



Assessment of Serum Level of Ghrelin in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Objectives: to study the relationship between serum level of ghrelin and cachexia in patients with COPD. **Background:** Chronic obstructive pulmonary disease is a major cause of chronic morbidity and mortality throughout the world. COPD involves several systemic features. The link between ghrelin, cachexia and COPD needs to be clarified.

Materials and methods: Serum levels of ghrelin were measured in 60 COPD patients (divided into 3 groups according to their BMI) and 30 control subjects using human ghrelin ELISA kits. **Results:** There was a significant increase in ghrelin level in COPD patients compared to control subjects. There was significant increase in serum ghrelin level in underweight COPD subgroup compared to normal weight and overweight subgroups. Serum levels of ghrelin in COPD patients were affected by the level of pulmonary obstruction. **Conclusions:** Serum level of ghrelin is increased in COPD patients, and is positively correlated with BMI and disease severity.

Received: 20 April 2017

Accepted: 5 June 2017

Available online: 1 July 2017

Keywords

- Ghrelin
- COPD
- Cachexia

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Introduction

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease, characterized by persistent air flow limitation which is usually progressive and associated with enhanced chronic inflammatory response in the airway and the lung to noxious particles and gases (1). Cachexia, which is a catabolic state characterized by weight loss and muscle wasting, occurs frequently in patients with COPD and is also an independent risk factor for death in these patients (2). In addition to excessive energy expenditure on exertive breath and the inflammatory response, patients with COPD, especially those with acute exacerbations, usually have a worse appetite and ingest less food (3, 4). Appetite is regulated by a variety of peptides; one of the most important peptides studied in cases of COPD is ghrelin.

Ghrelin is a 28-amino acid peptide mainly synthesized in X/A cells of the oxyntic glands in the mucosa layer of the gastric fundus. Ghrelin is present in human plasma in two forms: an inactive form known as deacylated ghrelin, and an active form, the acylated ghrelin synthesized under the action of ghrelin O-acyltransferase enzyme (GOAT) (5, 6). The acylation modification provides large hydrophobic groups, which are essential for activation of the growth hormone secretagogue receptor (7).

Acylated ghrelin is a potent stimulator of gastric secretion and motility (8). The circulating levels of ghrelin are elevated during fasting and before meals and decline postprandially, which implies that ghrelin plays a significant role in initiating

food intake (9, 10). Ghrelin is considered an orexigenic signal (gut-brain), that increases appetite and stimulates food intake by activating hypothalamic neuropeptide Y/agouti-related peptide-containing neurons, which also express GHS-R type 1a (GH secretagogue receptor 1a), via the modulation of fatty acid metabolism (11, 12).

Given that ghrelin has GH-releasing activity, ghrelin may have beneficial effects in cachexia associated with COPD patients through a GH-dependent mechanism. As GH is an anabolic hormone, protein stores are spared at the expense of fat during conditions of caloric restriction (13). Meanwhile, as a GH-independent mechanism, ghrelin induces a positive energy balance by decreasing fat utilization (14), stimulating food intake and adipogenesis and lipogenesis in murine and human adipocytes (15), increasing cardiac output in healthy humans (16), and inhibiting sympathetic nerve activity (17).

Due to the above actions, ghrelin exhibits anti-cachectic actions and used in the treatment of cachexia caused by a variety of diseases including chronic obstructive pulmonary disease. Nagaya et al., studied the effect of exogenous ghrelin administration in cachectic patients with COPD and they found that repeated administration of ghrelin stimulated feeding, increased body weight and lean body mass and increased peripheral and respiratory muscle strength (18).

All these data suggest that we have to clarify the role of ghrelin in cases of cachexia associated with COPD patients. So, the aim of this work was to study the relationship between serum level of ghrelin and cachexia in patients with COPD.

Subjects and methods

The present study was conducted on 60 diagnosed patients with COPD admitted to Chest Department, Menoufia University Hospitals and 30 age matching healthy persons were used as a control group.

