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Relationship between Stress and Cortisol Levels among Presenters

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Abstract **Original Research Article**

***Background*:** Stress occurs when an organism's internal regulating balance (or homeostasis) is disrupted by external environmental stressors. The HPA axis and the sympathetic-adrenal-mefahhdullary (SAM) system mediate the stress response in clinical circumstances. The adrenal cortex produces cortisol when the HPA axis is activated. It organizes the body's responses to stress by modifying metabolism, immune responses, and inflammation such that the body expends the least amount of energy feasible. Cortisol is a hormone found in saliva, urine, sweat, and hair. It is proportional to blood cortisol and can be used to determine HPA axis activity. ***Aims*:** To determine the relationship between stress and cortisol levels among presenters by the difference in levels scored in pre and post presentation. ***Material and Methods*:** A cross-sectional descriptive study was conducted to achieve the aims of the study that has been conducted to identify the relationship between stress and cortisol levels for the presenters between 10th. Jan, 2022 and Jan, 20th. Mar, 2022. The study has been conducted by selecting a typical form of the non-probability (Purposive) sample. The size of the sample comprises 70 participants who are included in the study. ***Results*:** The relationship between stress and cortisol (r = 0.279, P-value = 0.01] was significantly positively correlated. The Cortisol levels were higher following the presentation [37.13±25.1] than they were prior [19.9±13.3], as shown. P-values for the paired t-test were less than 0.0001 for this finding. ***Conclusion*:** It was concluded Post-presentation stress was higher than pre-presentation stress. The pre and post-presentation cortisol levels were higher.

Keywords: Stress, Cortisol, Presenters and HPA.

INTRODUCTION

Stress is described as a state where an organism's internal regulation balance (or homeostasis) is interrupted as a result of real or perceived external environmental pressures. This could be biological, environmental, social, emotional, or psychological factors that cause the body to attempt to reestablish homeostatic equilibrium, a process known as stress reaction [1]. The stress response is mediated in clinical situations by two distinct systems: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system. Finally, triggering of the HPA axis leads to cortisol synthesis by the cortex adrenal gland. It is critical for maintaining the body's responses to stress by changing metabolism, immune tasks, and the inflammation reaction in order for the body to expend the least amount of energy possible to combat stress [2].

Cortisol is a hormone that is produced by the adrenal glands and is found in saliva, urine, perspiration, and hair. Salivary cortisol (sCort) levels are proportional to blood cortisol levels and can be used to assess the activity of the HPA axis. It is widely considered the gold standard for stress measurement, and numerous studies refer to it as "stress hormones." When the SAM system is activated, more catecholamines are produced, including epinephrine and norepinephrine, which change metabolism and cardio-vascular reaction, preparing the body to deal with physical and psychological stress [2]. In bodily fluids such as blood or saliva, biomarkers of the activation of these stress systems can be discovered. Saliva is very useful in stress studies because it may be collected quickly, readily, and non-invasively without generating unnecessary stress [3]. Teaching speaking, particularly for university students, is viewed as an "interesting and difficult task." Indeed, maintaining students' "engaged in speaking" throughout the session

needs a number of tactics. The author highlights that one of the most effective ways to support students in reaching the lesson's objectives is a thorough introduction of the new language at the lesson's early phase, dubbed "the presentation phase." Following that, pupils require "ample activities" to aid them in practicing the new language; this is referred to as the "practice phase." Lastly, pupils must practice communicating with one another in their newly acquired language: this is referred to as "the production phase." [4]. Speaking stress results in communication breakdowns, which affect an individual's personal, social, and emotional well-being [5]. The goal of the current study: to evaluate the cortisol concentrations, and stress levels among presenter before (20) minute presentation, to measure the cortisol concentrations, and stress levels among presenter after Presentation and to determine the relationship between stress, and cortisol levels among presenters by the difference in levels scored in pre and post presentation.

METHODOLOGY

A cross-sectional descriptive study was conducted to achieve the aims of the study that has been conducted to identify the relationship between stress and cortisol levels for the presenters between 10th. Jan 2022 and Jan, 20th. Mar 2022. The study has been conducted by selecting a typical form of the non-probability (Purposive) sample. The size of the sample comprises 70 participants who are included in the study. The setting of the study is located in the Continuous Education Center at University of Kufa in AL-Najaf AL-Ashraf City.

