

DISCUSSION

Chronic obstructive pulmonary disease (COPD) is a leading and increasing cause of morbidity and mortality worldwide, and is characterized by persistent airflow limitation that is usually progressive, and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases.⁽¹⁾ Clinical and functional heterogeneity is a hallmark of COPD. COPD has multiple extra-pulmonary effects which are contributing to the increased morbidity and mortality, such as nutritional abnormalities, cachexia, skeletal muscle dysfunction and osteoporosis. Also co-morbid associations such as cardiovascular diseases, diabetes mellitus, depression, and cancer have an impact on the patient's health status and interfere with disease management.^(1,122,315)

In addition to local pulmonary inflammation, systemic inflammation is adding to the complexity of the disease, characterized by increased circulating levels of inflammatory cells, cytokines and acute phase proteins that occur in both stable disease and during exacerbations, which are notably associated with pulmonary and systemic inflammation and up-regulation of anti-inflammatory markers.⁽³¹⁶⁾ Exacerbations of COPD are associated with worsening of lung function, decreased health-related quality of life, increased inflammatory burden and a significant impact on survival.^(66,68)

Leptin, initially discovered as a regulator of food intake and energy expenditure, is emerging as a pleiotropic cytokine involved in the recruitment, activation and survival of inflammatory cells.⁽³¹⁷⁾ Leptin has been comprehensively studied in the literature as a multifunctional hormone with versatile activities and complex counteractions with other cytokines and adipokines. The receptor for leptin (leptin-R) has been demonstrated to be present throughout the body, including the lung, suggesting the potential involvement of leptin in the pathogenesis of respiratory disorders. However, whether it represents a friend or a foe is not yet fully elucidated.^(260,298) The range of leptin's effects is likely to be complex, as different thresholds exist for several of leptin's actions.⁽²²⁰⁾

Patients' characteristics:

The present study enrolled forty patients with COPD (the study group A); all patients were recruited from chest department in Alexandria Main University Hospital, and all were males so as to increase homogeneity of the study population and to abolish the male to female difference in leptin measurements. The study group was subdivided into patients with stable COPD and patients during exacerbation, each subgroup included twenty patients. Results were compared with a twenty five age and sex matched healthy subjects as the control group B.

The subgroup A1 included twenty patients during exacerbation. The exacerbation state was identified according to GOLD (2014) definition by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medications.⁽¹⁾ The subgroup A2 included another twenty patients who were free of any symptoms of exacerbation for at least 8 weeks with no change in their regular daily medications.

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There was no statistically significant difference of the age between the two subgroups nor with control subjects ($p=0.317$). The mean \pm SD of the annual exacerbation rate was higher for the COPD exacerbation subgroup (2.5 ± 1.0 exacerbation/year) versus that of the stable subgroup (2.15 ± 0.93 exacerbation/year), but the difference was not statistically significant. Smoking status showed no significant difference between the two subgroups.

Functional status of the studied patients:

Studying the functional status revealed a statistically significant difference of the six-minute walk test (6MWT) results between the two studied subgroups of patients, with a mean \pm SD for COPD exacerbation subgroup of (211.80 ± 75.45 meters) and that for stable COPD subgroup was (248.25 ± 66.88 meters) ($p=0.049$). Results denoted the effect of exacerbations on the decreased physical performance of the patients, as exacerbations are known to be associated with reduced functionality and worsening of disease severity.

Six-minute walk test has emerged as a tool to assess functional capacity that has the ability to evaluate the global and integrated responses of all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, neuromuscular units, and muscle metabolism. Also 6MWT results correlate better with measures of quality of life.⁽³¹³⁾ A distance less than 350 meters is associated with increased mortality in chronic obstructive pulmonary disease.⁽³¹⁸⁾

These results were in concordance with Ayedh et al (2013)⁽³¹⁹⁾ who studied the change in 6MWT as an indicator of exercise capacity to assess post exacerbation recovery of exercise capacity and its relation to stable status. Results showed that there was a significant decline of the of the walking distance after exacerbation compared to the baseline stable state, that recovered to baseline by 1 week of exacerbation, denoting a decrease in physical performance with exacerbation.

The present study revealed a significant positive correlation of 6MWT measures with multiple severity parameters in both subgroups. In COPD exacerbation subgroup, 6MWT results were positively correlated with predicted FEV1% ($r=0.559$, $p=0.0103$) and negatively with both scores of mMRC dyspnea scale ($r=-0.509$, $p=0.021$) and severity assessment by GOLD staging ($r=-0.474$, $p=0.034$), reflecting the ability of 6MWT to express the functional impairment during exacerbations and its effect on patients' performance, and also the ability of 6MWT to reflect disease severity during exacerbation.

The results were in agreement with a study by Blankenburg et al (2013)⁽³²⁰⁾ who assessed the course of COPD exacerbations in relation to functional parameters including 6MWT; 6MWT was measured on admission, prior to discharge and after a 4-weeks stable period. 6MWT was significantly increased from exacerbation to stable status, and after 4 weeks of outpatient treatment in clinically stable patients. That increment in the walk distance correlated negatively with advancing severity of COPD according to GOLD stages. The study demonstrated that 6-min walk test is a suitable parameter to assess the course of COPD exacerbations.

The stable COPD subgroup showed also a positive correlation between 6MWT results and the predicted FEV1% ($r=0.558$, $p=0.010$), confirming the relationship between 6MWT and the various degrees of disease severity and functional performance in the stable status as well,

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and highlighted the value of 6MWT in assessing functionality of stable COPD patients and its role as a simple evaluation of disability caused by respiratory impairment.

Those results agreed with Chen et al (2012)⁽³²¹⁾ who studied the relationship between the 6-minute walk test and pulmonary function tests in stable chronic obstructive pulmonary disease. The 6MWT results were correlated positively with the spirometric parameters FEV1% and FEV1/FVC, and negatively with RV/TLC. The study denoted that as the airflow limitation was progressively developing, the exercise tolerance of the patients was impaired more and more seriously.

Similarly, in a study by Fujimoto et al (2011)⁽³²²⁾ who retrospectively investigated the association of six-minute walk distance with data of spirometry and measurements of diffusing capacity of the lung for carbon monoxide (DLCO) from a total of 130 patients with COPD. Results showed 6MWT was significantly associated with FEV1% predicted, IC, TLC and DLCO % predicted, suggesting that impairment of pulmonary function at baseline might be involved in reduction of functional exercise capacity as assessed by 6MWT.

