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Morning Salivary Cortisol Associates with Elevated Serum Leptin Levels in Jordanian Young Men with Olive Pollen Induced Allergic Rhinitis

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Authors' contributions

Author MAS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author WHT contributed to data analysis, drafting the article, and final approval of the version to be published. Authors ABZ, AAJ, and AS managed the analyses of the study. Authors KAK, OA, and RK managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Recent studies have shown independently inter-correlations between allergy, obesity, leptin hormone, and stress markers. However, these findings were unclear and contradictory. Thus the aim of the present study is to evaluate diurnal levels of salivary cortisol and DHEA in sample of Jordanian young men with history of olive pollen-induced

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allergic rhinitis in relation to serum levels of leptin.
Methodology: 130 university male students aged (21.98±1.78) years, were divided into two groups (59 allergic and 71 non allergic). Fasting blood samples were collected and tested for blood glucose, lipid profile, serum leptin, and salivary stress hormones (cortisol and DHEA).
Results: Allergic subjects showed significantly higher means of serum leptin ($p<0.0001$), LDL ($p<0.0001$), Total cholesterol ($p=0.001$), and BMI ($p= 0.004$). Also BMI and Body weight significantly correlated with serum leptin in all subjects of the study. Stronger correlation was observed in allergic subjects ($r = 0.650$; $r = 0.589$) compared with non allergic subjects ($r = 0.349$; $r = 0.383$) respectively. Simple linear regression analysis showed that morning salivary cortisol (p=0.006) and midnight salivary DHEA (p=0.015), were significantly correlated in allergic subjects with the serum leptin levels concentration.
Conclusion: These results revealed an association between the morning salivary cortisol and elevated serum leptin levels in Jordanian young men with olive pollen induced allergic rhinitis.

Keywords: Allergy; stress; obesity; salivary cortisol; DHEA; leptin; lipid; Jordanian.

1. INTRODUCTION

Olive pollen is one of the most important causes of seasonal respiratory allergy in Mediterranean countries [1]. This can be seen clearly in Jordan, where the prevalence of olive pollen induced-allergy has risen dramatically among Jordanians in the last 10 years [2] [3]. On the other hand, previous studies reported epidemic proportions of type 2 diabetes and cardiovascular diseases in Jordan [4,5,6]. The major risk factors that are directly linked to these three diseases included obesity and stress [7,8,9].

Consequently, some local studies have demonstrated an elevation of obesity hormone leptin in Jordanian male youth with a parental history of diabetes [10] or those with dyslipidemia [4,11]. However, there are no data to support this correlation among Jordanian allergic subjects.Cross-sectional studies on young people and children showed a positive correlation between allergy and obesity [12,13,14] where obesity increases the risk of allergy [15] or they worsen each other [16].

Although it is thought that leptin is an important mediator of airway disease in obesity [17], its mechanism of action have not been clarified.

On the other hand, several studies have correlated allergy with stress levels using salivary cortisol [18,19] and Dehydroepiandrosterone (DHEA) [20] hormones. Both hormones are accurate tools for testing adrenocortical function and stress indicator in infants and young children [21]. Prior studies have conducted under allergens effects, but nevertheless, their observations were controversial in their relation to cortisol. Studies have shown independence [22], negative [23,24], or positive [18,25] correlation of cortisol levels with IgE mediated allergies in different ages. Similarly, despite the high interest in DHEA replacement therapy, as an important immune modulator [26], actual data from human studies are lacking and there were some inconsistent reporting [27,28]. On the basis of associations between obesity and allergy shown in prior reported studies, we hypothesize that diurnal cortisol variability as a marker of stress would be associated with higher BMI and elevated leptin levels in sample of Jordanian young men with history of olive pollen induced allergic rhinitis.

2. MATERIALS AND METHODS

2.1 Study Design and Participants

The present work was a cross sectional study carried out in the Applied Science University, Amman, Jordan during the period from January to April 2010. This study was performed using a protocol for the protection of human subjects approved by the Applied Science University Ethical Committee no DRGS-2007-7. A diagnosis of allergic rhinitis was made according to the [29] via direct interview with medical consultant team at nursing faculty staff. Student also filled out questioner including anthropometric and clinical characteristics. One hundred and thirty nursing students with a mean age of 21.3 ± 1.25 years (range 18–24) categorized into two groups: allergic (n=71) and non allergic(n = 59). The majority of Jordanian nursing students in the college were males (more than 90 %), therefore our study was conducted on male participants. However, female participant students (n=5) were excluded from the study because of the discrepancy between the numbers of males and females. To avoid confounding factors known to affect leptin and salivary hormones levels, subjects with chronic diseases (n=4) such as diagnosed cardiovascular diseases, cerebrovascular disease, dyslipidemia, stable hypertension treated by drugs, chronic hepatic disease, renal problems, or taking any kind of medications during the previous two months were excluded.

