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Study of plasma orexin-A level in COPD patients during acute exacerbation

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ABSTRACT

Background: Orexin-A is a neuropeptide that regulates excitement, alertness and appetite.
Aim of the work: To study the plasma level of orexin-A in patients with COPD during acute exacerbation and its relation with both body mass index and body fat percentage in these patients.
Subjects, methods: This study included 80 subjects who were classified into two groups: Group (I): included 50 patients with COPD in acute exacerbation. Group (II): included 30 apparently healthy subjects as a control group. Plasma orexin-A levels, body mass index (BMI), body fat percentage (BFP), oxygen saturation (SaO_2), partial pressure of arterial oxygen (PaO2) and partial pressure of arterial carbon dioxide ($PaCO_2$) were recorded for all patients. Each group was subdivided according to their body mass index into: underweight with BMI less than 18.5), normal weight (BMI more than 18.5 and less than 25) and overweight (BMI more than or equal 25 and less than 30).
Results: Orexin-A was found to be higher in different COPD weight groups (underweight, normal weight and overweight) when compared with those of control group. Overweight patients in group I had higher values Plasma orexin-A when compared with underweight and normal weight in the same group. Orexin-A increased significantly with increased BMI, $PaCO_2$ and BFP and had also significant negative correlation with PaO_2 and SaO_2.
Conclusion: Patients with COPD during acute exacerbation had higher values of plasma orexin-A when compared with normal subjects and plasma orexin-A correlated positively with BMI and BFP in these patients.

Introduction

Chronic obstructive pulmonary disease is common disease that can be prevented and treated. COPD progressively restricts airflow and is associated with chronic inflammatory reaction of the bronchi and the lung to harmful substances [1]. Lean body mass in *COPD* causes weakness in the respiratory muscles and this muscle weakness makes COPD patients at risk of respiratory failure [2]. Orexin-A is a member of a group of orexins which are present in the lateral hypothalamus [3]. Orexin-A administration causes a dose-dependent eating and accelerates metabolism [4]. Orexin-A promotes vigilance and regulate lipid metabolism and it also takes part in diabetes mellitus and obesity [5], with a strong and long lasting effect on appetite. Orexin-A can be measured in humans [3]. In animal studies orexin-A can pass to brain tissue, so elevation of its plasma level might lead to appetite control and energy loss [6]. Previous studies detected low values of orexin-A in COPD, with lower levels more prominent in underweight patients. However, plasma orexin-A is not yet studied in patients with COPD during acute exacerbation [7].

Aim of the work

To study the plasma level of orexin-A in patients with COPD during acute exacerbation and its possible relation with both body mass index and body fat percentage in these patients.

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Subjects and methods

This case-control study included 80 subjects; classified into two groups. Group (I): 50 COPD patients in acute exacerbation, admitted in Damanhur Chest Hospital in the period from October 2016 to June 2017. Their diagnosis was based on history of exposure to risk factor, clinical examination, radiology and spirometry [8]. Group (II): 30 apparently healthy persons as a control group, all of them were nonsmokers. Each group was subdivided according to BMI into: those with underweight (UW) (with BMI less than 18.5), those with normal weight (NW) (BMI more than 18.5 and less than 25), and those with overweight (OW) (BMI more than or equal 25 and less than 30) [9]. Ethical Approval of this study was obtained from the Ethical Committee of the Faculty of Medicine, Benha University. Each subject signed a written informed consent before being enrolled in this study.

Inclusion criteria for group I: COPD patients with increased shortness of breath; the main symptom of exacerbation. Other symptoms include increased phlegm, along with increased coughing and wheezing [10].

Exclusion criteria: Patients with bronchial asthma, obstructive sleep apnea, malignancy, heart failure, diabetes mellitus, other endocrine diseases or patients with psychiatric problems.

All subjects were subjected to the following: 1- History taking. 2- Physical examination (general & local). 3- BMI calculation: BMI = weight in kilograms/(height in meters)^2. 4-BFP: in males = (1.2× BMI) + (0.23× age) −16.2, and in females = (1.2× BMI) + (0.23× age) −5.4 [9]. 5- Laboratory investigations including CBC, blood sugar, liver and kidney function. 6- P-A and left lateral plain chest X-ray. 7- Spirometry pre- and post-bronchodilator. 8- Arterial blood gases (ABG) (for group I only). 9- Measurement of plasma orexin-A level by enzyme-linked immunosorbent assay (ELISA).

