Serum Leptin and Nitric Oxide in Chronic Obstructive Pulmonary Disease

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ABSTRACT

Objective: *Unexplained weight loss is common in patients with chronic obstructive pulmonary disease (COPD). Leptin not only is a critical regulator of body weight and appetite, but also serves as an immune-modulator. Nitric oxide (NO) is a potent relaxant of bronchial and pulmonary artery and leptin has a regulatory role in its synthesis. In the present study, the association of serum leptin levels with nitric oxide metabolites (NO) in COPD was investigated.* ***Methods****: Serum leptin and NO levels were measured in COPD patients [males (n=18) and females (n=15)] above forty years old compared with control group (n=30) in the same age matched group. Serum leptin levels were measured by enzyme linked immune sorbant assay (ELISA) technique. NO level was measured by spectrophotometric method.* ***Results****: Serum mean leptin level was significantly lower in COPD male group (9.7 ± 4.1 pg/ml) and female group (11.1 ± 3.7pg/ml) than corresponding control group (male: 12.7 ±1.4 pg/ml and female: 15.1 ± 1.5pg/ml) (p <0.01 in both). Also, serum nitric oxide (nitrite and nitrate) in COPD male (18.4 ± 3.7 μmol/L) and female group (15.9 ± 5.6 μmol/L) which was lower than corresponding control group (male: 21.2 ±1.9 μmol/L and female: 24.2 ± 2.5 μmol/L) (p <0.01 in both).* ***Conclusions****: low serum leptin associated with COPD is related with low BMI. Associated low nitric oxide serum level may be related to the pathogenesis of COPD.*

Abbreviations:

BMI *Body mass index*
COPD *Chronic obstructive pulmonary disease*
ELISA *Enzyme linked immune sorbant assay*
NO *Nitric oxide*

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive disease characterized by a decrease in the airways diameter that increases their resistance to airflow[(1)]. Unexplained weight loss, commonly observed in patients with COPD, is clinically important because it is an independent risk factor of mortality in these patients[(2)]. Cachectic patients with COPD show abnormalities of the autonomic nervous system, neuroendocrine function, and energy expenditure[(3)]. However, its pathophysiologic mechanisms are poorly understood[(4)].

Leptin is a peptide hormone secreted mainly by white adipose

tissue. Its level is regulated by several factors as starvation and hormones such as insulin[5] and ovarian sex steroids[6] stimulate leptin secretion. Also, Glucocorticoids[7] and testosterone[6] have an inverse relationship with leptin. It acts on hypothalamic centers to regulate food intake and energy expenditure. Leptin is, also, involved in the regulation of various other physiological processes including carbohydrate and lipid metabolism, gastrointestinal and cardiovascular function, inflammation, immune response and reproduction[8]. Serum leptin concentration is proportionated to the amount of white adipose tissue and is markedly increased in obese individuals[9].

The reported rise of blood leptin concentrations following acute infection and in chronic inflammation suggests that leptin may actively participate in the immune network and host defense[9]. Indeed, leptin levels are rapidly increased by many acute phase cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6)[10].

Nitric oxide (NO) is produced by endothelial cells and the terminal guanidine nitrogen atom(s) of L-arginine are the physiological precursors of endothelium-derived NO[11]. ***Masaki et al. (1989)*** showed that endogenous NO was a potent relaxant of isolated canine tracheal and bronchial smooth muscle and pulmonary artery[12]. NO is thought to relax smooth muscle by increasing cGMP[13] or by opening Ca^{2+}-activated K^+ channels which promotes a relaxation response[14]. Endogenous NO may attenuate mast cell mediator release, resulting in reduced leukocyte adhesion and vascular leakage in inflammatory airways disease[15].