2.2 Body Mass Index (BMI)

On the day of evaluation, Height (cm) and weight (kg) and BMI (kg/m^2) of participated students were recorded and then grouped according to the body mass index BMI: normal weight students, 20 ≤BMI <25; overweight students , 25 ≤BMI <30 ; and obese group, BMI >30.

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2.3 Blood Glucose and Lipid Profile

Fasting venous blood samples were obtained, centrifuged and stored at -20℃ until assayed. Fasting blood glucose samples were collected at 8 a.m. Blood glucose was confirmed by using One Touch test strips (Lifescan; Johnson & Johnson, Palmitas, CA). Triglycerides, total cholesterol and high density lipoprotein cholesterol (HDL) was determined using enzymatic colorimetric kits (Linear Chemicals, Barcelona, Spain). Low density lipoprotein cholesterol (LDL-C) was calculated from the equation recorded in a previous study [30].

2.4 Leptin

Fasting serum leptin samples aliquoted and stored in polypropylene vials at −20 ℃ until analysis which was performed after two weeks. Samples were assayed with an enzyme immunoassay kit (DRG Diagnostics, Marburg, Germany), according to the manufacturer's instructions at Al-Khalidy medical centre laboratories, Amman, Jordan.

2.5 Salivary Cortisol and DHEA

Salivary cortisol and DHEA were collected from the Participants at morning between the hours of 8 and 9 AM and at evening between the hours 11 and 12 PM 23:00 p.m and 24:000. To collect salivary samples, participants were provided with a Salivette sampling

device (cotton) along with both verbal and written instructions for usage. The instructions stated that participants were to collect saliva themselves. Participants were asked to drool passively through a straw into a tube, which then were kept on ice in order to precipitate mucins, and then centrifuged (10,000 × g, 15 min, 4 C). The supernatant (1mL) was collected and stored at -20ºC until the day of assay which was after two weeks. Salivary cortisol and DHEA were measured by an enzyme-linked immunosorbent assay (ELISA) (SLV-2930 and SLV-3012, respectively, DRG International, Inc., USA) at Ibn Alhytham Hospital laboratories, Amman, Jordan. The limits of detection of this assay were 1.48 pmol/l for salivary cortisol and 0.324 pmol/l for DHEA.

2.6 Statistical Analysis

The statistical analyses were performed using a statistical software package SPSS , version 19.0 for Windows (Chicago, IL, USA). T test statistical analysis was used to compare the differences of demographic and clinical findings between the means of the two study groups.The Pearson analysis was used to find if there is any correlation between participant's characteristics and serum leptin levels. We used simple linear regression, to evaluate the effect of serum leptin levels as independent variable (IDV) on the salivary hormone (Corisol and DHEA) levels as dependent variables (DVs).

3. RESULTS

A total of 130 male university students participated in the study. Of these participants, 59 (45.4%) currently had olive pollen induced allergic rhinitis and 71 (54.6%) had no history of the disease. Table 1 show the anthropometric and clinical variables of the participants grouped by history of olive pollen induced allergic rhinitis. Among allergic group there was 21 % smokers (n=15), while in the non allergic group the percentage of smokers was 49 % (n=29). The mean of ages for all subjects was 21.98±1.78 years and ranged from 18-24 years.

Values for mean BMI were significantly higher in allergic subjects ($p<0.005$.) compared with non-allergic subjects. Mean leptin levels was higher in allergic group compared to non allergic (13.22 ± 8.89 vs 7.27 ± 4.94) ng/ml, ($p<0.0001$).

The mean (±SEM) values of serum low density lipoprotein cholesterol LDL-C ($p<0.0001$) and total cholesterol ($p=0.001$) in allergic study group was significantly higher than the corresponding mean values in non-allergic group. No significant differences were observed in the mean values for age, weight, height, fasting blood glucose, HDL-C ($p =0.747$) or Triglycerides TG ($p = 0.215$) between the two study groups.