However, contradictory results were noted by Fastenau et al (2013)⁽³²³⁾ who studied the relationship between functional exercise capacity and daily physical activity in patients with mild to moderate COPD by assessing the six-minute walk distance. There was no correlation observed between 6MWD and FEV1% predicted, results might be explained by the narrow range of severity of the studied patients in the mentioned study; where the mean \pm SD of FEV1% was (74 \pm 14), indicating mild to moderate COPD, which might affect the lost relationship between the lung function and the exercise capacity.

Assessing the scores of the mMRC dyspnea scale in the current study showed a higher mean for the COPD exacerbation subgroup (2.60 \pm 0.59) versus (2.35 \pm 0.74) for the stable group, however the difference was not statistically significant, which might be due to the nature of comparison in the present study which was between two different groups of patients with different baseline symptoms, rather than following the same patients from exacerbation to stable state.

The current study revealed a number of significant correlations of the mMRC scores in both studied subgroups that reflected its ability as an important measure of the various aspects of disease severity. In COPD exacerbation subgroup, mMRC scores showed a significant negative correlation with predicted FEV1% ($r=-0.729$, $p=0.002$) and FEV1/FVC ($r=-0.625$, $p=0.003$), reflecting the value of the mMRC assessment in reflecting severity of airflow limitations during exacerbations and its association with disease severity. Scores of mMRC was positively correlated with assessment of disease severity by GOLD stages ($r=0.802$, $p<0.001$) and negatively correlated with the distance measured by the 6MWT ($r=-0.509$ at $p=0.021$), denoting that mMRC scores were not only reflecting disease severity during exacerbations but also reflection the patients functional status and exercise capacity.

Those results are in concordance with a study by Inal-Ince et al (2005)⁽³²⁴⁾ who studied functional capacity in severe chronic obstructive pulmonary disease. The scores of mMRC showed significant negative correlations with the measured distance by 6MWT and the predicted FEV1%, denoting the relationship between of the level of breathlessness and the functional capacity of the patients.

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In the stable COPD subgroup, scores of mMRC showed also a significant negative correlation with FEV1/FVC ($r = -0.459$, $p = 0.041$) and a positive correlation with the assessment of disease severity by GOLD stages ($r = 0.497$, $p < 0.025$). The results denoted the value of mMRC dyspnea scale as a clinical method to evaluate dyspnea in stable status as well as during exacerbations and its association with the degree of airflow limitation and disease severity.

Those results were in agreement with a study by Mhler et al (1998) ⁽³²⁵⁾ who assessed ratings of dyspnea with mMRC scale in comparison with various physiological functions, mMRC scores were correlated significantly with lung function parameters including FVC and predicted FEV1%. Results suggested that the clinical evaluation of patients with COPD should include the routine measurement of dyspnea in addition to spirometry. Also similar results were noted in a study by Chhabra et al (2009) ⁽³²⁶⁾ who assessed relationships between scales of dyspnea and multiple measures of physiological impairment in stable COPD. Score of mMRC showed significant positive correlations with post-bronchodilator FEV1% predicted.

However, contradictory results were reported by Bestall et al (1999) ⁽¹⁵⁰⁾ who studied mMRC dyspnea scale as a method of categorizing disability in COPD for rehabilitation purpose. Results showed that predicted FEV1% was not associated with grades of mMRC. However, the studied patients in the mentioned study were recruited from an outpatient pulmonary rehabilitation program, since rehabilitation is designed to improve exercise performance and reduce disability, that difference might be attributed to the benefit from a rehabilitation program with decreased disability in the studied patients.

Body mass index and its relation to disease severity:

The present study revealed no statistically significant difference between the three groups regarding BMI. Comparing the BMI within the stable COPD subgroup in relation to the severity assessment by GOLD stages revealed a statistically significant difference of BMI values between different stages ($F = 11.209$, $p = 0.001$), with lower values of BMI associated with increased severity. Also comparing the BMI in relation to the combined COPD assessment classification groups revealed a statistically significant difference of BMI in the different stages ($F = 3.770$, $p = 0.044$). BMI showed a significant negative correlation with severity assessment by GOLD stages ($r = -0.605$, $p = 0.004$) in stable COPD patients. Results were suggesting a relationship between the poor nutritional status as reflected by the low BMI and the increase in disease severity. In addition, BMI showed a significant negative correlation with scores of mMRC dyspnea scale ($r = -0.476$, $p = 0.033$) in the stable COPD subgroup, confirming its relation to the decline in functional status of the patients.

The results were reflecting the importance of the BMI as a nutritional parameter in assessing the disease severity. COPD is known to cause anorexia, and cachexia is one of the extrapulmonary manifestations of COPD. The decreased BMI with the increased stage of COPD demonstrated the link between disease severity and poor nutritional status of the patients. Furthermore, in a meta-analysis by Cao et al (2012) ⁽³²⁷⁾ about the association between body mass index and mortality in patients suffering from chronic obstructive pulmonary disease, the findings indicated that reduced BMI was associated with an increased risk of mortality in COPD. A higher risk of death was found not only in underweight patients, but also in those with normal

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BMI. The meta-analysis concluded that for patients with COPD, being overweight or obese had a protective effect against mortality.

The present study results agreed with a study by De et al (2012)⁽³²⁸⁾ who assessed BMI as one of the important parameter to assess functional status of patients with COPD, and to assess the prevalence of underweight among clinically stable COPD patients and relationship of BMI with increasing severity of COPD as assessed by GOLD stages. Results showed that with increasing severity of COPD, a significant decline in mean BMI was observed ($p=0.0001$), concluding that BMI decreases further with increasing severity of COPD.

The same findings had been noted by Montes de Oca et al (2008)⁽³²⁹⁾ who studied factors influencing BMI in COPD populations, findings showed that aging, current smoking, and severe COPD (GOLD stages III and IV) were associated with lower BMI.

However, contradictory results were noted in a study by Ischaki et al (2007)⁽³³⁰⁾ who studied body mass and fat-free mass indices in stable COPD and their relation with variables expressing disease severity. BMI was not statistically different among patients' different stages of COPD, however, the mentioned study showed that the fat-free mass reflected the staging of the disease, presenting the highest values in those stages where minimal airflow limitation and obstruction existed, denoting the relation between the nutritional status and the disease severity .

Spirometric assessment:

Spirometric assessment of the two studied subgroups of patients showed no statistically significant differences between different parameters, which might be due to the fact that comparison was between two different groups of patients with different functional baselines, rather than following the same population during disease progression.