Although leptin receptors are expressed in endothelial cells[16] but its effect on NO generation is controversial. In vitro, leptin stimulates endothelial NO production[17,18] and induces NO-mediated smooth muscle relaxation[19,20]. The results of in vivo studies are less clear. Some authors have observed the involvement of NO in leptin-induced vasodilatation[21], whereas others have not[22,23]. In addition, leptin may induce NO-independent smooth muscle relaxation[21]. ***Frühbeck and Gomez-Ambrosi (2001)*** observed increased plasma concentration of nitric oxide metabolites (nitrites and nitrates) following leptin administration[24]. Apart from endothelial cells, leptin may stimulate NO synthesis in other tissues such as adipocytes[25], macrophages[26] and the central nervous system[27]. In addition, plasma NO level is affected by factors other than NO production such as dietary nitrates and renal excretory function.

The present study was undertaken to investigate the association of plasma leptin levels and NO metabolites (nitrites plus nitrates) levels in COPD.

PATIENTS & METHODS

The study population included 33 patients with COPD (18 males and 15 females) and 30 healthy persons as a control group, of average age (> 40 years). The patients are with clinically stable mild to severe COPD (FEV_1

30-80% predicted) (GOLD **I-III**)[28]. Forced expiratory volume in 1s (FEV1) was calculated from the flow volume curve using a spirometer (Jaeger SN.:-692669 German) applied before and 15 min after inhalation of a β_2-agonist via a metered-dose inhaler. FEV_1 was expressed as a percentage of reference values[29]. Exclusion criteria included cardiovascular diseases, renal, metabolic, hormonal diseases, smoking, alcohol or receiving any drug therapy such as lipid lowering drugs, vitamins, antioxidants, or antihypertensive drugs. Past history of respiratory disease other than COPD was excluded. The studied population was taken from outpatient clinic of the respiratory diseases, Sohag University Hospital, Sohag Faculty of Medicine, Sohag University, in the period from March 2006 to January 2007.

Sample Collection and isolation of plasma

Blood was obtained from all subjects in the overnight fasting state (from 9:00 PM on the previous night) by venipuncture at 9.00 AM. Blood was collected in glass tubes containing ethylenediaminetetra acetic acid. Blood was centrifuged at 1600*g* for 15 min at 4°C, and plasma was collected and stored at -70°C until analysis.

Leptin Assay

The DSL-10-23100 ACTIVE® Human Leptin ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay (30).

Nitric oxide metabolites Assay

NO levels were measured as the concentration of nitrate plus nitrite in the plasma in two step process. The first step is the reduction of nitrate (NO_3) to nitrite (NO_2) by nitrate reductase. The second step is the addition of the Griess Reagent which converts nitrite into deep purple azo compound. The sample was deproteinized with ZnSO4, and the concentration of nitrite was measured spectrophotometrically at 540 nm using the Griess reaction with commercial kit (Cayman chemical, catalog No 780001, Germany).

Statistical Analysis:

Values were expressed as mean, medians (range) and ± standard deviation (SD). For both serum leptin and NO in comparisons of controls and COPD patients, unpaired Student's *t*-test was used. Differences with p values≤ 0.05 were considered significant by using SPSS software (release 10.0).

RESULTS

Thirty three patients (male n= 18 and female n=15) above forty with mild to severe COPD (FEV_1 range 30-80% predicted) were included in the present study. The average age in years in COPD was 40.1 - 48 in male and 40 -49 in female and in control group was in male 40.1 - 48 and in female 40.2–55.1. Baseline characteristics are shown in table 1

Table (1): Demographic and clinical data of the COPD patients and control group

	Control (n= 30)		COPD (n= 33)	
	Male	**Female**	**Male**	**Female**
Age	43.3 ± 2.6	44.5 ± 4.2	43.3 ± 3.7	42.2 ± 2.2
Weight	67.4 ± 5.9	97.1 ± 7	60.2 ± 10.6*	70.1 ± 10*
Height	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.03	1.7 ± 0.1
BMI (Kg/M^2)	25.1 ± 2.1	28.1 ± 3.6	19.2 ± 3.5 *	21.4 ± 3.2 *
Ex-smoker	-	-	14	-
(%)FEV_1	83.8 ± 2.8	82.4 ± 1.9	42.1 ± 5.3*	41.7 ± 4.3*
(%)FVC	99.1 ± 4.6	96.3 ± 3.7	67.5 ± 2.4 *	67.5 ± 2.3 *
FEV1/FVC	85 ± 6	86 ± 3	62 ± 8*	62 ± 7*

