Research Article

Effects of Serum Leptin on Lung Functions in Obese and Non Obese Adults

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Abstract |

Background: Leptin is a hormone produced by adipose tissue. Its level is directly associated with the total mass of fat cells in the body. Higher leptin levels are associated with decreased lung functions.

Objective: To determine serum leptin levels in our obese and non obese population and to correlate the body mass index (BMI), and serum leptin levels with pulmonary function tests (PFT's).

Methods: This case control analytical study was conducted at the Institute of Chest Medicine, King Edward Medical University, Lahore, Pakistan. One hundred and twenty individuals – 60 obese (30 males, 30 females) 60 non-obese (30 males, 30 females) fulfilling the inclusion criteria were enrolled through non-probability purposive sampling. The data was collected from the medical students, employees of KEMU/Mayo Hospital and the attendants of the patients presenting to the hospital. Informed written consent was taken from all subjects. The demographic information of these subjects like name, age, sex, height, weight, and BMI were recorded. Spirometry of all the subjects was performed on Spirolab iii. Fasting blood samples were taken for the measurement of serum leptin levels.

Results: The mean serum Leptin in obese was 16.59 ± 13.56 ng/ml and in non-obese cases,it was5.58 ± 6.30 ng/ml with p-value < 0.0001.In obese cases significant negative correlation was found in serum leptin with Forced Vital Capacity (FVC) (r = - 0.459, p-value < 0.0001) and with Forced Expiratory Volume (FEV1) (r= - 0.369, p-value 0.004).

Conclusions: It is concluded that serum leptin level was higher in obese subjects as compared to the non-obese subjects and increase in serum leptin level was associated with decrease in lung functions (FVC and FEV1).

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Introduction

Leptin is a 16kDa hormone which is non glycated protein having 167 amino acids. It is mostly produced in the adipose tissue.[1] The gene responsible for its production is the obese gene (Obgene) which is located on the chromosome number 7 in human fat cells. Main function of Ob gene is to control the balance between intake of food and energy utilization.[2] Other than the fat tissue, leptin is also produced in the gastric fundic mucosa, placenta, type II alveolar pneumocytes and lung macrophages. When leptin is released in blood, it circulates till it reaches its receptor (Ob-Rb) present mostly in the hypothalamus and some in other tissues of the body.[3] Hypothalamus has two types of neurons which

respond to the levels of leptin. Type 1 neurons synthesize the appetite suppressing peptides such as alpha melanocyte stimulating hormone.[4] Type 2 neurons synthesize the appetite stimulating peptides such as neuropeptide Y. By communicating with these two neurons in the hypothalamus, leptin regulates food consumption and the energy utilization of the body. When the serum leptin rises, it decreases appetite by activating type 1 neurons to produce the appetite suppressing peptide. The central leptin receptor activation also enhances sympathetic system which stimulates energy utilization in adipose tissue.[5] Conversely, during starvation the fat mass decreases which lowers the leptin levels and reduce the energy expenditure thus restoring the fat depots. This is how leptin maintains the balance in a relatively narrow range between the food intake and the energy expenditure thus avoiding starvation and overeating.[6]

Serum level of leptin is directly related to the mass of adipose tissue present in our body. In obese people the serum leptin levels are increased on account of presence of large amount of fat cells in their body. Major role of leptin is to send a message to the brain about the fat stores of the body so that it can make necessary changes in energy intake and energy expenditure.[7] Increased leptin should result in less intake of food and more utilization of fat stores. But this hyperleptinemia fails to do so in obese subjects and this state has been called the leptin resistance. Leptin resistance appears to be common in extremely obese persons but rare in nonobese. The arcuate nucleus of hypothalamus is the main site of leptin resistance because it is most sensitive to the circulating leptin.[8]

Leptin hormone is also produced by alveolar type II pneumocytes and lung macrophages. The leptin receptors that are mostly present in hypothalamus are also found in lung tissues and alveolar cells. Studies have shown that leptin acts as a respiratory stimulant and also takes part in lung development. This hormone is essential for the postnatal develop-ment of lungs and the remodeling of parenchymal tissue. It is also associated with increased surface area of the alveolar tissue thus affecting both the structural and functional ability of respiratory system. In a mature lung, leptin acts as a stimulant of ventilation exhibiting increased breathing, minute ventilation and tidal volume. It is also associated with increased arterial Partial Pressure of Carbon dioxide ($PaCO_2$) and decreased hypercapnicventilatory response even before the onset of obesity.[9]

Spirometry is the best technique to determine the lung functions and to diagnose and treat different respiratory complications. This method is used to measure few lung functions such as Forced vital capacity (FVC) which is the volume of air forcefully exhaled from the point of maximum inspiration. It also measures Forced Expiratory Volume (FEV1), the volume of air expired during the first second of the above mentioned procedure. The FEV1/FVC ratio is calculated by computing both measurements. The instrument used in spirometry is called spirometer.[10]

In this study, we investigated the association between total body fat, and serum leptin level with the dynamic lung volumes (FVC, FEV1). This study will help in understanding the relationship of obesity and serum leptin levels with the respiratory problems in Pakistani population.

