader, which provided the final cortisol concentration in µg/L

To ensure consistency across both groups, the same saliva collection and analysis procedures were applied to the non-tribal control group.

Additionally, the study included the use of the EliKine™ Mouse TNF-α ELISA Kit (calibration range: 15.6 pg/mL-1000 pg/mL, detection limit: 8 pg/mL) and the Rat CRP ELISA Kit (calibration range: 150 µg/L-2400 µg/L, detection limit: 15 µg/L) to explore inflammatory markers associated with stress.

Statistical Analysis

Data analysis was performed using SPSS software version 26.0. The concentration and optical density values of cortisol in both groups were expressed as mean±standard deviation. To compare the significant differences in cortisol levels between the two groups, the Mann-Whitney U-test was employed, given the non-parametric distribution of the data. A p-value <0.05 was considered statistically significant.

Additional Measures for Data Integrity

- To control for confounding variables such as occupational stress, environmental factors and dietary habits, participants completed a brief questionnaire to document these variables
- To minimize technical bias, all ELISA analyses were performed in duplicate and internal controls were utilized to validate assay precision

By ensuring rigorous sampling techniques, proper handling of samples and appropriate statistical analysis, this study aimed to deliver reliable and meaningful insights into stress-related cortisol differences between Malayali tribal and non-tribal populations.

RESULTS

The present study included a total of 44 participants, divided equally into two groups: the Malayali tribal group (n = 22) and the non-tribal control group (n = 22).

The mean Optical Density (OD) value in the Malayali tribal group was 0.37±0.008, while the mean OD value in the non-tribal group was significantly lower at 0.28±0.003. Similarly, the mean cortisol concentration in the Malayali tribal group was 13.44±2.15 µg/L, whereas the non-tribal group exhibited a significantly lower mean cortisol concentration of 8.73±1.85 µg/L.

Statistical analysis using the Mann-Whitney U-test demonstrated a statistically significant difference ($p<0.05$) between the two groups. The results confirmed that the Malayali tribal population exhibited significantly higher cortisol levels than their non-tribal counterparts, indicating a heightened stress response in the tribal group.

In terms of optical density, the non-tribal group displayed significantly lower OD values compared to the tribal group, further reinforcing the observed disparity in cortisol levels (Table 1).

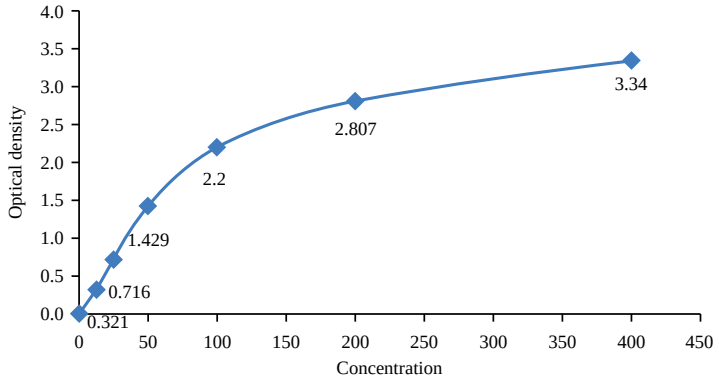

Figure 1: Graph showing the relationship between cortisol concentration levels and optical density

Table 1: Mann whitney U test revealing the significant differences between control group and tribal group in cortisol concentration and optical density

Population	Tribal	Non tribal	Mann Whitney U-value	p-value
Optical density	1.38±0.88	0.28±0.003	19.59	0.048*
Conc µg/l	13.44±2.15	8.73±1.85	27.03	0.029*

The ELISA assay results revealed a non-linear relationship between optical density and cortisol concentration. At lower cortisol concentrations, there was a steep increase in OD values, indicating heightened assay sensitivity in this range. Conversely, at higher cortisol concentrations, the curve plateaued, suggesting a reduced change in OD increased (saturation point). This predictable pattern aligns with typical ELISA assay behavior, highlighting consistent assay performance with optimal sensitivity at lower cortisol concentrations and stable detection at higher levels (Figure 1).

These findings collectively emphasize that the Malayali tribal population experienced significantly elevated stress levels compared to the non-tribal group. This disparity may be attributed to socio-environmental stressors such as limited healthcare access, economic hardship and environmental instability, which are common challenges faced by indigenous communities.

DISCUSSION

Key Findings

This *in vitro* study aimed to compare salivary cortisol levels between the Malayali tribal population in Thiruvannamalai and a non-tribal control group. The results demonstrated that the Malayali tribal group exhibited significantly higher cortisol concentrations (13.44±2.15 µg/L) and mean optical density (0.37±0.008) compared to the non-tribal control group, which recorded lower cortisol levels (8.73±1.85 µg/L) and optical density values (0.28±0.003). Statistical analysis using the Mann-Whitney U test confirmed a significant difference between the two groups ($p<0.05$).

The ELISA assay demonstrated a non-linear relationship between optical density and cortisol concentration. At lower cortisol concentrations, the assay showed high sensitivity, reflected by a steep increase in OD values. Conversely, at higher concentrations, the curve plateaued, indicating a saturation point where OD values stabilized despite further increases in cortisol levels. This result aligns with known ELISA assay behavior and confirms the assay's accuracy and reliability.