Age in years, weight in Kg, height in meter
** means that P <0.05 between these value and corresponding same sex in control group).*

Leptin was detected in serum samples of control group and COPD patients (fig.1A). The mean value of serum leptin was significantly lower in COPD patients (10.3 ± 3.9 ng/ml) than that in control group (13.9 ± 1.9 ng/ml). Also, the mean of serum NO was significantly lower in COPD patients (16.9 ± 5.2µmol/L) than that in control group (22.7 ± 2.6µmol/L) (figure 1B).

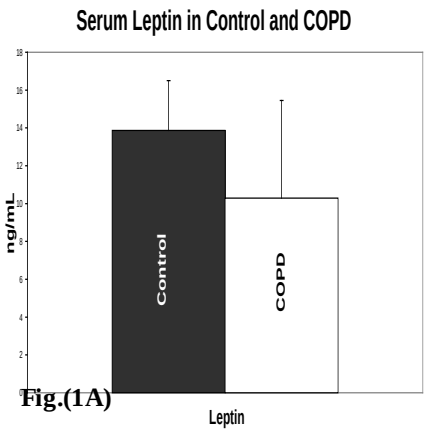

Fig.(1A)

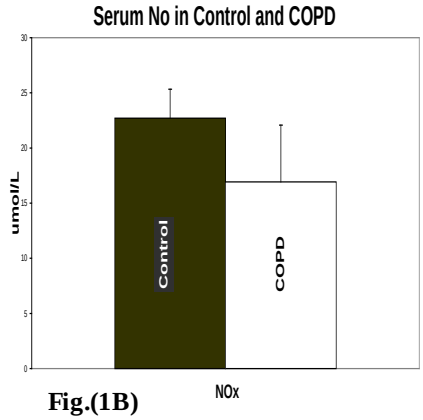

Fig.(1B)

Figure (1): Serum leptin (1A) and NO (1B) in control (Black columns) and COPD (white columns) groups.

In COPD patients, serum leptin and NO levels were significantly lower in both male and female groups compared to the mean of both male and female control group (Table 2). To compare the serum leptin level to the change in the BMI, levels of serum leptin was divided on BMI.

Leptin / BMI of each case and that value had no significant change in male and female control group compared to the corresponding sex of COPD group (Table 2)

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Table (2): Biochemical characters of Control and COPD groups.

	Control		*COPD*	
	Male	*Female*	*Male*	*Female*
Leptin	12.7 ± 1.4	15.1 ± 1.5	9.7 ± 4.1*	11.1 ± 3.7*
Leptin/BMI	0.4 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.2
No	21.2 ± 1.9	24.2 ± 2.5	18.4 ± 3.7*	15.9 ± 5.6*

Serum leptin in ng/ml, NO in µmol/L.
** means that P <0.05 between these value and corresponding same sex in control group.*

DISCUSSION

Recent studies point to the role of bioactive mediator secreted by white adipose tissue such as leptin. The short forms of leptin receptor are expressed in choroid plexus, kidney, gonads, liver, lung and vascular endothelium, where they mediate leptin transport into the brain[8]. The role of leptin on the lung was demonstrated to have a pro-inflammatory effect in the lung, although such effects have been reported in other systems ***(31 and 32)***, and also promotes lung growth[33].

Level of serum leptin was for a long time controversially associated with COPD patients. ***Noriaki et al. (1999)*** and ***Takabatake et al. (1999)*** found that serum leptin values were significantly lower in patients who had COPD than in control subjects[34&35]. But, ***Creutzberg, et al. (2000)*** found that plasma leptin was elevated in exacerbating COPD patients[36]. Leptin regulates appetite and increased circulating leptin may contribute to the anorexia observed in these patients. Leptin is, also, directly up-regulated in inflamed COPD lung[16].

