Chettinad Health City Medical Journal
Volume 11, Issue 2 - 2022, Pg. No. 3-8
Peer Reviewed & Open Access Journal

Research Article

A Comparative Study on Influence of Stress on Diurnal Variations in Salivary Cortisol Levels among Selected Employed and Unemployed Women

Anees F Thabassum[1], K Begum[2], A Fathima[3]

[1]Assistant Professor, Department of Clinical Nutrition and Dietetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, India.
[2]Professor (Rtd), Department of Studies in Food Science and Nutrition, Manasagangothri, University of Mysore, Mysore, India.
[3]Chief Dietician, Department of Clinical Nutrition and Dietetics, Narayana Hospitals, Mysore, India.
DOI: https://doi.org/10.24321/2278.2044.202210

I N F O

Corresponding Author:
Anees Fathima Thabassum, Department of Clinical Nutrition and Dietetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, India.
E-mail Id:
aneesshazi@gmail.com
Orcid Id:
https://orcid.org/0000-0002-8735-8034
How to cite this article:
Thabassum AF, Begum K, Fathima A. A Comparative Study on Influence of Stress on Diurnal Variations in Salivary Cortisol Levels among Selected Employed and Unemployed Women. Chettinad Health City Med J. 2022;11(2):3-8.

Date of Submission: 2022-05-31
Date of Acceptance: 2022-06-20

A B S T R A C T

Background: Secretion of stress hormone cortisol correlates with the intensity of stressors and their period of occurrence, and may have serious implications on health in the long run.

Aim: This study explores the diurnal variations of the salivary cortisol levels among selected employed and unemployed women.

Method and Material: From a population of 400 employed and 272 unemployed women, 40 women (20 employed and 20 unemployed) were selected. In order to compare the cortisol levels between women without stress and with stress, 5 each from employed and unemployed groups with normal stress scores and 15 from each group with mild to moderate stress scores based on the DAS (Depression, Anxiety and Stress Score) were included in the study.

Results: Employed women exhibited two peaks in the hormonal profile, a sharp peak in the early morning (CAR-cortisol awakening response) with the mean cortisol level of 14.7 ± 5.9 nmol/l which slowly dropped to 10.1 ± 4.7 nmol/l in the noon and increased again by evening to 11.8 ± 4.6 nmol/l. Among the unemployed women, a single peak appeared for CAR that is 12.6 ± 3.5 nmol/l, which gradually declined to 8.1 ± 2.3 nmol/l by evening time.

Conclusion: The extent of stress is exhibited by multiple peaking of cortisol levels among employed women. Unemployed women with stress had markedly high levels of cortisol compared with their counterparts with no stress. It is of greater concern that the employed women with stress have markedly high cortisol levels which could be an indication of health issues in the longer run.

Keywords: Depression, Anxiety, Stress (Psychological), Cortisol, Diurnal Rhythm

Introduction

Women whether employed or home-makers experience stress as they are burdened by carrying out the monotonous roles fixed for them. Stress has been considered as an outcome of constant environmental pressure and an individual's personality which create an imbalance between the level of demand placed upon them and their capability to meet those demands. Stress is a mild form of mental state that continued to occur for a long period and if it is more severe, it may lead to symptoms related to anxiety and depression. Any type of pressure such as environmental, physiological, or personality trait causes hormonal changes, triggering the secretion of stress hormones.[1] Stressful life events induce activation of the hypothalamic-pituitary-adrenal axis which stimulates the production of cortisol that influences health.[2] The level of its secretion correlates with the level of pressure and period of occurrence of pressure. Cortisol exerts an extensive range of outcomes on the mind and body and influences the reproductive, thyroid, and immune systems. The body prepares for a "fight or flight" response during stress, while doing so, it can inhibit the production of other hormones.[3]

Stress and Cortisol

Based on the circadian rhythm, cortisol levels are higher in the morning than in the evening. However, an extended higher level of cortisol has been found to be linked with numerous negative effects, ranging from deterioration in cognitive function, such as memory or emotional adjustment to immune function disorders and hypertension.[4] Prolonged stress over time leads to an impaired response of the adrenal gland. Elevations in adrenal hormone levels due to chronic exposure to various levels of emotional and physical stressors often cause disorders ranging from anxiety to infertility. Long-standing stress would cause reduced adrenal hormone output leading to adrenal insufficiency. An array of syndromes and illnesses can be the outcome of the maladaptive response to stress augmented with disturbances in the functioning of the HPA axis. Either condition results in health problems, hence cortisol levels have been considered to be an indicator of stress.[2] Cortisol is considered to be an important pre-clinical biological marker of ill-health.[5-7] However, it is worthwhile to mention here that cortisol because of its catabolic nature causes serious health problems.