Multiple studies had reported the association of acute exacerbation of chronic obstructive pulmonary disease with worsening airflow obstruction and lung hyperinflation, and improvement following the acute exacerbations. In a study by Parker et al (2005)⁽³³¹⁾ who assessed physiological changes during exacerbations of COPD, improvements in FEV1% , FRC and lung hyperinflation had been noted after repeated testing over a period of 60 days after recovery, concluding that COPD exacerbations are characterized by worsening airflow obstruction and lung hyperinflation, and improvement of dyspnea following acute exacerbations was associated with reduction in lung hyperinflation and consequent increase in expiratory flow rates. However, the mentioned study was following the same group of patients throughout the course of the disease which is not the case in the present study that compared two different groups of patients with probably different baselines.

Staging of the studied patients:

Assessment of severity according to GOLD stages based on predicted FEV1% revealed no significant differences between the two studied groups. For COPD exacerbation subgroup, one patient was GOLD stage I (5.0%), five patients were GOLD stage II (25.0%), seven patients were GOLD stage III (35.0%) and seven patients were GOLD stage IV (35.0%). As for the stable COPD subgroup, three patients were GOLD stage II (15.0%), six patients were GOLD stage III (30.0%) and eleven patients were GOLD stage IV (55.0%).

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As regard the combined COPD assessment classification, the present study revealed no statistically significant difference between the two patients' subgroups. Interestingly, there was a difference showed between percentages of the very severe cases in the two studied subgroups according to each classification. The percentage of the very severe stage (GOLD IV) within COPD exacerbation subgroup based on predicted FEV1% was (35.0%) of the patients (7 patients with GOLD stage IV), and from the combined COPD assessment view, percentage of the very severe stage (Group D) turned out to be (75.0%) of the patients (15 patients were group D with high risk and more symptoms). That observation denoted the higher ability of the combined COPD assessment classification to detect patients with the highest risks, as it takes in consideration spirometric criteria, patients' symptoms and risk of exacerbation as well. Similar finding was noted in the stable subgroup; with percentage of the very severe cases by GOLD staging was (55%) (11 patients with GOLD IV) and that for the combined assessment system was (70%) (14 patients group D).

BODE index scores and relations; a comprehensive measurement of COPD complexity:

The current study revealed the wide scope of BODE index in assessing COPD patients, whether is stable state or during exacerbation. Previous studies had speculated that the staging based solely on the FEV1% incompletely describes the complexity of COPD and that it has become desirable to evaluate the patient more comprehensively if we are to impact on outcomes.⁽¹⁵³⁾

Although the current study revealed no statistically significant difference between the two studies groups as regard BODE scores, BODE index score in the COPD exacerbation subgroup showed a positive association with assessment of disease severity according to GOLD staging based on FEV1% ($r=0.884$, $p<0.001$) and the combined COPD assessment classification as well ($r=0.680$, $p=0.001$). There was a difference between the views of the two previously mentioned staging classifications especially when it comes to assess severe patients. BODE index showed a positive correlation with both staging systems despite that difference, a finding that implied the ability of BODE index with its multicomponent nature to express heterogeneity and complexity of COPD disease. The same results were detected in the stable subgroup where BODE index correlated positively with assessment of severity according to GOLD staging ($r=0.837$, $p<0.001$) and with the combined COPD assessment system ($r=0.733$, $p<0.001$). The current study showed a significant positive correlation between FEV1/FVC ratio and the BODE score in both exacerbation and stable subgroups ($p=0.004$ and $p=0.003$ respectively), a result was confirming the ability of BODE index to reflect disease severity in exacerbation and stable status as well.

Those results agreed with observations by Sarioglu et al (2010)⁽³³²⁾ who studied relationships between the BODE index and multiple parameters in stable COPD patients including GOLD staging based on FEV1%. BODE index showed to be positively correlated with COPD stages as classified according to (GOLD) and also with the number of exacerbations and hospitalizations.

In COPD exacerbation subgroup, BODE index was positively correlated with CRP ($r=0.499$, $p=0.025$), a result was linking the higher BODE scores with the higher grades of

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systemic inflammation and further denoting the ability of BODE index to reflect even the underlying inflammatory nature of the disease. In COPD, the low-grade systemic inflammation showed to be associated with increased risk of cardiac injury, and it has been implicated as the potential bridge between COPD and cardiovascular diseases and cancer which are leading causes of morbidity and mortality in COPD.⁽¹¹²⁾ In addition, Man et al (2006)⁽³³³⁾ had speculated that higher levels of CRP were associated with cardiovascular causes of mortality including coronary heart disease, stroke and myocardial infarction, and also cancer specific causes of mortality. The current study results were confirming the link between the BODE index as a mortality assessment tool and the upgraded systemic inflammation characterizing COPD exacerbation, that was presented by the higher levels of CRP, with its positive association with BODE scores.

The current study results were in concordance with results of De Torres et al (2006)⁽¹⁰²⁾ who studied CRP in relation to multiple prognostic parameters in COPD including the BODE index, the study had observed that CRP levels correlated independently with multiple important prognostic variables including the BODE index.

Similar results were conducted in a study by Sarioglu et al (2010)⁽³³²⁾ who studied BODE index in relation to quality of life and inflammatory cytokines in stable COPD patients. There was a significant relationship between BODE index and COPD stage as classified according to (GOLD) classification, serum CRP levels and BODE scores were also correlated. There were significant correlations also between BODE scores and disease duration, number of exacerbations, SGRQ scores, denoting its relation to various prognostic and follow up parameters of COPD and systemic inflammation.

CRP levels and relations in the studied groups:

Beside the important role of CRP as a marker of systemic inflammation in COPD, it also has a significant role as a predictor of future outcome, and it has been used also to follow up response to treatment.^(106,112,334) In the present study, levels of CRP showed a statistically significant difference between the three studied groups ($p=0.001$). Within the COPD exacerbation subgroup, CRP level mean \pm SD was (37.66 ± 42.24 mg/l), and it was significantly higher than the other two groups. Also CRP levels were significantly higher in patients with stable COPD (12.41 ± 13.91 mg/l) than in the control group (2.47 ± 1.63 mg/l) with ($p=0.001$). Results reflected the up-regulation of systemic inflammation characterizing COPD exacerbation, which was represented in the significantly higher level of CRP during exacerbation than in both stable state and control subjects, reflecting the increased inflammation from the stable status to exacerbations, and highlighted the role of CRP as a biomarker of systemic inflammation.