High serum leptin levels and obesity (high body weight) are associated with female than in male in both control and COPD. Serum leptin is produced by adipose tissue leptin, so its level is higher in women than in men[37]. Also, the expression of serum leptin is higher in female than male and this may be due to the effect of sex hormones where it can be inhibited by testosterone and increased by ovarian sex hormones[6].

Circulating leptin levels are lower in COPD patients compared with healthy controls but that level was associated with low body weight probably due to reduced white adipose tissue. Apart from its satiety effect, leptin is, also, recognized to induce an inflammatory response[38]. Elevated serum leptin in COPD patients was partially explained by acute exacerbation of COPD[36] or systemic glucocorticosteroid administration[39]. The low serum leptin in COPD may be related to have one of the following reasons. First, hypoxic COPD patients were found to have impaired glucose

tolerance and hyperinsulinemia[5]. Second hypoxic gonads and low female sex hormone[6] but it is most probably related to low body weight. In addition, leptin potentates expression of nitric oxide synthase in murine macrophage[40].

The significant association between low total NO and COPD may be related to the pathogenesis of COPD. Bronchial constriction[41], increasing of pulmonary vascular resistance with pulmonary hypertension[42] and decreasing in ciliary beat frequency[43] with accumulation of intra-bronchiolar stick secretion are the pathology with COPD. NO had many pharmacological effects on the airways, first, it relaxed the pulmonary artery which preconstricted with hypoxia[12]. NO was shown to induce pulmonary vasodilatation[44].

Endogenous NO modulates cholinergic neurotransmission by inhibiting acetylcholine release[41] or reducing the release of cyclo-oxygenase products[40] to perform its bronchodilator action on airway smooth muscle. Nonselective β_2-adrenoceptor agonist isoprenaline was shown to up-regulate the ciliary beat frequency by a NO-dependent mechanism[43]. An increase in ciliary beat frequency would aid in the clearance of harmful substances contained within the mucus mesh.

In conclusion, in spite of low circulating leptin in COPD, that change is related to low BMI. Low serum leptin is associated with low concentrations of serum NO in these patients. Low level of serum NO (not leptin) has a role in the pathogenesis of COPD. Role of serum NO in the prognosis and progression of COPD needs further study.

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دور هرمون اللبتن وأوكسيد النيتريك فى حالات ضيق الشعب الهوائية المزمن

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قسمي الكيمياء الحيوية الطبية*و الأمراض الصدرية**بكلية الطب البشرى– جامعة سوهاج

إن من أهم مظاهر ضيق الشعب الهوائية المزمن الدال على تطور هذا المرض هو نقصان الوزن المستمروحيث أن هرمون اللبتن من أهم الهرمونات التى لها دور ليس فقط فى التحكم بالوزن من خلال تأثيره على بعض مراكز المخ بل له دوراً أيضاً فى تنشيط الجهاز المناعى. أما أوكسيد النيتريك فله دور في ارتخاء الشعب الهوائية والشريان الرئوى والذى يعتمد فى تخليقه على هرمون اللبتن.قد اجريت هذه الدراسة لمعرفة تأثير ضيق الشعب الهوائية المزمن على هرمون اللبتن وأوكسيد النيتريك من خلال إختيار عدد ثلاثة وثلاثون مريضاً يبلغ أعمارهم أكثر من الأربعين عاماً بعد عمل كافة الفحوصات والتحاليل للتأكد من وجود ضيق بالشعب الهوائية المزمن لديهم ومقارنتهم بعدد ثلاثون متبرعاً من الجنسين.قد وجدو انخفاضا ملحوظا في هرمون اللبتن وأوكسيد النيتريك فى مرضى ضيق الشعب الهوائية المزمن عن غير المصابين.قد تم مقارنة هرمون اللبتن بوزن كل مريض على حدة ووجد أن هذا النقص فى الهرمون يمكن إرجاعه إلى نقص فى وزن المريض المصاحب لهذا المرض وقد صاحبه نقصفي أوكسيد النيتريك بالدم الذى يعتقد أن له دوراً فى حدوث ضيق الشعب الهوائية المزمن.