Patients and Methods

This case control analytical study was conducted at the Institute of Chest Medicine, King Edward Medical University Lahore, Pakistan. Sample size was One hundred and twenty individuals – 60 obese (30 males, 30 females) 60 non-obese (30 males, 30 females). Sample size was calculated by taking confidence level=95%, power = 90% and mean difference for spirometric parameters for obese and non-obese individuals (obese 3.55 + 0.36 L, non-obese 3.75 + 0.28 L).[11] it was non-probability purposive sampling.

The study groups were divided into obese and non-obese groups. Subjects 20-40 years of age, Non - smokers, healthy, mentally fit of both genders were included. Body Mass Index (BMI) values between 18.5 and 22.9 Kg/m^2 were considered non- obese. BMI greater than or equal to 25 kg/m^2 was considered obese. Subjects with history of asthma, chronic bronchitis, interstitial lung disease, respiratory tract infection or on treatment of any of these conditions and those who were unable to complete spirometry were excluded from the study. The data was collected at the Institute of Chest Medicine, King Edward

Medical University/ Mayo Hospital, Lahore from the medical students, employees of KEMU/ Mayo Hospital and the attendants of the patients presented to the hospital. Informed written consent was taken from all subjects. Subject's identity was kept confidential. History of present and past medical conditions including chronic bronchitis, asthma, occupations exposures to dusts and physical examination were carried out to include or exclude subjects from the study. The demographic information of these subjects like name, age, sex, height, weight and BMI were recorded on proforma.

Spirometry was performed on Spirolab III. All the subjects were asked to come between 10 a.m. to 1.00 p.m. with light breakfast and to avoid tight clothing. The procedure was explained to the subjects. Nose clips were used to avoid air leak. Disposable mouth piece was used for each subject. Subjects were asked to inhale maximally, close the lips tightly around the mouthpiece and immediately blow the air out with full force until no air came out from the lungs. Same procedure was done again and three acceptable and reproducible results were recorded. Results were called acceptable which started at maximum inspiration, without any hesitation at start and expiration completed without any pause. Reproducible results were those in which maximum variation of two best reading of FVC and FEV1 were less than 200 ml. Predicted values were calculated from the subject's data like sex, age, ethnic group, weight and height. Results were interpreted with predicted values.

Early morning fasting blood samples were collected. Serum leptin concentration was measured using a sensitive human leptin immunoenzymatic assay kit (Diasource human Leptin EASIA, KAP 2281). It is a Sandwich-Assay performed on the microtiter plate using two specific and high affinity antibodies. The leptin in the samples binds to the first antibody coated on the micro titer plate and is immobilized. In the next step, the second specific anti-Leptin Anti-body binds to the immobilized leptin. The amount of substrate turnover is determined calorimetrically by measuring the absorbance at 450nm against a reference filter set at 650nm.The extent of absorbance is directly proportional to the serum leptin concentration.[12] After taking the blood samples, they were centrifuged and serum was stored at -20 degrees centigrade. The freezed samples were then thawed and placed in the vortex machine before using so that the serum was thoroughly mixed. From each sample, 50 microliters was taken by pipette and placed in the wells of the micro titer plate. After that, anti-leptin antibody conjugate and incubation buffer was added to each well and incubated for two hours on the horizontal shaker. Then the plates were washed and chromogenic solution was added to each well and again incubated for 30 minutes on the horizontal shaker. Finally, stop solution was added and the plate was placed in the Micro titer plate reader Dia 710 (Diamate UK).

Data was analyzed using Statistical Package for the Social Sciences (SPSS) software, version 20. Independent't' test was used to compare the FVC, FEV1 and FEV/FEV1 between obese and non obese groups. Pearson correlation was used to assess linear correlation between anthropometric variables like BMI, serum leptin and lung volumes. The p-value of ≤ 0.05 was considered statistically significant.

Results

The mean age of all cases in this study was 28.92 ± 6.34 years, while mean age in obese and non- obese cases was 32.18 ± 5.89 years and 25.65 ± 4.98 years.

The mean serum leptin in obese and non-obese cases was 16.59 ± 13.56 ng/ml and 5.58 ± 6.30 ng/ml. The mean serum leptin was significantly higher in obese population when compared to non-obese cases, p-value < 0.0001 (Table 1).

The mean serum leptin level of male patients in obese group was 9.5±7.9ng/ml and in female patients was 23.6±14.4ng/ml and mean serum leptin level of male patients in non obese group was 3.3±4.5 ng/ml and in female patients was 7.9± 7.1ng/ml.

The mean FVC in obese cases was 3.18 ± 0.92 L and in non-obese cases was 3.68 ± 0.93 L with p value of 0.021. The mean value of FEV1 in obese was 2.83 ± 0.82 L and in non-obese cases was 3.19 ± 0.71 L with p value 0.011 and mean FEV1/FVC ratio in obese cases was 84.73 ± 11.86 and in non-obese cases it was 90.27 ± 6.89 with p value of 0.002. The mean FVC, FEV1 and FEV1/FVC ratio was significantly lower in obese cases when compared to non-obese cases, p-value < 0.05.