Results were in concordance with a study by Bathoorn et al (2009)⁽³³⁵⁾ who studied inflammatory parameters during COPD exacerbations compared to stable disease. Results showed that systemic inflammation increased during exacerbations as assessed by blood total leukocyte and neutrophil counts, and serum CRP and IL-6. These biomarkers were particularly increased in case of bacterial exacerbations, speculating that inflammation increased from a stable phase of disease to an exacerbation, and confirming the association of serum biomarkers including CRP with exacerbations especially during bacterial exacerbations.

Similarly, in a study by Lacoma et al (2011)⁽³³⁶⁾ who investigated biological markers including CRP in COPD patients in stable phase, patients undergoing an exacerbation, and

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patients with pneumonia. CRP showed significant differences among the three patient groups, being higher in patients with pneumonia, followed by patients with exacerbation ($P < 0.0001$). CRP levels decreased one month after the exacerbation episode. In addition, a study by Dev et al (1998)⁽³³⁷⁾ showed that CRP elevation was observed in patients having exacerbation with proven infections and also in those where infection was not proven, the study implicated that CRP is of value in the assessment of exacerbations of COPD, and that the measurement of serum CRP may provide an additional objective indicator of infection.

In the present study, patients with stable COPD showed a significantly higher level of CRP than controls ($p=0.001$) reflecting its role as a biomarker of low-grade systemic inflammation characterizing COPD, also the difference detected between stable patients and patients during exacerbations denoted the role of CRP in following up response to treatment throughout the course of the disease. The results obtained are consistent with previous studies, indicating the presence of systemic inflammation in patients with stable COPD, Karadag et al (2008)⁽³³⁸⁾ had studied serum CRP in stable COPD patients versus control subjects, CRP was significantly higher in stable COPD patients than in control subjects ($p<0.001$), denoting its role as a valid biomarker of low-grade systemic inflammation.

Similarly, Pinto-Plata et al (2006)⁽³³⁹⁾ had studied CRP in stable COPD patients to answer the question what if CRP rise in COPD was related directly to COPD and its associated systemic inflammation or secondary to other factors such as concomitant ischemic heart disease (IHD) or smoking status. Results showed that CRP levels were raised in COPD patients without clinically relevant IHD and independent of cigarette smoking, furthermore CRP was reduced in patients with COPD using inhaled corticosteroids, concluding that CRP may be a systemic marker of the inflammatory process that occurs in patients with COPD.

In addition, CRP levels showed a number of significant correlations in patients with COPD exacerbations. CRP was negatively correlated with predicted FEV1% ($r=-0.469$, $p=0.037$), a result denoted the link between the decline in lung function, the disease severity and the increased systemic inflammation during exacerbations.

Also CRP showed a significant negative correlation with the measured distance by 6MWT ($p=0.037$) in patients with COPD exacerbations, a result further demonstrated the relationship between systemic inflammation and the impairment in the patients' functional capacity and physical performance. CRP level was positively correlated with BODE score ($r=0.449$ at $p=0.025$), and that denoted the effect of the increased systemic inflammation during exacerbation on the higher morbidity and mortality reflected by BODE index.

Results were in agreement with Long et al (2011)⁽³⁴⁰⁾ who studied correlations among the levels of CRP and interleukin-18 (IL-18), quality of life, and pulmonary function in acute exacerbation and stable COPD versus controls, results showed that serum levels of CRP and IL-18 in the acute exacerbation group were significantly higher than those in the stable group and the control group. The serum levels of CRP and IL-18 in the exacerbation group and the stable COPD group were negatively correlated with FEV1% and FEV1/ FVC, denoting that serum levels of CRP and IL-18 might be related to the lung dysfunction in the patients with COPD.

Similar findings were reported by Urboniene et al (2008)⁽³⁴¹⁾ who studied patients with COPD, asthma, and controls in relation to pulmonary function tests, C-reactive protein

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concentration measurement, and smoking history. Results showed that In COPD patients, C-reactive protein level correlated negatively with FEV1% predicted, in contrast, there was no correlation between C-reactive protein level and analyzed parameters in asthmatics and control group.

In patients with stable COPD, the present study showed a weak negative correlation in between CRP and predicted FEV1% ($r=-0.394$, $p=0.086$) that did not reach statistical significance, which might be attributed to lack of variability of patients' stages as assessed by FEV1% within this subgroup, as those patients lacked GOLD stage I, and 55% of the patients were GOLD stage IV, that uneven distribution might be responsible for the weak association between their predicted FEV1% and levels of CRP.

However, patients with stable COPD showed a significant negative correlation between levels of CRP and the distance measured by 6MWT ($r=-0.454$, $p=0.045$), that was implying a link between the decline of functional status and physical performance of the patients with the low grade systemic inflammation in the stable state, as systemic inflammation had been implicated in the muscle weakness and in the pathogenesis of tissue depletion in COPD.⁽⁹³⁾

The present study findings were consistent with the results of De Torres et al (2006)⁽¹⁰²⁾ who studied CRP levels in 98 stable COPD patients in relation to multiple clinical parameters including FEV1% predicted, MMRC, 6 MWT and BODE scores. CRP levels correlated significantly with all parameters, denoting that CRP levels are raised in stable COPD patients and supporting the fact that when lung function worsens, CRP levels increase.

Similar observations also were reported by Aksu et al (2013)⁽¹⁰⁷⁾ who studied relationship between serum CRP levels and different clinical parameters in stable chronic obstructive pulmonary disease considering the impact of smoking behavior and biomass exposure; CRP levels did not differ significantly according to smoking status or biomass exposure, moreover, COPD cases due to biomass exposure who never smoked also had higher CRP levels compared to healthy controls. CRP levels significantly correlated with mMRC scores and 6 minute walk distance. The study speculated that systemic inflammation is inherent to COPD independent of ever-smoking status and correlates with disease severity.

Leptin as an inflammatory cytokine in COPD:

COPD exacerbations had been studied as inflammatory events, with several airway and systemic inflammatory markers increasing. Previous studies showed increases in airway neutrophils when stable that increased further at exacerbation. Systemic inflammation seemed to be associated with a direct correlation between the degree of airway inflammation and the size of the systemic acute-phase response, especially in association with bacterial and viral infection.⁽⁶⁸⁾

The present study revealed a statistically significant difference of leptin levels between the two studied groups of patients, with mean leptin level \pm SD for COPD exacerbation subgroup was (38.25 ± 26.87 ng/ml) and that for the stable subgroup was (9.25 ± 6.10 ng/ml) ($p<0.001$). In healthy controls, the mean leptin level \pm SD was (5.3 ± 3.05 ng/ml). Leptin levels showed a statistically significant difference between COPD exacerbation subgroup and controls ($p<0.001$) and also between stable COPD group and controls ($p=0.025$). Results showed that leptin level

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was significantly higher in stable COPD than healthy controls and it was much higher during exacerbations.