Salivary Cortisol:

A. Principle Cortisol:

The Human Cortisol has been pre-coated on a micro-ELISA plate. Human Cortisol in samples or Standard competes for binding sites on the Biotinylated Detection Ab specific for Human Cortisol with a predetermined amount of Human Cortisol on the solid phase supporter. Excess conjugate and unbound sample or standard are rinsed from the plate, and each microplate well is incubated with Avidin conjugated to Horseradish Peroxidase (HRP). After that, each well is filled with a TMB substrate solution. The enzyme-substrate reaction is halted by the addition of stop solution, and the spectrophotometric color change is determined.

B. Kit Cortisol Components:

Part	Size
The Microwell Plate - 96-well microplate coated with antibodies (8 wells, 12 strips)	1 plate
The Reconstituted standard - lyophilized, 400 ng/ml	2 vials
The Concentrated Antibody conjugated to biotin [100X) - 120 ul/vial	1 vial
The Streptavidin-HRP solution concentrated [100X) - 120 ul/vial	1 vial
The Antibodies conjugated to biotin 14ml/vial diluent	1 bottle
The Diluent for Streptavidin-HRP - 14 ml/vial	1 bottle
The Buffer for Washing Consolidate [25x) - 30 mL per vial	1 bottle
12 ml/vial Substrate Solution	1 bottle
12 ml/vial Stop Solution	1 bottle
Seals for Plate Covers	4 pieces

C. The Reagents Preparation:

1. **The Wash Buffe**r

To prepare 600mL of Wash Buffer, 30mL of Wash Buffer Concentrate has been diluted with 570mL of the distilled water.

2. Standard/Specimen

1.0 mL Standard and Sample Diluent were added to the standard, and it was allowed to stand for 10 minutes before gently inverting it several times. After it has completely dissolved, a pipette was used to thoroughly mix it. This reconstitution yields a 400 ng/mL working solution. Then, as needed, make serial dilutions. The following is the recommended dilution gradient: 200, 100, 50, 25, 12.5, 6.25, and 0 ng/mL, respectively:

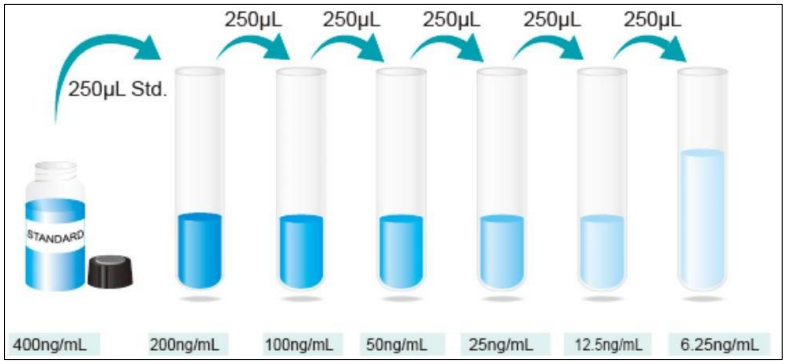

Figure 3.1: The Eppendorf tubes

3. The Working Solution:

In the clean plastic tubes, the concentrated Biotin-Conjugate solution 1:100 is diluted with the Biotin-Conjugate antibody Diluent and the concentrated Streptavidin-HRP solution 1:100 with the Streptavidin-HRP Diluent.

D.Procedure:

1. The first two columns filled in with the standard working solution: Each solution concentrations is added twice, one to each well, next to each other (50 uL for each well). The remaining wells are filled with the samples (50 uL for each well). Immediate addition of 50 mL of Biotinylated Detect Ab working solution to each well. On the plates, the sealant contained in the kit is applied and the incubation was for 45 minutes at 37°C.
2. The solution from each well Aspirate and decant and replace it with 350 µL of wash buffer. After 12 minutes of soaking, aspirated or decanted the solution from each well is and pat dry with clean absorbent paper. This step is repeated three times more.
3. Each well filled with 100 µL of the HRP Conjugate working solution. The plate is sealed using the Plate sealer. At 37°C, the incubation was for 30 minutes.
4. The solution is aspirated or decanted from each well and the washing operation contains five times as in step 2.
5. Each well is filled with 90 µL of Substrate Reagent. The plate is resealed with a new plate sealer. And the incubation was for approximately 15 minutes at 37°C. The plate is kept away from the direct sunlight.
6. Each well is filled with 50 µL of the Stop Solution.
7. Using a microplate reader set to 450 nm to determine the optical density (OD value) of each well simultaneously.