Biomarker for Stress - Salivary Cortisol

Salivary cortisol testing is a highly known technique used to diagnose Cushing's disease (hypercortisolism). As it is reliable, non-invasive, and convenient, it is much preferred over serum or urine testing. In the blood, only 1-15% of cortisol is free and biologically active as most of it is bound to carriers. Saliva can be used to measure the free fraction of cortisol and reliably reflects the amount of free cortisol circulating in the blood.[8,9] Multiple sampling over the course of the day is convenient - twice a day sampling (morning and bedtime), or four times during a day (within one hour of waking, mid-day, evening and bedtime).[10,11]

Hence, this study was undertaken to assess the diurnal variations among women (employed and unemployed) using salivary cortisol as a biomarker. It was considered important to relate hormonal responses among women with and without stress, as cortisol is a biomarker for stress.

Materials and Method

Study Design and Population

This was a population-based cross-sectional study conducted in Mysore city for a period of 2 years. A total of 672 (400 employed and 272 unemployed) educated and married women aged 25-40 years participated in the study. Employed women from various fields such as accounts/ administration, healthcare, and teaching were purposefully selected depending on their desire to participate in the study. Written informed consent was obtained from the participants after explaining to them the details of the study. The study was approved by the Institutional Ethics Committee. Inclusion criteria drafted were age between 25 and 40 years, for employed women - employed for more than 2 years, for unemployed women - homemakers, not involved in any income generating activities, educated (graduate), non-pregnant and menstruating, married, with at least one child, and no mental disorders. Exclusion criteria were age above 40 years, having any ongoing treatment for chronic diseases, on steroids, oral contraceptive pill, and those with psychiatric problems.

Assessment of Depression, Anxiety and Stress Score

A 42-item self-administered questionnaire - DASS - Depression, anxiety, and stress score by Lovibond PF and Lovibond SH[12] was used to measure the three negative emotional states - depression, anxiety, and stress. Respondents were instructed to record their responses on a 4-point scale based on the degree to which each of the 42 statements was experienced over the past week. Higher scores on each subscale demonstrated increasing severity of depression, anxiety, and stress. Individual respondent scores on the DASS subscales were explained by changing them to z-scores and comparing them to the normal values as given in the DASS manual. Based on the stress scores, the participants were classified as normal if the score was 0-14, mild if it was 15-18, moderate if it was 19-25, severe if it was 26-33, and extremely severe if it was more than 33.

Selection of the Subjects

From the total population, participants who showed

ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202210

interest to participate in the salivary cortisol estimation were explained in detail about the study. In order to get a homogenous population, age, socio-economic status, family status, and level of stress (as per DASS) were considered as criteria for selection. Forty subjects were finally selected (after considering the prevalence of stress and the statistical significance) (20 employed and 20 unemployed women) from the volunteers for the study, among whom 5 participants each from employed and unemployed women were normal (with no stress) and 15 from each group had stress.

Salivary Cortisol Estimation - Collection and Storage of the Saliva Sample

The procedure to collect saliva samples was explained and each participant was handed over 3 sterile coded plastic containers (5 ml capacity). The time of collection of the sample was specified as per the guidelines given by Saxbe DE.[13] The saliva samples were collected 3 times in a day, early morning sample (within 30 minutes of awakening), afternoon sample (before lunch), and evening sample (before leaving the workplace in case of employed and before evening tea around 5 pm in case of unemployed women). Passive drool technique was employed to collect un-stimulated saliva which pools on the floor of the mouth. This technique maintains the consistency of the sample collected.

Instructions given to the Participants Before Saliva Collection

- Avoid consumption of foods high in sugar, acid, and caffeine immediately before sample collection
- Maintain normal activities
- Avoid consumption of any major meal before 60 minutes of sample collection
- Remove food residues by rinsing the mouth with water. A 10 minutes wait is required to avoid sample dilution before sample collection

After Saliva Collection

Participants were advised to freeze the samples immediately after collection. In the case of employed women, early morning samples were frozen, while the rest of the samples were collected from the office premises by the investigator and were immediately taken to the lab for freezing.

Analysis

In Vitro Diagnostic Test Kits (Diagnostics Biochem Canada Inc.) were used to determine cortisol by enzyme immune assay.

Statistical Analysis and Interpretation

The data obtained were subjected to statistical analysis using XLSTAT 18.06 software package. Parametric and non-parametric analyses and descriptive statistics such as percentages and means ± SD were used to report the results. Associations between the variables were tested using Student's "t" test.