After having a written consent, each patient underwent:

1. Medical history taking.
2. Physical examination (general and local).
3. Electrolytes and lipid profile.
4. Routine laboratory investigations: Complete blood count, liver profile, kidney profile and plasma glucose level.
5. Plain Chest x ray (posterior-anterior and lateral views).
6. Measurement of pulmonary functions for group I to assess severity of COPD using VIASYS spirometer: these include the forced expiratory volume in the first second (FEV1) pre and post administration of a bronchodilator, forced vital capacity (FVC), FEV1/ FVC ratio and peak expiratory flow rate.

- Morning pre-bronchodilator spirometry found that the FEV1/ FVC ratio is less than 70% in all COPD group.

- Post-bronchodilator spirometry of the FEV1 after 15 minutes of inhalation of 400 µg salbutamol delivered by metered dose inhaler, observed that the improvement of the FEV1 didn't exceed 12% (19).

- Patients were classified according to GOLD classification (1) (based on post-bronchodilator FEV1) into:

- Stage I: mild COPD FEV1 \geq 80% normal.
- Stage II: moderate COPD FEV1 50-79% normal.
- Stage III: sever COPD FEV1 30-49% normal.
- Stage IV: very sever COPD FEV1 <30% normal, or <50% normal with chronic respiratory failure present.

7- Measurement of body mass index (BMI). Already diagnosed COPD patients were subdivided into three subgroups according to body mass index:

- a) Normal weight COPD patients with BMI 18.5-24.9 (20 patients).
- b) Overweight COPD patients with BMI over 25 (20 patients).
- c) Underweight COPD patients with BMI less than 18.5 (20 patients).

8. Serum ghrelin level was measured for all subjects using Human Ghrelin Enzyme Immunoassay Kit (20).

Statistical analysis

The data collected were tabulated & analyzed by SPSS (statistical package for the social science software) statistical package version 20 on IBM compatible computer. Quantitative data were expressed as mean \pm standard deviation ($X \pm SD$) and analyzed by applying t test for comparison between two groups of normally distributed variables, while for comparison between two groups of not normally distributed variables Mann-Whitney test was applied and Kruskal–Wallis test

for comparison between more than two groups. Qualitative data were expressed as number and percentage (No & %) and analyzed by applying chi-square test and for 2×2 table and one cell has expected number less than 5 fisher's exact test was applied. Spearman correlation was used for no normally distributed quantitative variables or when one of the variables is qualitative. P value > 0.05 is considered insignificant.

Results

The range of age for COPD patients group was ranged between 44 to 69 years with mean value of 56.48±6.55 years and for controls group was ranged between 40 to 67 years with mean value of 54.3±8.88 years. The range of BMI for COPD patients group was between 16.4 to 31kg/m² with mean value of 23.06±4.42 kg/m² and for controls group was between 21.1 to 24.6 kg/m² with mean value of 23.59±0.95 kg/m². Also the table shows that there were 58 male patients and two females among the COPD patients while among control subjects there were 27 males and three females. Statistically there were no significant difference between both COPD patients and controls as regards their age, BMI and sex (p > 0.05) Table I.

In the COPD patients group, 48 patients were smokers and 12 patients were ex-smoker and in control group there was 23 smokers and 4 nonsmokers. There was non significant difference between COPD patients and controls as regards their smoking status (p > 0.05) Table II.

There was an increase in the ghrelin level in COPD patients compared to control group. The mean ± standard deviation of ghrelin level in COPD patients was 744.16±686.30 (ng/L) and that

of normal subjects was 0.22 ±0.05 ng/L. This difference was highly significant (p < 0.001)

Table III.

The ghrelin level in underweight COPD patients' subgroup was increased compared to either the normal weight or the overweight subgroups. The mean ± standard deviation of ghrelin level in COPD subgroups was 1527.25±467.2 ng/L in underweight cases, 697.26±161.12 ng/L in normal weight cases and was 7.97±7.33 ng/L in overweight cases. This difference was statistically highly significant (p < 0.001) Table IV.

There was highly significant difference between serum levels of ghrelin in correlation to level of obstruction as the highest level was detected in severe obstruction and decreased as obstruction get better Tables V and VI.