Pearson correlation equation was used to see the relationship between BMI, serum leptin levels and lung volumes FVC, FEV1 and FEV1/FVC ratio.

Among obese cases we found significant negative correlation of BMI with FVC (r value -0.329, p value 0.010) while FEV1 also had significant negative correlation with BMI (r value -0.281, p value 0.046). There was significant positive correlation seen between BMI and FEV1/FVC ratio (r value 0.293, p value 0.023). In non obese cases there was no significant correlation between BMI and FVC, FEV1 and FEV1/FVC ratio (P value >0.05).

In obese cases significant negative correlation was found in serum leptin with FVC (r = - 0.459, p-value < 0.0001) and with FEV1 (r= -0.369, p-value 0.004). In non-obese cases there was no significant corre-lation of serum leptin with FVC (r = -0.027, p-value 0.081) and with FEV1 (r = -0.081, p-value = 0.069) and Serum Leptin with FEV1/ FVC ratio (r = 0.163, p-value = 0.158)(Table 2)

Table 1: *Serum Leptin Level in Study Population*

	Obesity	Mean	S.D	Mini-mum	Maxi-mum	p-value
Serum Leptin (ng/ml)	***Obese (n=60)***	16.59	13.56	2.29	51.92	***<0.0001****
	Non Obese (n=60)	5.58	6.30	0.45	25.64	
	Total (n=120)	11.08	11.89	0.45	51.92	

SD Standard Deviation, * P Value < 0.05 is Significant

Table 2: *Pearson Correlation Coefficient Between BMI, Serum Leptin and Lung Functions*

	Obesity		FVC (liters)	FEV1 (liters)	FEV1/ FVC
Body Mass Index (kg/m^2)	**Obese *(n=60)***	Pearson Correlation	-0.329	- 0.281	0.293
		p-value	***0.010****	***0.046****	***0.023****
	Non-obese *(n=60)*	Pearson Correlation	-0.117	-0.120	0.108
		p-value	***0.371***	***0.363***	***0.411***
Serum Leptin (ng/ml)	**Obese *(n=60)***	Pearson Correlation	-0.459	-0.369	0.174
		p-value	***<0.0001****	***0.004****	***0.183***
	Non-obese *(n=60)*	Pearson Correlation	-0.027	-0.081	0.163
		p-value	***0.081***	***0.069***	***0.158***

Discussion

Obesity has been associated with markers of systemic and vascular inflammation. Leptin is a hormone which is primarily secreted by the adipose tissues and its level in blood is directly associated with the proportion of adipose tissue present in the body. Higher leptin levels are associated with decreased lung function.

In our study mean leptin values were higher in obese subjects as compared to non obese (P value < 0.0001%). These results are comparable with the study of Lee H J et al.[13] which showed that BMI is directly associated with serum leptin levels and morbidly obese persons have highest serum leptin level and more chances of leptin resistance. Another study by Considine RV et al.[14] showed that mean serum leptin concentration in the 139 obese subjects was 31.3±24.1 ng per milliliter, as compared with 7.5±9.3 ng per milliliter in the normal-weight subjects (P<0.001) and they showed that serum leptin levels are directly associated with BMI (r = 0.85, P<0.001).These results are comparable with our results.

This study showed that when the BMI increases, the lung functions like FVC (r value -0.329, p value 0.010), FEV1 (r value -0.281, p value 0.046) significantly decreases. These results are consistent with the study of Rehmanet al[15] done on medical students showed that subjects with BMI <23% had better FEV1 and FVC (p values 0.025 and 0.026) as compared to overweight and obese individuals. A study by Bankeyet al[16] done on 120 subjects showed that increase in BMI is associated with deterioration in lung functions, FVC and FEV1.Another study by Al- Badretal[17] reported that obese individuals have significant inverse relationship between BMI and lung functions, FVC and FEV1

Higher leptin values were associated with statistically significant decrease in FVC (r = - 0.459, p-value < 0.0001) and with FEV1 (r= -0.369, p-value 0.004). These results are comparable with the study of Sin et al[18] which reported that individuals with impaired lung functions had higher serum leptin levels and there was an inverse relationship between serum leptin level and FEV1.

Hickson DA et al[19] examined 4,697 participants in

the Jackson heart study about the relationship of serum leptin level with lung functions FVC, FEV1 and FEV6. They reported inverse relationship between serum leptin level and lung functions (P value <0.001). These results are comparable with our results.

In this study we found negative association of BMI, and serum leptin with dynamic lung volumes, FVC and FEV1 in men and women of adult Pakistani population. The Strength of our study is the recruitment of subjects of both genders who were healthy without co-morbidity. Our study had the limitation of using only dynamic lung volumes and static lung volumes like functional residual capacity and total lung capacity were not assessed which are also significantly decreased in obesity.

We recommend conducting larger studies including both dynamic and static lung volumes to better understand the effects of serum leptin on lung functions.

Conclusion

It is concluded from the results of this study that

- Serum leptin level was higher in obese subjects as compared to the non-obese subjects.
- Increase in serum leptin level was associated with decrease in lung function.

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