Strengths and Limitations

A major strength of this study was its robust methodology, including the use of standardized ELISA kits and a blinded analysis procedure to minimize bias. Additionally, matching participants by age and gender improved the validity of the comparisons between the two groups. The study also minimized diurnal variations in cortisol by scheduling all saliva collection sessions in the afternoon, enhancing the consistency of results.

Another key strength was the inclusion of the Malayali tribe, a marginalized and hard-to-reach community that has been underrepresented in stress biomarker research. The study's insights contribute valuable data to an area with limited published research.

However, the study also had some limitations. The sample size (n = 44) was relatively small, which may limit the generalizability of the findings to the broader Malayali tribal population. Additionally, the cross-sectional design captures cortisol levels at only one time point, preventing an assessment of long-term stress trends. Furthermore, the study lacked a comprehensive analysis of socioeconomic variables, lifestyle factors and environmental stressors, which could have further explained the elevated cortisol levels observed in the tribal group. Future research incorporating these variables would provide a more comprehensive understanding of stress physiology in tribal populations.

Comparison with Similar Research

The findings of this study are consistent with previous research highlighting elevated cortisol levels in populations exposed to chronic socio-environmental stress. For example,

a study by Kumaraguru *et al.* [17] similarly reported increased cortisol levels in marginalized and tribal populations, attributing these changes to external stressors such as economic instability and healthcare limitations.

Likewise, research by Obulareddy *et al.* [18] demonstrated that heightened cortisol levels are linked to increased psychological stress, which has been shown to contribute to periodontal diseases. Additionally, a study by Walls *et al.* [19] observed that individuals with elevated cortisol levels had experienced major stressful events, with tobacco usage identified as a significant contributing factor.

The findings of Kanagaraj *et al.* [20] further reinforce the results of the present study, where marginalized groups like the Narikuravar tribes of Chennai were reported to experience elevated cortisol levels due to socio-economic challenges, which adversely impacted their daily lives.

In a broader comparison, research by Berger *et al.* [21] investigated cortisol differences between indigenous and non-indigenous groups. Similar to the present study findings reported that indigenous groups exhibited a significantly heightened cortisol awakening response, attributed to chronic environmental stressors and socio-cultural marginalization [21].

Explanation of Key Findings

The elevated cortisol levels observed in the Malayali tribal population are likely driven by multiple stressors, including limited healthcare access, economic instability and cultural marginalization. Prolonged exposure to these conditions may have triggered persistent activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in chronic cortisol secretion [22].

The higher mean optical density observed in the tribal group further supports the presence of sustained physiological stress. The chronic activation of the HPA axis and prolonged cortisol elevation can lead to long-term health risks, including cardiovascular issues, immune suppression and metabolic disturbances [23,24].

Conversely, the lower cortisol levels in the non-tribal group suggest a comparatively reduced stress burden. Improved socioeconomic stability, better access to healthcare and reduced environmental uncertainties may have contributed to the lower cortisol concentrations observed in this group.

The ELISA assay's non-linear correlation between cortisol concentration and optical density was consistent with expected biological behavior. The assay demonstrated optimal sensitivity at lower cortisol levels and saturation at higher concentrations, ensuring reliable detection across a broad concentration range [25].

Implications and Action Needed

The findings of this study underscore the urgent need for targeted interventions to reduce chronic stress in the Malayali tribal population. Policymakers should prioritize efforts to:

- Improve economic opportunities for tribal communities
- Enhance healthcare access with culturally appropriate services
- Introduce educational initiatives that promote mental well-being and resilience

In particular, mental health support programs tailored to the unique socio-cultural context of the Malayali tribe are essential. Such programs should integrate traditional practices with evidence-based interventions to foster acceptance and engagement within the community [26].

Future research should focus on:

- Conducting longitudinal studies to assess cortisol fluctuations over extended periods
- Expanding sample sizes to improve the generalizability of findings
- Incorporating qualitative data such as interviews and surveys to explore the psychological and social dimensions of stress in tribal communities

These strategies would provide a more comprehensive understanding of stress patterns and guide the development of effective interventions.

CONCLUSION

This study demonstrates a significant difference in salivary cortisol levels between the Malayali tribal population of Thiruvannamalai and a non-tribal control group. The Malayali tribal group exhibited higher cortisol concentrations and greater optical density values, suggesting elevated stress levels compared to their non-tribal counterparts.

The findings point to multiple factors contributing to heightened stress in the tribal group, including socioeconomic hardships, restricted healthcare access and environmental instability. Conversely, the non-tribal population's comparatively lower cortisol levels reflect improved social and economic stability.

The study highlights the critical need for targeted interventions to address stress-related health risks in tribal populations. Key strategies include expanding healthcare services, promoting mental health support and developing community-based stress management programs that align with the cultural values of the Malayali tribe.

Future research should adopt longitudinal study designs with larger sample sizes to assess cortisol patterns over time and evaluate the impact of targeted interventions. Additionally, exploring the role of cultural practices, dietary habits and social networks in stress management would provide a more holistic understanding of stress physiology in indigenous groups.

Addressing these disparities through policy reforms and culturally relevant interventions will play a vital role in reducing stress-related health risks and improving the overall well-being of indigenous communities.

Ethical Statement: Ethical clearance was obtained from Institutional ethics committee, Saveetha University with the number SRB/SDC/UG-1997/24/PHD/211.

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