Discussion

This work was carried out on 60 diagnosed patients with COPD. They were subdivided into three equal subgroups according to body mass index: Normal weight COPD patients with BMI 18.5-24.9, overweight COPD patients with BMI over 25 and underweight COPD patients with BMI less than 18.5. 30 non COPD, age matching healthy persons were studied as a control group. Serum ghrelin was measured for all subjects.

In the present study despite there is no significant difference between both groups as regard cigarette smoking and this may be due to group selection, still smoking one of the most important risk factors for development of COPD as it produces a large amount of reactive oxygen species (ROS) that induces an oxidant burden in smokers (21–25) and disrupts the oxidant–antioxidant mechanisms that

Table (I): Comparison between the studied groups as regards age, BMI and sex

Parameters	The studied groups				Test of significant	P Value
	COPD N = 60		Controls N = 30			
Age (years) X ± SD Range	56.48±6.55 44 – 69		54.3±8.88 40 – 67		t- test 1.19	0.24
BMI (Kg/m ²) X ± SD Range	23.06±4.42 16.4 – 31		23.59±0.95 21.1–24.6		t- test 0.88	0.38
	No	%	No	%		
Sex						
Male	58	96.7	27	90	FE	0.33
Female	2	3.3	3	10	1.69	

X = Mean, SD = Standard Deviation, t = Student t test and FE = Fisher Exact test

Table (II): Comparison between the studied groups as regards their smoking status

Smoking	The studied groups				X ²	P value
	COPD patients N = 60		Controls N = 30			
	No	%	No	%		
Smoker	48	80.0	23	76.7	4.15	0.13
Ex smoker	12	20.0	5	16.7		
Non smoker	0	0.0	2	6.7		

X² = Chi square

Table (III): Comparison between the studied groups as regards their serum ghrelin level (ng/L)

Ghrelin level (ng/L)	The studied groups		U test	P value
	COPD patients N = 60	Controls N = 30		
X ± SD	744.16±686.30	0.22 ±0.05		
Range	1.33 – 2122	0.15 – 0.31	7.71	<0.001

X = Mean, SD = Standard Deviation and U= Mann Whitney U test

Table (IV): Comparison between serum ghrelin levels (ng/L) among COPD patient's subgroups

Ghrelin level (ng/L)	COPD patients sub groups			K test	P value
	Underweight N = 20	Normal weight N = 20	Overweight N = 20		
X ± SD	1527.25±467.2	697.26± 161.12	7.97±7.33	51.1	<0.001
Range	740.5 – 2122	310 – 910.58	1.33 – 23.11		

X = Mean, SD = Standard Deviation and K = Kruskal Wallis test

Table (V): Correlation between serum ghrelin level (ng/L) and degree of pulmonary obstruction according to the FEV1%

	Ghrelin level (ng/L)	
	R	P value
Level of obstruction	+ 0.57	<0.001

(r) = Spearman correlation

Table (VI): Correlation between serum ghrelin serum level (ng/L) and level of pulmonary obstruction among COPD patient's subgroups

Level of obstruction	Ghrelin level (ng/L) among COPD patients subgroups			
	Underweight N = 20	Normal weight N = 20	Overweight N = 20	Total N=60
	X ± SD	X ± SD	X ± SD	X ± SD
Mild obstruction	886.7±126.6	590.5±396.6	1.7±0.5	768.2±271.4
Moderate obstruction	1547.6±423.3	770.3±51.8	11.5±9.4	1160.2±675.4
Moderate to severe obstruction	1690.7±388.8	684.7±141.7	6.45±4.8	743.9±660.0
Severe obstruction	2122.0	640.9	-----	235.6
Very severe obstruction	-----	910.6	-----	910
K	6.43	4.25	4.61	14.1
P value	0.09	0.37	0.10	0.007

K = Kruskal Wallis test

plays a major role in triggering and promoting chronic inflammation of airways occurring in COPD (26).

ROS induced by cigarette smoke were also involved in the alterations in mitochondrial network morphology present in the airway smooth muscle and airway epithelial cells (27) these alterations causes mitochondrial dysfunction which has been found in oxidant-induced lung damage of COPD patients exposed to chronic cigarette smoke (28).