Results

Sociodemographic Characteristics of the Subjects

A total of 672 women aged 25 to 40 years participated in the study, among whom 400 were employed (EW) and 272 women were housewives (UEW). Housewives were not involved in any income-generating activities. The majority of women in both the groups were in the age group of 25-34 years. More than 50% (56% employed and 52% unemployed) of women practised Hinduism, followed by those practising Islam (28% employed and 34% unemployed) and Christianity (16% employed and 14 % unemployed). Information on the educational qualification of subjects revealed that 60% and 63% of women were graduates (employed and unemployed respectively). Nuclear families were predominant among the study groups (71% employed and 73% unemployed), which reflects the current trend of the family structure. Figure 1 depicts the job profile of the participants, 32% were in the teaching profession, 24% were from the healthcare sector, and 44% were in administrative jobs. It also provides details about the breakups in job positions under each of the occupation groups.

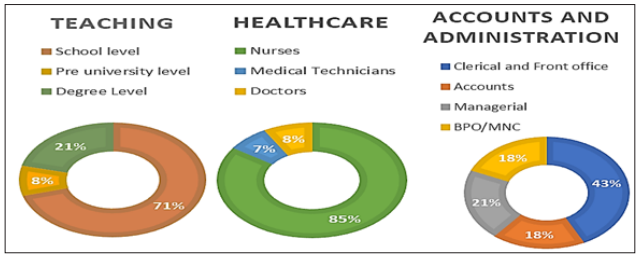

Figure 1.Information on the Job Profile of the Employed Women

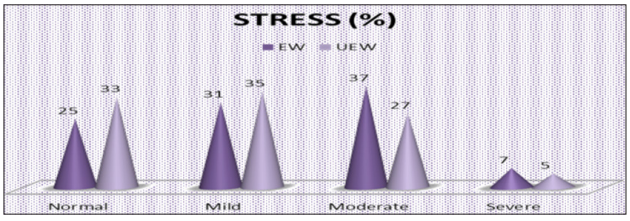

Figure 2.Stress Levels among Women (Employed Women (EW) n = 400, Unemployed Women (UEW) n = 272)

Prevalence of Stress among Women Participants

DASS is a recommended tool used to assess stress, anxiety, and depression among normal individuals. Figure 2 suggests the presence of stress in both employed and unemployed women. Mild and moderate forms of stress

ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202210

occurred in a relatively higher proportion of women with the per cent occurrence among the two groups of women being essentially similar. A small but higher percentage of unemployed women were normal (no stress) (33%) as compared to the employed (25%).

The diurnal variations in salivary cortisol levels (Table 1) among employed women with no stress (normal) revealed a higher circulatory level in the morning with a cortisol awakening response (CAR) of 6.7 ± 1.3 nmol/l which gradually dropped to 5.4 ± 0.74 µg/ml and 4.5 ± 1.1 nmol/l in the afternoon and evening respectively. These values were higher than those of the unemployed normal women (no stress) whose mean CAR value was 6.0 ± 1.0 nmol/l which dropped to 3.9 ± 1.2 and 3.9 ± 1.3 nmol/l in afternoon and evening samples. This clearly indicates that the circulatory levels of stress hormones are higher (cortisol) among employed women even in those without stress (normal) according to the DASS score.

Similar differences in cortisol levels were noted among employed and unemployed women with stress. Employed women had markedly higher levels of cortisol as compared to unemployed women. Employed women exhibited two peaks in the hormonal profile, a sharp peak in the early morning (CAR) with the mean cortisol level of 14.7 ± 5.9 nmol/l which slowly dropped to 10.1 ± 4.7 nmol/l in the noon and increased again by evening to 11.8 ± 4.6 nmol/l. Among the unemployed women, a single peak appeared for CAR corresponding to 12.6 ± 3.5 nmol/l, which gradually declined to 8.1 ± 2.3 nmol/l by evening time. The Cortisol Awakening Response - CAR was higher in both the groups which eventually reached the basal level at night. Generally, cortisol exhibits a robust diurnal pattern. Its levels peak between waking and 30-45 minutes post waking (CAR) and, decline during the day (in the absence of stressors to stimulate cortisol secretion) until a trough is reached at around midnight. Normal subjects had a mean salivary cortisol level of 15.5 ± 0.08 nmol/l at 08:00 hr and 3.9 ± 0.2 nmol/l at 20:00 hr.[14] Among employed women, it was evident that the extent of stress is exhibited by multiple peaking of cortisol levels. It could be possible that individuals with markedly high-stress levels having multiple cortisol peaks would continue to have high basal cortisol levels, thereby the CAR is significantly higher.[15] Our results are supported by other studies where among office workers, elevated Csal (salivary cortisol) levels in the evening have been linked to stress symptoms, whereas a high daytime Csal level was associated with higher stress scores in teachers.[16,17] Hence our results have brought to light that employed women have relatively higher cortisol levels even among those with no obvious stress.