Ventilatory function assessment: (Spirometry)

The procedure was performed using Schiller Spirovit SP-10 (USA) according to Miller et al. [11]. The best of three trials was taken. If FEV1/FVC < 70%, reversibility test was done.

Reversibility testing: After a dose of 200 μg salbutamol administered, via pressurized metered dose inhaler, the best of three additional trials after 15 min was recorded. Post-bronchodilator FEV_1/FVC and post-bronchodilator FEV1 were calculated. If post-bronchodilator change in FEV1 < 12%, the patient was considered to have COPD.

Plasma orexin-A measurement

A venous blood sample (2ml) was taken from each subject on ethylene diamine tetra-acetic acid (EDTA). Centrifugation of samples for 10 min at 1000×*g* was done. Plasma was separated and stored at -80 °C. Repeated freeze-thaw cycles were avoided. Hyperlipidemic, icteric or hemolyzed samples were excluded. In-vitro double-antibody sandwich ELISA was used to measure human plasma level of orexin-A in all subjects using a commercial kit; Shanghai Sunred Biological Technology, China (with the assay range; 7 ng/L–2000 ng/L and its sensitivity; 5.125 ng/L). The optical density was measured at 450 nm by TECAN Infinite F50 ELIZA Reader (Singapore) with calculation of orexin-A values via the standard curve by Magellan Tracker software (Tecan Trading AG, Switzerland) [12].

Data management: The data were arranged in tables and further analysis was done by using the computer program SPSS (Statistical Package for Social Science) version 16. Student's *t*-test was used for comparison between two groups and anova "F" test was used if there were more than two groups. Correlations between data were performed by using Pearson correlation coefficient "r" test. P value is considered to be statistically significant if ≤0.05 [13].

Results

Out of fifty COPD patients (group I), 42 were males and 8 were females. The mean age in group I was (62.06 ± 7.59) years. The mean body mass index was (29.41 ± 8.81) kg/m^2. In group II (30 subjects), 28 were males while 2 were females. The mean age of controls was (61.93 ± 6.56) years. The mean body mass index was (24.88 ± 4.36) kg/m^2. There were statistically significant differences between group I and group II as regards body weight, BMI and BFP being statistically higher in group I, however, there was no statistical significant differences regarding age and sex (Table 1). Plasma orexin-A mean level was statistically higher in group I when compared with group II (77.03 ± 26.06 vs. 24.08 ± 10.03 ng/L respectively) (Table 2). In group I; patients were classified according to BMI into: UW nine patients; orexin-A ranged from 25.47 to 88.98 with Mean ± SD (49.19 ± 23.58), NW eight patients; orexin-A ranged from 56.23 to 96.49 with Mean ± SD (72.86 ± 12.31) and OW thirty-three patients; orexin-A ranged from 43.44 to 119.90 with Mean ± SD (85.63 ± 23.86). In group II (according to BMI), there were; two UW persons; orexin-A ranged from 19.55 to 25.07 with Mean ± SD (22.31 ± 3.90), fourteen NW persons; orexin-A ranged from 7.50 to 39.55 with Mean ± SD (23.41 ± 11.06) and fourteen OW persons; orexin-A ranged from 7.09 to 38.75, with Mean ± SD (24.99 ± 9.95). Overweight subjects in both groups (case and control) had higher values of plasma orexin-A levels when compared with those with normal weight, who in turn, showed higher plasma orexin-A levels when compared with those with under weight. Plasma orexin-A levels in OW, NW and UW of the case group were higher than their corresponding subdivisions of the control group (Table 3). In group I,

Table 1
Statistical comparison between studied groups as regards personal data, BMI and BFP.