Our study revealed that, serum ghrelin level was significantly higher in COPD patients than control subjects. Serum ghrelin level was higher in underweight subgroup than normal weight and in normal weight higher than overweight subgroup. The results suggested that the plasma ghrelin level is elevated in response to a cachectic state and ghrelin may have a role in the compensatory mechanism in malnutrition status of COPD.

This is in agreement with previous studies that showed increased ghrelin concentrations in COPD

group compared to the control subjects and in underweight COPD than in normal weight or obese patients with COPD (29 - 31).

It is increasingly recognized that COPD involves several systemic features, particularly in patient with severe disease (32). Cachexia is defined as a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. Anorexia, inflammation, and increased muscle protein breakdown are frequently associated with cachexia (33). Cachexia occurs frequently in patients with COPD (2).

Ghrelin is a natural ligand for the growth hormone (GH)-secretagogue receptor 1a (GHSR1a). It induces a positive energy balance and weight gain by decreasing fat utility and stimulating appetite through stimulation of GH (34). Ghrelin strongly stimulates GH secretion in humans, several folds more potently than GHRH (35). GH regulates IGF-I levels, promotes anabolism, and increases muscle strength (36).

In a multicenter, randomized, double-blind, placebo-controlled trial conducted to investigate the efficacy and safety of adding ghrelin to pulmonary rehabilitation (PR) in cachectic COPD patients it showed that ghrelin administration improved symptoms and respiratory muscle strength. While the results were considered most likely to have been due to ghrelin treatment, the precise mechanism that underlies the improvement of exercise performance or symptoms remains unclear. They suggested that repeated ghrelin administration may have beneficial, sustained effects after administration on symptoms through

GH-dependent and/or –independent mechanisms (37).

Results of the present study reveal a significant positive correlation between serum levels of ghrelin and the level of obstruction as the highest level was detected in severe obstruction and decreased as obstruction get better. It is well known that severe cases of COPD are associated with severe inflammatory process and weight loss.

Pulmonary and systemic inflammation might contribute to weight loss in patients with COPD (38). The inflammation causes activation of inflammatory cells and release of various inflammatory mediators such as IL-8, IL-6 and TNF- α . These mediators can destroy lung structure and promote the inflammatory response of neutrophils (39, 40). Both ghrelin and its receptor were shown to be expressed in human immune cells, and ghrelin acts on both immune cells and endothelial cells to block the expression of pro-inflammatory cytokines induced by inflammatory stimuli such as leptin, TNF- α and endotoxins (41, 42). These data ensure the anti-inflammatory effect of ghrelin in patients with COPD and provide a GH-independent mechanism to prevent weight loss.

Xu et al., found that plasma ghrelin was negatively correlated with BMI and percentage of body fat in the COPD patients and was positively correlated with TNF- α and IL-6 in the underweight patients (30). Also Deveci et al found that TNF- α and IL-6 levels were statistically higher in COPD patients and for cachectic COPD patients but serum ghrelin levels were statistically lower; so there was a positive correlation between serum ghrelin levels and body mass index in patients with COPD (43).

It has been reported also that the systemic inflammatory response increases the activity of neurons expressing pro-opiomelanocortin and release of melanocyte-stimulating hormone (MSH), resulting in decreased appetite and increased degradation of nutrients or tissue. The melanocortin system is a key site for the action of ghrelin, where agouti-related peptide competitively inhibits activation of MSH on downstream neurons and hence ghrelin increases the appetite (44).

On the other hand reduced ghrelin secretion in obese patients was found to be an adaptive mechanism to a long-term positive energy balance. Although circulating plasma ghrelin levels are low in obese people, a lack of postprandial ghrelin suppression was observed, which could contribute to increased food intake in these people (45).

Conclusions

From the present study we can conclude that serum level of ghrelin is increased in COPD patients, it was higher in the underweight patients than in the normal weight patients with COPD and have significant relation to the severity of COPD. So serum ghrelin level may be a useful indicator for malnutrition in COPD patients and may be involved in the pathogenesis of COPD by affecting the body energy metabolism.

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