3.1 Correlation of Allergic Rhinitis with Obesity and Lipid Profile

The data indicates that irrespective of the levels of leptin in both groups are mediated by subject's BMI and body weight in both subject groups and the relationship seems to be more stronger in allergic group (pearson correlation coefficient: 0.650 and 0.589 vs. 0.349 and 0.383) respectively (Table 2). Significant correlation ($p=0.002$) was observed between leptin and total cholesterol when the two groups (non allergic and allergic) were combined together (Table 2).

Table 1. Participant's characteristics of the two study groups subdivided by the history of allergic rhinitis (Mean ± SD)

	Non- Allergic Mean ± SD	Allergic Mean ± SD	t-test	p-value
Age(yr)	22.54 ± 1.48	22.61 ± 1.85	3.402	0.01
Fasting blood glucose mg/dl	87.57 ± 6.44	85.98 ± 9.42	1.080	0.282
Weight (kg)	76.23±12.75	82.49±15.17	2.44	0.16
Height(cm)	175.9 ± 5.33	174.84 ± 6.66	- 0.971	0.333
BMI	24.63 ± 2.4	26.85± 4.68	2.914	0.004
Leptin (ng/ml)	7.27 ± 4.94	13.22 ± 8.89	4.723	0.000
TG(mg/dL)	121.42 ± 4.97	134.41 ± 58.56	1.248	0.215
Chol(mg/dL)	166.83 ± 31.35	188.13 ± 30.85	3.536	0.001
HDL(mg/dL)	50.46 ± 8.37	49.9 ± 8.75	- 0.323	0.747
LDL(mg/dL)	92.83 ± 30.41	117.43 ± 29.44	4.241	0.000
Morning Salivary cortisol nmol/L	9.04 ± 3.76	8.71 ± 3.79	- 0.923	0.358
Midnight Salivary cortisol nmol/L	3.86 ± 1.74	4.17 ± 2.00	0.840	0.403
Morning Salivary DHEA nmol/L	1.66 ± 0.49	1.65 ± 0.26	- 0.139	0.889
Midnight Salivary DHEA nmol/L	0.94 ± 0.41	1.05 ± 0.36	1.258	0.212

Table 2. The pearson correlation between participant's, characteristics and serum leptin levels in subjects with and without history of olive pollen – induced allergy and in all participants irrespective of normality of serum level of leptin

	Non Allergic		Allergic		All	
Variable	**r**	***p* -Value**	**r**	***p* -Value**	**r**	***p* -Value**
Age	- 1.33	0.280	0.053	0.733	0.110	0.249
FBG	0.067	0.623	0.064	0.639	0.067	0.463
Weight	0.383**	0.001	0.589**	0.000	0.534**	0.000
Height	0.258	0.034	- 0.063	0.661	0.016	0.862
BMI	0.349**	0.004	0.650**	0.000	0.576**	0.000
TG	0.001	0.993	0.163	0.252	0.146	0.134
Total Cholesterol	0.174	0.222	0.248	0.066	0.294**	0.002
HDL-C	- 0.163	0.259	-0.016	0.911	-0.110	0.264
LDL-C	0.199	0.161	0.241	0.074	0.163	0.185
Morning Salivary cortisol	0.051	0.694	0.403**	0.006	0.188	0.054
Midnight Salivary cortisol	-0.016	0.906	-0.045	0.779	0.006	0.954
Morning Salivary DHEA	-0.125	0.350	-0.047	0.757	-0.072	0.472
Midnight Salivary DHEA	- 0.049	0.729	0.366*	0.015	0.207	0.042

*** Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

3.2 Correlation of Serum Leptin with Salivary Stress Hormones in Allergic Rhinitis Subjects

To establish a relationship between leptin serum levels and subjects' stress hormones, cortisol and DHEA, linear regression analysis was carried out considering the following dependent variables: morning salivary cortisol , midnight salivary cortisol , morning salivary DHEA and midnight salivary DHEA (Table 3). A simple linear regression analysis showed that two levels of all diurnal salivary hormones in this study were correlated with serum leptin levels in allergic subjects (Table 3).The strongest correlation with serum leptin levels was morning salivary cortisol ($p<0.005$) in allergic group. Midnight DHEA also show a correlation with serum leptin in allergic subjects but less than that observed with morning cortisol ($p<0.005$).