Table 1.Diurnal Variations in Salivary Cortisol Levels among the Selected Employed and Unemployed Women

Stress Level	Diurnal Assessment	Mean ± SD Values of Salivary Cortisol Levels in Employed Women (nmol/l) (n = 20)	Mean ± SD Values of Salivary Cortisol Levels in Unemployed Women (nmol/l) (n = 20)	"t" Significance	P Value
Normal (n = 5)	Early morning	6.7 ± 1.3	6.0 ± 1.0	+ 0.89	0.197
	Afternoon	5.4 ± 0.74	3.9 ± 1.2	+ 5.1	0.025
	Evening	4.5 ± 1.1	3.9 ± 1.3	+ 0.88	0.215
With stress (n = 15)	Early morning	14.7 ± 5.9	12.6 ± 3.5	+ 1.31	0.125
	Afternoon	10.1 ± 4.7	9.1 ± 1.7	+ 0.9	0.221
	Evening	11.8 ± 4.6	8.1 ± 2.3	+ 2.88	0.004

Table 2.Comparison of Diurnal Changes in Salivary Cortisol Levels among Employed and Unemployed Women Without Stress (Normal) and With Stress

Employment Status	Diurnal Assessment	Mean ± SD Values of Salivary Cortisol Levels in Women Without Stress (nmol/l) (n = 5)	Mean ± SD Values of Salivary Cortisol Levels in Women With Stress (n = 20)	"t" Significance	P Value
Employed	Early morning	6.7 ± 1.3	14.7 ± 5.9	-2.907	0.0047
	Afternoon	5.4 ± 0.74	10.1 ± 4.7	-2.201	0.020
	Evening	4.5 ± 1.1	11.8 ± 4.6	-3.498	0.0012

ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202210

Unemployed	Early morning	6.0 ± 1.0	12.6 ± 3.5	-4.127	0.0003
	Afternoon	3.9 ± 1.2	9.1 ± 1.7	-6.243	< 0.0001
	Evening	3.9 ± 1.3	8.1 ± 2.3	-3.890	0.0005

In order to ascertain a statistically significant difference in the levels of salivary cortisol, a student "t" test was carried out to compare the levels between the 2 groups - employed and unemployed women (normal and with stress). Results from Table 2 reveal a significantly higher level of salivary cortisol among employed women with stress at all three timings (early morning p = 0.0047, afternoon p = 0.020, and evening p = 0.0012). While comparing unemployed women (normal vs with stress) too, a significantly higher level of salivary cortisol was noticed among unemployed women with stress at all three timings (early morning p = 0.0003, afternoon p < 0.0001, and evening p = 0.0005).

Discussion

Employed women with stress have markedly high cortisol levels which could be an indication of health issues in the longer run. Unemployed women also experienced stress, thereby their cortisol levels were markedly high as compared to their counterparts with no stress.

According to the Harvard Business Review[18] report on the Nielsen survey conducted on 6500 women from 21 nations including India, the highest proportion of stress was found among Indian women. Among them, 87% were stressed most of the time and 82% had no time to relax. They concluded that Indian women perform different roles in daily life, like a job along with household tasks, causing higher stress. Employment options for Indian women are booming but conventionalism and household expectations are the determinants of stress.

A comparative study by Adam EK[19] on mothers of children aged 2 years (both working and full-time homemakers) revealed that maternal relationship activity and increased home and job demands were aligned with more significant morning cortisol levels accounting for 40% of the variance in the cortisol levels of working mothers and a significant dip in the cortisol level accounting for 43% of the variance in cortisol slopes throughout the day.

Lovell B et al.[15] reported that female undergraduate students in the high-stress group had flattened diurnal cortisol slopes and high mean diurnal output exhibited by HPA hyperactivity in the evening. They also reported experiencing recurrent health issues with notably greater frequency.

Dahlgren A et al.[14] reported that elevated evening levels of cortisol were linked with expressions of stress and low-grade self-assessed health. Izawa S et al.[16] reported that cortisol levels increased during the period of stress; however, the CAR decreased after the period of stress. Evolahti A et al.[20] concluded that middle-aged women with high demands at work presented with significantly high cortisol levels. Continually high cortisol levels in response to chronic stress may lead to central deposition of body fat, dyslipidaemia, high blood pressure, and insulin resistance.[21]

Conclusion

Stressors in women's life vary in nature, frequency, and stages of their occurrence, and may change according to one's psychological sagacity. Hence, women displayed significantly different patterns (especially afternoon and evening levels) of neuroendocrine reactivity in response to stress. Also, the evening and morning levels of cortisol appear to be high in employed women (with and without stress).

Stress and health impairments linked to it are intrinsic issues prevailing among humans. It is imperative to understand the biological mechanisms linked with stress and disease. However, in humans, it is a challenging task to identify and understand the mechanisms of stress response leading to dysregulation in homeostasis.

Acknowledgement

We acknowledge all the participants for their kind cooperation.

Financial Support

UGC-Maulana Azad Fellowship for Minorities.

Conflict of Interest: None

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ISSN: 2278-2044
DOI: https://doi.org/10.24321/2278.2044.202210