In addition, the present study revealed a statistically significant positive correlation between leptin levels and the inflammatory marker CRP in both exacerbation ($r = 0.605$, $p = 0.005$) and stable subgroups ($r = 0.447$, $p = 0.048$). CRP showed a statistically significant difference between the three studied groups ($p = 0.001$).

That higher leptin level noted in the COPD exacerbation subgroup in addition to its positive correlation with CRP could be attributed to a role of leptin during the exacerbation event as a part of the systemic inflammation sequence. That surge of leptin levels during exacerbation in comparison with the stable state seemed to follow the same manner of multiple inflammatory cytokines during the up-regulation of the systemic inflammatory response during exacerbation. In addition, the higher level of leptin in the stable state –in comparison with healthy subjects– and its correlation with CRP also spotted the light on its role as an inflammatory cytokine related to the low grade systemic inflammation status in COPD. As multiple evidences exist to support the fact that the systemic inflammation is seen in stable COPD and when there is an exacerbation, systemic inflammatory markers get worse. ⁽⁸⁸⁾

The current study results were in concordance with multiple studies that investigated the role of leptin during COPD exacerbation and also the stable state. Creutzberg et al (2000) ⁽³⁰⁰⁾ had investigated the course of the energy balance in relation to leptin and other inflammatory markers in 17 male patients with severe COPD during the first 7 days of hospitalization for an acute exacerbation. Leptin level was significantly higher in the patients with COPD on day 1 compared with its concentration in the healthy subjects ($p = 0.006$). Leptin level was still significantly higher on day 7 of the exacerbation than in healthy controls ($p = 0.017$). Leptin concentration decreased gradually throughout the exacerbation. In addition, leptin was found to be positively correlated with soluble TNF-R55 ($r = 0.65$, $p = 0.041$).

Krommidas et al (2010) ⁽³¹⁰⁾ reported similar results in assessing leptin and adiponectin in 63 COPD patients hospitalized for acute exacerbations. All systemic inflammatory biomarkers including CRP, tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and leptin were elevated during admission compared to resolution and stable phase 8 weeks later. Leptin showed a higher level on admission than the resolution state and also 8 weeks later ($p < 0.001$). The study concluded that leptin may represent an inflammatory biomarker during exacerbation of COPD. Also leptin presented a significant positive associations during admission with CRP ($p = 0.025$), IL-6 ($p = 0.001$) and TNF- α ($p = 0.006$). Also in a stable state, leptin levels showed significant positive associations with IL-6 ($p = 0.013$) and TNF- α ($p = 0.022$).

Similar observations were reported by Kythreotis et al (2009) ⁽⁹⁷⁾ who studied serum leptin, insulin like growth factor I (IGF-I), TNF- α , interleukin 1 β (IL-1 β), interleukin 6 (IL-6) and interleukin 8 (IL-8) levels in 52 COPD patients with acute exacerbation on admission to hospital (day 1) and two weeks later (day 15) in comparison with healthy subjects. Circulating leptin levels were high on day1 of the exacerbation and remained elevated on day15 in COPD patients compared to healthy subjects. Serum leptin levels were significantly higher in COPD patients on (day 1) than in healthy controls ($p < 0.001$). Serum leptin levels were also significantly higher on (day 1) admission compared to day 15 ($p < 0.05$). A positive correlation was observed between leptin and TNF- α on (day 1) ($r = 0.620$, $p < 0.001$). This correlation

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between TNF- α and leptin was true even when patients with chronic bronchitis and emphysema were studied separately ($p < 0.001$ and $p = 0.014$ respectively).

Furthermore, Leptin had been studied as a component of systemic inflammation in multiple disorders and from different aspects as well. From a structural perspective, leptin is a member of the cytokine family that includes IL-6, IL-12, IL-15, granulocytes-colony stimulating factor, and leptin receptor belongs to the class-I cytokine receptor family, which also includes the receptors of IL-6, IL-12, prolactin and growth factor. Leptin also has been shown to augment the release of cytokines IL-6, TNF- α from activated peripheral blood mononuclear cells and macrophages.⁽³⁴²⁾ Matarese et al (2005)⁽³²⁵⁾ had reviewed the direct actions of the leptin on adaptive immune responses including T helper-1 (TH1) and T helper-2 (TH2) responses, focusing mainly on cytokine pattern secretion, suggesting that leptin may influence growth, differentiation and also T-cell activation by interacting with T-cell co-stimulatory antigens. In neutrophils, leptin induces chemotaxis and the release of reactive oxygen species. Also leptin has a wide regulatory action on natural killer (NK) cells by affecting proliferation, differentiation, activation and cytotoxicity.

A previous study had revealed that IL-6 and leptin concentrations were dramatically elevated in critically ill patients suffering from acute sepsis compared with healthy subjects.⁽³⁴³⁾ Moreover, leptin was found to play a role in the pathogenesis of autoimmune disorders including rheumatoid arthritis and systemic lupus erythromatosis (SLE).⁽²²⁴⁾ Those findings give further evidences for an inflammation-related role for leptin in several diseases; the present study suggested such a role in COPD specifically.

However, contradictory results were reported by Takabatake et al (1999)⁽³⁴⁴⁾ in a study investigated serum leptin levels in patients with COPD versus healthy controls, the study reported that serum leptin levels were significantly lower in COPD patients than healthy controls. However, this could be attributed to that the BMI and the percent of body fat in the studied patients of the mentioned study were significantly lower the healthy controls, where BMI was ($18.1 \pm 2.7 \text{ kg/m}^2$) for the studied patients versus ($22.8 \pm 2.2 \text{ kg/m}^2$) for the controls ($p < 0.0001$), which might suggest a relation of this result to the studied patients' lower BMI. As leptin is known to be linked primarily to the fat mass, the mentioned study might reveal that relationship rather than reflecting the inflammatory status of COPD.

Different observations were noted in a study by Çalikoglu et al (2004)⁽²⁹⁹⁾ who studied leptin levels in patients with stable COPD and during exacerbation versus healthy controls, leptin levels were lower in stable patients than controls but this difference was not statistically significant ($p = 0.65$). However, leptin levels were significantly higher in patients with exacerbation than in both stable-disease and control group ($p < 0.001$).