Studied Variables			Groups		Test	P Value
			Group (I) (n = 50)	Group (II) (n = 30)		
Age (years)		Mean ± SD	62.06 ± 7.59	61.93 ± 6.56	0.076[a]	0.94
Sex	Male	n (%)	42(84.0%)	28(93.3%)	0.762[b]	0.383
	Female		8(16.0%)	2(6.7%)		
Height (meters)		Mean ± SD	1.65 ± 0.06	1.76 ± 0.07	6.58[a]	0.001*
Weight (kg)		Mean ± SD	88.32 ± 18.49	77.33 ± 18.23	2.59[a]	0.012*
BMI (kg/m^2)		Mean ± SD	29.41 ± 8.81	24.88 ± 4.36	3.06 [a]	0.003*
BMI (kg/m^2)	Underweight	n (%)	9(18.0%)	2(6%)	9.357[b]	0.009*
	Normal		8(16.0%)	14(46.7%)		
	Overweight		33(66.0%)	14(46.7%)		
BFP		Mean ± SD	38.55 ± 10.33	19.5 ± 5.99	9.2[a]	0.001*

BFP: body fat percent, BMI: body mass index, [a]: student *t* test, [b]: Chi square test, *: significant

Table 2
Statistical comparison between studied groups as regards plasma orexin-A level.

Studied Variable		Groups		Student "t" test	P Value
		Group (I) (n = 50)	Group (II) (n = 30)		
Plasma orexin-A (ng/L)	Mean ± SD	77.03 ± 26.06	24.08 ± 10.03	10.64	0.001*

* Significant

Table 3
Comparison between underweight, normal weight and overweight subdivisions of the studied groups regarding plasma orexin-A level.

Studied Variables	Group (I) (n = 50)			Anova "F" test	P value
Orexin-A (ng/L)	Underweight (n = 9)	Normal weight (n = 8)	Overweight (n = 33)		
Range	25.47–88.98	56.23–96.49	43.44–119.90	9.459	<0.001*
Mean ± SD	49.19 ± 23.58	72.86 ± 12.31	85.63 ± 23.86		
	Group (II) (n = 30)				
	Underweight (n = 2)	Normal weight (n = 14)	Overweight (n = 14)		
Range	19.55–25.07	7.50–39.55	7.09–38.75		
Mean ± SD	22.31 ± 3.90	23.41 ± 11.06	24.99 ± 9.95	0.113	0.894
P_1	0.015*	<0.001*	<0.001*		

* *Significant, P:* comparison between underweight, normal weight and overweight subjects of the same group, p_1 comparison between underweight, normal weight and overweight cases with their corresponding in the controls.

Table 4
Correlation between plasma Orexin-A with ABG parameters, BMI and BFP in group I.

Studied Variables	Plasma Orexin-A (ng/L)	
	"r"	P Value
PaO_2 (mmHg)	−0.374	0.007*
$PaCO_2$ (mmHg)	0.645	<0.001*
SaO_2 (%)	−0.356	0.011*
BMI (kg/m^2)	0.695	<0.001*
BFP (%)	0.631	<0.001*

PO_2: partial pressure of arterial oxygen, $PaCO_2$: partial pressure of arterial carbon dioxide, SaO_2: oxygen saturation, BMI: body mass index, BFP: body fat percentage, *: significant.

there were statistical significant positive correlations between plasma orexin-A level from one side and BMI, $PaCO_2$ and BFP from the other side but statistical significant negative correlations between plasma orexin-A level and both PaO_2 and SaO_2 (Table 4).