Statistical Analyses

Descriptive statistics: (Frequency and percentage tables; mean and standard deviation). Inferential Statistics: Paired t-test was used to find the significant difference in cortisol level between pre and post presentation for the same group [6].

RESULTS

(Table 1.) Shows that the highest percentage of the age group [30-39) years old with (48.57%), females' participants (58.57 %), who are married (64.29%); and who are living in urban area (97.14 %); with bachelor education level (51.43 %). Table 2. Shows the frequency distribution of studied sample according to presentation data, which explains the highest percentage of the specialty are: those that have scientific specialty (60 %); within his subject matter (68.57 %). In addition, the same table shows that most presenters do previously similar presentation (55.71%) and most of them has been taken more than two hours to prepare the presentation (40 %), without depending on a ready template (68.57 %), most participants have been taken a quarter an hour to review the presentation [32.86%). With the exception of six presenter suffering from (three Diabetes Mellitus, one Hypertension, one Psoriasis, one Diabetes Mellitus and Hypertension), the presenters did not have a chronic disease (91.43%). (Table 2.) Shows that about [10%) of presenters in the current study have high stress in the pre presentation, while about (42.86%) of them have high stress in the post presentation. (Table 3.) shows that stress assessment in Post presentation was higher than those in Pre presentation for overall stress items, this result was statistically high significant according to paired t-test and P-value was <= 0.001. And shows that the level of cortisol was high in Post presentation in comparison with Pre presentation [37.13±25.1] and [19.9±13.3] respectively. This result was statistically significant according to paired t-test and P-values was <0.0001.

Table 1: The Frequency distribution of the sample under study according to Demographic Data

Demographic data		**Freq.**	**%**
Age groups (Years)	<= 29	25	35.71
	30 – 39	34	48.57
	40 – 49	8	11.43
	50 and more	3	4.29
	Mean±SD(Range)	32.59±7.8[22-65)	
Gender	Males	29	41.43
	Females	41	58.57
Marital status	Single	22	31.43
	Married	45	64.29
	Divorced	3	4.29
	Widowed	0	.00
	Separated	0	.00
Monthly income	Enough	49	70.00
	Enough to some extent	15	21.43

Demographic data		Freq.	%
	Not enough	6	8.57
Residence	Urban	68	97.14
	Rural	2	2.86
Educational level	Bachelor	36	51.43
	High Diploma	0	0.00
	Master	17	24.29
	Doctorate	15	21.43
	Post-doctoral	2	2.86
Total		70	100%

Table 2: The Frequency distribution of Stress overall Questionnaire Items pre and post presentation

Stress overall Questionnaire Items		Pre Presentation		Post presentation	
		Freq.	%	Freq.	%
Stress Assessment	Low stress	63	90.00	40	57.14
	High stress	7	10.00	30	42.86
Total		70	100%	70	100%

***Cutoff value=2; when mean of score <=2 stress is low, when mean of score >2 stress is high**

Table 3: The Comparisons of parameters between pre and post presentation using paired t test.

Parameters	Pre Presentation			Post presentation			T-test (df=69)	P-value (Sig.)
	Mean	SD	Assess.	Mean	SD	Assess.		
Overall Stress Items	1.74	0.29	Low	1.89	0.36	Low	-4.933	0.001**
Cortisol Concentration(ng/ml)	19.9	13.3	N/A	37.13	25.1	N/A	-4.962	<0.0001