Different results were noted by Kythreotis et al (2009)⁽⁹⁷⁾ in a study of correlations between leptin and the inflammatory markers TNF- α , IL8 and IL 6 in COPD patients during exacerbations. Positive correlations were found on day 1 of admission but no significant correlations were found on day 15. Despite leptin level remained elevated on day15 in COPD patients compared to healthy subjects. That difference might be related to the different composition of the studied group in the mentioned study that contained 9 women, in addition to the different mean BMI of the studied patients ($25,6 \pm 4,1 \text{ kg/m}^2$).

Leptin relations with parameters of disease severity during exacerbations:

The current study observed positive correlations between leptin levels and a number of severity parameters within the COPD exacerbation subgroup; including severity assessment by GOLD stages with ($r=0.689$, $p=0.001$), the combined COPD assessment groups ($r =0.484$, $p=0.031$) and scores of BODE index ($r =0.834$, $p<0.001$). Leptin also showed a negative correlation with FEV1% predicted ($r= -0.718$, $p<0.001$), results were suggesting a relationship between the higher leptin level and the increased severity of the disease during exacerbation, as presented by the association of the increased leptin with the worsening airflow limitations and higher degree of disease severity, the correlation with BODE index further linked the leptin rise during exacerbation to a worsening morbidity and a higher risk of poor outcome.

Leptin also showed important correlations with parameters expressing functional capacity and physical performance in patients during exacerbations. It was negatively correlated with 6MWT measurements ($r = -0.680$, $p=0.001$) and positively correlated with scores of mMRC dyspnea scale ($r =0.641$, $p=0.002$), which might link the worsening of the patients' functional status during exacerbations with the detected higher leptin level, suggesting a relationship between the leptin surge during exacerbation and the decline in the patients' functional capacity. The previous results were going hand by hand with similar correlations observed between CRP and other severity parameters in the exacerbation group, including a positive correlation between CRP and BODE score ($r =0.499$, $p=0.025$) and a negative correlation between CRP and both FEV1% predicted ($r= -0.46$, $p =0.037$) and mMRC dyspnea scale ($r=-0.56$, $p=0.010$). Taken together, the present study suggested a broader role of leptin during exacerbations of COPD as an inflammatory marker and also as a marker of disease severity.

In concordance with the present study, similar observations were reported by Liang et al (2013) ⁽³⁴⁵⁾ who studied leptin and interleukin-6 (IL-6) expression in a total of 51 patients with COPD. Leptin and IL-6 levels were determined in patients with an acute exacerbation of COPD, stable COPD and in healthy controls. Results showed that the levels of leptin and IL-6 in the serum and sputum and the WBC counts of patients with acute exacerbations were higher than those in healthy controls ($P<0.05$), and these values in exacerbation patients were increased compared with those of stable COPD patients. These variables were inversely related to FEV1% predicted, suggesting that leptin levels are associated with the severity of inflammation and that leptin expression increases with increased severity.

Similarly, Rubinsztajn et al (2012) ⁽³⁴⁶⁾ had studied 67 patients with COPD that had been subdivided according to their severity by GOLD stages. Body composition, leptin and adiponectin were studied in relation to GOLD stages. Results showed that serum leptin was statistically different between GOLD stage II and GOLD stage IV and it showed a positive correlation with lung hyperinflation and also with a decreased 6MWT.

In addition, leptin in the airways of COPD patients had been investigated, Bruno et al. (2005) ⁽²¹²⁾ had studied leptin expression in bronchoscopic bronchial mucosal biopsies in COPD patients and current smokers, results showed increased leptin expression in bronchial mucosa of chronic obstructive pulmonary disease patients than in smokers and normal subjects, and it was associated with airway inflammation and airflow obstruction. Similarly, in a study by Broekhuizen et al (2005) ⁽²⁹⁸⁾ leptin was investigated in induced sputum in 14 male patients with

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moderate COPD and correlated with other inflammatory cytokines, leptin was detectable in induced sputum of 10 COPD patients. A significant relationship was found between sputum leptin and sputum CRP ($r=0,943$; $p<0.001$) and total TNF- α in sputum ($r =0.690$, $p<0.01$).

On the genetic level, Hansel et al (2009) ⁽²⁹⁶⁾ had studied 429 European Americans randomly selected patients with COPD. Lung function was measured annually over 5 years; the study identified genetic variants in the leptin receptor gene significantly associated with lung function decline, identifying the leptin receptor gene as a novel candidate gene for COPD.

Leptin, BMI and health status interrelationships in stable COPD:

Interestingly, the present study observed no correlation between leptin level in the COPD exacerbation subgroup and the body mass index ($r=-0.189$, $p=0.425$), in contrast to the stable patients subgroup, that showed a positive correlation between BMI and leptin levels ($r=0.671$, $p=0.001$) and also the control group ($r=0.409$, $p=0.042$). In Addition, the previous correlation of leptin with multiple severity parameters in the exacerbation group had been lost in patients with stable COPD, indeed, it had been reversed for some items, including a negative correlation between GOLD stages and leptin ($r=-0.466$, $p=0.038$) and a negative correlation between BODE scores and leptin ($r=-0.527$, $p=0.017$).

Those different correlations might suggest different role for leptin in the studied patients with stable COPD, the negative correlation with disease severity by GOLD stages suggested that a higher leptin was associated with a less severe disease in contrast to what had been observed in the exacerbation subgroup, also the negative correlation with BODE scores suggested that maintaining a higher leptin level in such patients was in favor of less mortality in contrast to the COPD exacerbation subgroup too.