Finally our results clearly indicates that serum leptin may explain the variation of the morning salivary cortisol levels in allergic subjects more than midnight salivary DHEA (R^2: 0.403 vs 0.134) Table 3.

Table 3. Effect of serum leptin on stress hormones in study participants stratified by allergic rhinitis

Salivary	Non Allergic : Normal (n=71)				Allergic (n=59)			
Hormone	R2	Slope	SE	P value	R2	Slope	SE	P value
Morning Cortisol	0.003	0.041	0.103	0.694	0.162	0.159	0.55	0.006
Midnight Cortisol	0.000	-0.005	0.045	0.906	0.002	- 0.009	0.032	0.779
Morning DHEA	0.016	-0.013	0.014	0.350	0.002	- 0.001	0.004	0.757
Midnight DHEA	0.049	-0.004	0.011	0.729	0.134	0.014	0.006	0.015

R R^2: R square, SE: Regression Coefficient (SE): Standard Error

4. DISCUSSION

In this cross-sectional study, we found a correlation between the morning salivary cortisol levels and elevated serum leptin of Jordanian young men with history of olive-pollen induced allergic rhinitis. We noted a significant correlation of BMI and body weight to serum leptin levels in all subject groups. Also significant correlations between allergy history of the subject and elevation of LDL and cholesterol levels were observed.

Although some studies have reported that serum leptin levels in allergic rhinitis and mild asthma were similar to normal groups [31] , recent studies have pointed out that increased leptin in obese individuals potentially explains the mechanism by which obesity leads to an exacerbation of asthma [32];[33], but data does not show a scientific firm mediates the link between the two conditions [34]; therefore, we could examine serum leptin, BMI and lipid profile parameters as potential mediators and/or effect modifiers of our observed associations [10];[11]. Elevation of serum levels of LDL-Cholesterol and total cholesterol in obese subjects of our study confirm previous reports [35]; [36]; [37]; [38]. Overall, these findings support our hypothesis that elevated obesity hormone (leptin) levels, associated

with elevated stress hormone levels (cortisol) seems to be associated with cardiovascular risk factors like high LDL and total cholesterol in obese children and adolescents .

The long-term effects of dysregulation of the neuroendocrine stress system is thought to be one potential mechanism through which individuals are predisposed to metabolic disorders [39] like obesity.

A major strength of our study is that it explains for the first time the powerful impact of leptin levels on stress in olive pollen –induced allergic rhinitis (r = 0.159). The physiology of leptin effects on HPA axis may explain these findings. Under stress the action of leptin is augmented by CRH secretion in the lungs [40,41] leading to selective release of proinflammatory and allergic mediators including leptin, which acts on a variety of immune cells that are involved in allergic response [33]. Furthermore, some studies conducted that, leptin may pushes the immune system toward a Th2 pattern by the induction of IL-6 production [42] or via IL-13 secretion [43], which stimulates B lymphocytes to produce IgE [44] . Interestingly, a previous study [45] showed that, obesity, and menopause act on three interrelated mechanisms, where an increase in leptinergic (obesity) signaling relative to HPA signaling would decrease cellular smooth muscle concentration of cAMP and promote muscle contraction in adults with the onset of asthma. This explain why the circadian rhythm of cortisol secretion becomes even more important at morning, since it is well known that the symptoms of asthma worsen overnight particularly in the early hours of the morning [46]. Of course, our study has a number of limitations to be kept in mind for future work. First, although we found significant associations between morning cortisol levels and leptin in allergic subjects we did not measure IgE levels and some involved hypersensitivity mediators like IL-6 and IL-13 .Second, in this study we did not measure serotonin or sex steroid hormones like testosterone where some investigators have suggested that serotonin and reproductive hormones might be involved in the causal pathway. Third, we did not measure expiratory reserve volume (ERV) for participants although our study design was based on allergic rhinitis patients.

In conclusion, our results demonstrated an association between morning salivary cortisol with elevated serum leptin levels in Jordanian young men with rhinitis allergy.

CONSENT

All authors declare that written informed consent was obtained from the participants for publication of this work.

ETHICAL APPROVAL

Ethical approval for the study was granted by the Faculty of Science, The University of the Applied Science Ethics Committee. All experiments and procedures were performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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