Discussion

In the current study, there was statistical significant difference between group I (cases) and group II (controls) regarding BMI distribution as it was statistically higher in group I ($p < 0.05$). Our result was not matched with that of Maria et al. who revealed that underweight and normal weight were more common in COPD patients than overweight [14]. Our study disagreed with Zhou et al. who performed 2 population-based studies (a cross-sectional study and a subsequent four-year cohort study); they revealed that low BMI is a systemic manifestation of COPD and an important risk factor for the disease [15]. These different results could be explained by the fact that the present study had fewer numbers of cases which may be non-representative. Our study showed that there was a highly statistical significant difference between group I and group II as regards plasma orexin-A level ($p < 0.01$), being higher in COPD patients during acute exacerbation when compared with normal subjects. This result was in agreement with Zhu et al. who studied plasma orexin-A levels in patients with COPD suffering from type II respiratory failure; plasma orexin-A level was higher in patients suffering from COPD when compared with controls [16]. On the other hand, this work disagreed with Matsumura et al. who studied the role of plasma orexin-A in regulating body composition in stable COPD, they stated that plasma orexin-A level was significantly lower in COPD than in control subjects [7]. This difference could be explained by the fact that COPD patients in the current study were hospitalized and in acute exacerbation while COPD patients in the study of Matsumura et al. [7] were not hospitalized and were in stable state. COPD patients during exacerbation had an increased energy use [17] and consume little food when compared with patients with COPD in stable state and as a result there is stimulation of prepro-orexin gene with subsequent elevation of plasma orexin-A level [18]. This disturbed energy balance could explain the higher values of plasma orexin-A in patients with COPD in the present study. In the current study, there was a highly statistical significant difference between cases and controls as regards BFP distribution ($p < 0.01$) as it was statistically higher in group I. Increased BFP in group 1 might be due to increased plasma orexin-A level in group 1 which stimulated glucose influx in 3T3-L1 adipocytes and the increased energy absorption was stored as lipids, orexin-A thus increased lipid formation [19]. It also might inhibit lipid catabolism [20]. Our study showed that there was statistical significant difference between UW, NW and OW subjects in both case and control groups regarding plasma orexin-A level and it was significantly higher in COPD patients ($p < 0.05$). These results were in agreement with Zhu et al. who revealed that plasma orexin-A level in UW, NW and OW subgroups of COPD patients were higher than the corresponding subgroups of normal subjects [16]. The current study coincided also with those of Kawad et al. who revealed that plasma levels of orexin-A and leptin were elevated in overweight children when compared with those who have normal weight. Their study was performed to measure plasma orexin-A and to evaluate its relationship with other anthropometric and metabolic markers. Forty-seven overweight children were included in their study. Plasma orexin-A level was higher in OW children (17.0 ± 0.4 ng/L) than in NW children (13.5 ± 1.1 ng/L) [21]. Our study didn't coincide with Adam et al. who revealed that plasma orexin-A had lower values in overweight persons. In their study, plasma orexin-A and leptin levels were studied in people with BMI between 19.8 and 59 kg/m^2. Obese subjects had lower values of Plasma orexin-A [22]. As an explanation for this difference, plasma orexin-A levels might be affected by the underlying disease

in different studies. In the present study, plasma orexin-A level showed significant positive correlation with BMI in patients with COPD. This result was in concordance with Zhu et al. who revealed that plasma orexin-A level increased significantly with increased BMI in COPD patients with type II respiratory failure [16]. Similar result were obtained by Matsumura et al. who found that orexin-A levels correlate positively with BMI and fat mass values in COPD patients [7]. In the present study, there was statistical significant positive correlation between plasma orexin-A levels and BFP in COPD cases. This result agreed with Zhu et al. who revealed that plasma orexin-A levels correlate positively with BFP in type II respiratory failure COPD patients [16]. This result could be explained by that: 1- In obesity, elevated lipids and triglyceride in peripheral blood led to increased hypothalamic orexin-A gene expression [23]. 2- The presence of orexin-A receptors in fatty tissues with its role in lipid formation [19]. In the present study, there was a statistical positive correlation between plasma orexin-A levels and $PaCO_2$ in COPD patients, a finding that didn't coincide with Zhu et al. [16] and Igarashi et al. [24] who revealed no correlation. Also, Igarashi et al. found no correlation between plasma orexin-A level and $PaCO_2$ in patients with obstructive sleep apnea [24]. These different results could be explained by different patient categories included in these studies. In the current study, there was statistically significant negative correlation between plasma orexin-A levels and both partial pressure of arterial oxygen and partial pressure of arterial carbon dioxide in patients with COPD. Our results were in agreement with Zhu et al. who revealed a negative correlation between orexin-A and both partial pressure of arterial oxygen and oxygen saturation in COPD patients with type II respiratory failure [16]. This result could be explained by the fact that orexin-A has a role in respiratory control by accelerating ventilation [25].

Conclusion

Patients with COPD during acute exacerbation had higher values of plasma orexin-A when compared with normal subjects and plasma orexin-A correlated positively with BMI and BFP in these patients.

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