That positive correlation with the body mass index detected in stable COPD patients, as well as the control group, demonstrated that patients with stable COPD had been able to maintain their normal leptin metabolism, that's primarily related to their BMI and the fat mass, by the same means it was physiologically regulated and correlated with BMI in the control subjects. That loss of correlation noted during exacerbation seemed to be related to the surge of leptin levels during the exacerbation event. In the stable subgroup, the ability to maintain that higher leptin level was in contrast to what had been studied regarding cachectic patients with COPD and cachexia in general. Studies of cachectic patients including COPD had showed lower levels of leptin than normal, which was mainly related to the lower body mass index and fat mass in such patients. As COPD is known to cause anorexia, nutritional deficiencies and pulmonary cachexia in which both fat mass and fat-free mass are depleted⁽³⁴⁷⁾, the higher leptin level in the stable state observed in the current study might denote that those patients are still able to maintain their normal physiological regulations of leptin and so their nutritional status and their fat mass, and might further suggest that they have not yet developed the catabolic/anabolic disturbances found in wasted COPD patients. ^(348,349)

The present study results showed a mean BMI for the stable subgroup of (20.71 ± 4.22 kg/m²), and there was a significant difference of the BMI in relation to the different stages within the stable patients subgroup, whether by GOLD stages ($p = 0.001$) or by the combined COPD assessment groups ($p=0.044$), where the lower BMI associated with increased disease severity. Maintaining a positive correlation with different BMI values within different stages of

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the stable group might confirm the maintained normal nutritional and metabolic status of those patients and so the negative correlations observed between leptin and BODE index and with severity assessment by GOLD stages, which were in favor of better overall general status, better progression and less mortality. All over results might suggest that maintaining normal leptin physiology in the stable state might act as a friend rather than a foe.

These results were in agreement with a study conducted by Creutzberg et al (2000)⁽³⁰⁰⁾ who studied leptin and other inflammatory markers in 17 male patients with severe COPD during the first 7 days of hospitalization for an acute exacerbation. On day 1 of the exacerbation, no significant correlations could be revealed between leptin and BMI. In contrast, on day 7 of the exacerbation, leptin positively correlated with fat mass after correcting for the influences of sex and age. The fact that no such relation was seen at day 1 of the exacerbation probably indicated a temporary dissociation, related to the exacerbation, of the normal feedback regulation of leptin by fat mass.

Similar findings also noted in a study by Çalikoglu et al (2004)⁽²⁹⁹⁾ in which leptin and TNF in males with COPD exacerbation, stable COPD and control group were studied. Leptin and TNF levels were significantly higher during exacerbation. Leptin levels were significantly correlated with the nutritional parameters in both control and stable groups. In stable group, there were significant correlations between serum leptin levels and BMI ($r = 0.454$, $p = 0.02$) and percent of fat mass ($r = 0.570$, $p = 0.002$). However, in patients with acute exacerbation, a correlation between leptin and nutritional parameters was not found, denoting that the inappropriately increased levels of leptin and TNF noted during acute exacerbations in patients with COPD may lead to changes in nutritional parameters and body weight in the course of the disease.

More or less similar findings were reported by Profita et al (2011)⁽³¹⁷⁾ in a study of levels of leptin, (TNF- α) and soluble form of intercellular adhesion molecule-1 (sICAM-1) in sputum and plasma of 27 smoker and former smoker patients with stable COPD. Plasma leptin was positively correlated with both patients' weight ($r = 0.69$, $p = 0.0006$) and BMI ($r = 0.63$, $p = 0.001$). A significant positive correlation was present between sputum leptin levels and FEV1/FVC% ($r = 0.39$, $p = 0.04$) in stable COPD patients, which was explained by a possible relationship between leptin and the resolution of airway infection in COPD patients.

Brusik et al (2012)⁽³⁰⁶⁾ studied relationship between resting energy expenditure (REE), disturbances in adipokines and weight loss in 44 patients with stable COPD. Results showed that from underweight to normal weight and obese patients, resting energy expenditure decreased ($p < 0.001$) and leptin levels increased ($p < 0.001$), and resting energy expenditure was inversely related to leptin level ($p < 0.001$) (which is opposite to the normal physiology), i.e. underweight patients showed an increased REE and a decreased leptin compared to normal weight-overweight patients. The study suggested a role of adipokines in energy imbalance in COPD-related cachexia and reported that since reductions in leptin levels reflect higher metabolic rate and adipose tissue depletion in underweight patients with COPD, low circulatory leptin concentrations might potentially serve as a biomarker of catabolism in clinical conditions associated with wasting.

Peng et al (2007)⁽³⁵⁰⁾ had investigated the potential roles of leptin and ghrelin in malnutrition in 53 patients with COPD versus control subjects. Plasma leptin levels were

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decreased, in underweight patients with COPD, and the levels were associated with nutritional parameters. After adjustment for nutritional parameters, leptin levels were elevated in COPD patients than control and correlated to TNF-alpha, suggesting that leptin might play a role in systemic inflammation of COPD. Another study by Broekhuizen et al (2005)⁽⁹¹⁾ of cachexia in COPD and its relation to inflammatory cytokines had showed that leptin was disproportionately lower in cachectic patients than in non-cachectic patients when corrected for the amount of fat mass.

In addition, Takabatake et al (2001)⁽³⁰⁷⁾ had studied another aspect of leptin and cachexia relationship in COPD, by investigating the physiologic significance of the circadian rhythm of circulating leptin and its relation to the pathophysiology of cachexia in COPD. Results demonstrated that circadian rhythm of leptin is absent in cachectic patients with COPD. The study speculated that low leptin concentration and the blunted diurnal variation in leptin could cause an alteration in the negative feedback to the hypothalamic-pituitary axes and that the loss of circadian variation in leptin level may have pathophysiologic significance for cachectic patients with COPD. Also inflammatory signaling in the hypothalamus had been implicated as a possible factor for the imbalanced energy homeostasis in cachexia syndrome.⁽²³⁴⁾

Those findings came in concordance with the results of the present study, the fact that patients with stable COPD were able to maintain normal correlation between leptin level and BMI suggested a maintained leptin signaling pathway and so maintained energy balance reflected on their nutritional parameters, that further reflected a better functional status and less morbid condition, as it had been shown by the negatively correlated leptin with both GOLD staging and BODE index within the stable COPD subgroup.

However, controversial results had been reported regarding the relations of leptin to different parameters in stable COPD specifically. Contradictory results to the present study had been reported by Yang et al (2006)⁽³⁵¹⁾ who assessed the role of serum leptin and tumor necrosis factor- α in malnutrition of male COPD patients, study demonstrated that serum leptin levels were significantly lower in weight-losing stable COPD patients than COPD patients without malnutrition and controls. There was a positive correlation between serum leptin levels and BMI in weight-losing COPD patients and it had been suggested that circulating leptin levels remain regulated even in patients in the cachexia status, and that leptin seemed not to play an important role in weight loss in patients with COPD. However, the mean BMI \pm SD of the underweight patient in the mentioned study was (19.66 \pm 1.39 kg/m²) which is still higher than the cut value of underweight patient (18.5 kg/m²) according to WHO classification.⁽³¹⁴⁾ The same study observed a higher but not statistically significant leptin level in exacerbation group, however there was a disappearance of correlation with BMI in the exacerbation group which might denote its inflammatory role during exacerbation.