DİSCUSSİON

Throughout the course of the current research's data analysis, the results in (Table 1.) showed that the highest percentage of people in the age group (30-39) years old had (48.57%). In terms of gender, the current study included mostly males (41.43%) and females (58.57%) who were married (64.29%). Hancock SB *et. al.* mentioned that the rates of prevalence were ranging from 14% to 48%, in which women usually having more public speaking anxiety (27% women) in comparison to men (14% men) [6]. Furthermore, the highest percentage in the studies above is agreed between the percentages, since the selection of the sample for both gender ranges from females to the highest percentage in the selection of samples, but the difference in the number sample size. For residence, presenters were living in urban area (97.14 %); with bachelor education level (51.43 %), this results agreed with a study about underscores the critical need for additional models and a theoretical framework that explain the non-linear, bi-directional, and dynamic nature of stress and PA interactions. Currently, there are either a dearth of theoretical models of stress and behavior or they are highly contextualized (e.g., work environments, urban life) [7]. According to (Table 2.), approximately 10% of presenters in the current study had high levels of stress before presentations, and post-presentation stress was equally high (42.86 %). A previous study of University of Murcia in Spain reported that the stress in the post presentation was high stress. These result was consistent with current study, which showed a real stressful situation after public oral presentations the speech was part of the final examination, and participants had to speak in a different language [3], and another previous study, conducted at Sweden's Institute of Stress Medicine/Department of Medical and Health Sciences, revealed that stress-related exhaustion developed as a result of long-term stress exposure to reflect general levels of psychological stress, and that overall stress ratings were categorized into low and high stress [8]. According to (Table 3.) shows that post-presentation stress assessments were greater than pre-presentation stress assessments for overall stress items, this result was significant by using the paired one group t-test. According to a previous study conducted at the University of Illinois at Urbana-Champaign, the adrenal gland releases adrenaline, along with other hormones such as cortisol, into the bloodstream, signaling the heart to pump harder, lead to hypertension, open breathing passages, and constricted blood vessels in the skin and small bowel in order to increase blood flow to major muscle groups during the stress reaction [9-10]. While another study conducted in Iraq found that students who are fearful of public speaking frequently exhibit a number of symptoms during a public performance setting, including palpitations, perspiration, gastrointestinal discomfort, diarrhea, muscle tension, and confused states [11]. Additionally, a previous study found a positive correlation between morning saliva cortisol and vocal symptoms, confirming that subjects with more cortisol concentrations either experienced more of the vocal symptoms included in the questionnaire or experienced them more frequently than subjects with lower cortisol concentrations. As mentioned by "the Revised Psychobiological Framework

for Voice Disorders", the composite variable of vocal symptoms had a positive association with morning saliva cortisol, confirming that subjects with higher cortisol concentrations either experienced a larger number of vocal symptoms or had more vocal symptoms. As proposed by the updated "Psychobiological Framework for Voice Disorders", a composite variable representing vocal symptoms demonstrated a strong positive association with salivary cortisol concentrations (P-value=0.001]. The post-presentation stress intensity showed significant increase compared to the pre-presentation stress intensity for the overall stress item, and this finding was statistically significant for high stress. Our findings corroborated those of previous studies conducted at the University of Bonn in Germany, which concluded that stress is statistically significant only for the first and second ratings. However, as illustrated in the figures, and because this is the time when the stress is greatest, these are the measuring times that must be focused on, it can be demonstrated that women were subjectively more stressed, or nervousness, and that this effect lasted up to 45 minutes. The effect sizes were all moderate to large, with the largest effect sizes occurring immediately prior to the presentation, which is typically when stress is at its peak [12]. Additionally, in same table shows that the level of cortisol was high in Post presentation in comparison with pre presentation [37.13±25.1] and [19.9±13.3] respectively. This result was statistically significant according to paired t-test and P-values was <0.0001. According to the paired t-test, this result was statistically high significant, with P-value was < 0.001. According to the paired t-test, this result was statistically highly significant, with P-value was<=0.0001. Our findings corroborated a prior study from the University of Iowa in America, which exhibited that the presenting stress experiment demonstrated that persons with higher baseline cortisol levels also had higher cortisol levels during acute stress, and vice versa [13-14]. Salivary cortisol concentrations were within the detectable range of the Enzyme-Linked Immunosorbent Assay (ELISA), ranging from 0.10 to 1.56 g/dl [14]. Cortisol levels increased to a high of around 6.50 nmol/L over the individual baseline level during the speech.

CONCLUSION

It was concluded that oral presentation of the participants has increased their stressful situation. Cortisol levels were elevated after doing the presentation compared to its levels before presentation.

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Conflict of Interest: Nil

Ethical Approval

This case-control study was approved by the medical ethics committee in the Faculty of Medicine/Kufa University (Reference#: MEC-15 on November 8, 2021].

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Author Contributions

Ali Majid Hassana: Corresponding author, data collection, manuscript concept, writing, results analysis. Ali J. Eidan: manuscript submission, revision and gallery proof. Murtadha Kanim Adea: Data collection, manuscript concept and writing.

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