Another study by Gaki et al (2011)⁽³⁵²⁾ had demonstrated a positive relationship between BODE index and leptin level in stable COPD patients in contrast to the present study, and that was explained by the possible role for leptin in the systemic component of COPD. However, the fat-free mass index in the mentioned study was negatively correlated with leptin, which is against normal physiological mechanisms of leptin, and that might denote that the studied patients were having a disturbed leptin signaling and metabolism and a higher catabolic/anabolic imbalance.

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Zhou et al (2013) ⁽³⁵³⁾ had conducted a meta-analysis to determine whether the level of leptin is related to the disease status of COPD, results showed that circulating leptin levels were correlated with the body mass index (BMI) as well as percent fat mass in stable COPD patients. The correlation coefficient tended to be weaker during exacerbation. A positive correlation between leptin and tumor necrosis factor (TNF- α) levels was found in COPD exacerbations, while it disappeared in patients with stable disease. The meta-analysis speculated that the normal regulatory mechanism of leptin is maintained in stable COPD patients despite weight loss, and that the additional correlation between leptin and TNF- α during exacerbations may suggest its valuable role in the evaluation of systemic inflammatory responses in COPD patients during exacerbation. Finally it's worth mentioning what was speculated by Ioffe et al (1998) ⁽²²⁰⁾ that the range of leptin's effects is likely to be complex, as different thresholds exist for several of leptin's actions.

Taken together, the current study suggested a role of the adipokine leptin as a multifunctional hormone with an important role in systemic inflammation characterizing COPD. Leptin was related to the low grade systemic inflammation in patients with stable COPD as well as during exacerbations. Leptin showed important associations with parameters of disease severity during exacerbations. In stable state, maintaining normal correlation between leptin and BMI as a nutritional parameter denoted a maintained physiological leptin regulations in contrast to cachexia state, which was in favor of less disease severity and better overall morbidity and mortality.

SUMMARY

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading and increasing cause of morbidity and mortality worldwide, and is characterized by persistent airflow limitation that is usually progressive, and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. The clinical and functional heterogeneity of COPD in addition to its multiple extra-pulmonary effects and associated comorbid conditions are further contributing to the increased morbidity and mortality. Inflammation occupies a central role in the pathogenesis of COPD. In addition to local pulmonary inflammation, systemic inflammation is adding to the complexity of the disease, characterized by increased circulating levels of inflammatory cells, cytokines and acute phase proteins that occur in both stable disease and during exacerbations.

BODE index has been introduced as a composite index having the ability to evaluate COPD more comprehensively, reflecting not only impairment in lung function, but also systemic consequences of the disease, disease progression, and mortality.

Leptin, initially discovered as an adipokine plays an important role in regulating energy balance, is emerging as a pleiotropic cytokine involved in the recruitment, activation and survival of inflammatory cells, and different studies had showed a growing role for leptin in COPD patients.

Aim of the work

The present study aimed to investigate the possible inflammatory role of leptin in COPD patients during exacerbation and stable status as well and to assess its relations to different disease aspects in stable state and during exacerbation. That was achieved by comparing leptin levels in stable COPD patients, patients during exacerbation and a healthy control group, and assessment of leptin relation to multiple disease parameters including CRP and BODE index

Methods

The present study enrolled 40 patients with COPD subdivided into twenty patients with stable disease and twenty patients during exacerbations. Results were compared versus twenty five healthy age matched subjects. All patients were males. Patients with BMI exceeding 30 (kg/m²) and those with comorbidities that might affect leptin as metabolic syndrome had been excluded. Fasting plasma level and the inflammatory marker CRP were assessed in all groups. The studied patients had been subjected to spirometric assessment, severity assessment by GOLD criteria based on predicted FEV1%, combined COPD assessment and assessment of BODE scores. Also routine laboratory investigations, chest x-ray had been done to all patients.

Results

Results showed a significantly higher level of leptin during COPD exacerbation than both stable patients and the control group ($p < 0.001$). CRP levels also were significantly higher during exacerbation than in the other two groups ($p = 0.001$). There was a significant positive association

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between leptin level and the inflammatory marker CRP in COPD exacerbation subgroup ($p=0.005$) and also in the stable subgroup ($p=0.048$). Results suggested a role of leptin during exacerbation as well as the stable state as an inflammatory marker related to the systemic inflammation status characterizing COPD, with higher level in stable COPD than controls and much higher levels during exacerbations.

In patients with COPD exacerbation, leptin levels showed a positive correlations with a number of severity parameters including severity assessment by GOLD stages based on FEV1% ($p=0.001$) and with the combined COPD assessment groups ($p=0.031$) as well as the BODE index scores ($p<0.001$). Leptin also showed a positive association with scores of mMRC dyspnea scales ($p=0.002$). There were significant negative correlations with predicted FEV1% ($p<0.001$) and 6MWT results ($p=0.001$). Overall findings suggested a link between a higher leptin level and a worsening functional status, higher severity and higher mortality during exacerbations.

Patients with stable COPD showed a positive correlation between their BMI values and leptin levels ($p=0.001$), and that was showed also in the control subjects. However no correlation was noted between BMI and leptin in the exacerbation subgroup, which might be attributed to a surge of the leptin level during exacerbation, suggesting a link between the higher levels of leptin and the upgraded systemic inflammation during exacerbation. As leptin is primarily related to the nutritional parameters as BMI and fat mass, and showed to be lower in cachectic patients, that maintained correlation with BMI in the stable patients in addition to the higher level than controls might denote the patients' ability to maintain normal energy balance especially with different BMI values noted within the stable subgroup.

In the stable subgroup, there was a negative correlation with severity assessment by GOLD staging ($p=0.038$) and with BODE scores ($p=0.017$), which was in favor of less severity and mortality in contrast to the exacerbation group.

Taken together, the present study suggested that maintaining a higher leptin in stable COPD patients in addition to the preserved correlation with BMI suggested a maintained normal leptin signaling pathway and the ability of those patients to maintain normal energy balance and nutritional status, and that further reflected a better general condition and less mortality as shown in the negative correlation between leptin level and both GOLD stages and BODE scores in the studied patients with stable COPD.

CRP showed a significant positive correlation in COPD exacerbation group with BODE scores ($p=0.025$) and was negatively correlated with FEV1% ($p=0.037$), denoting the relationship between the upgraded systemic inflammation during exacerbation and the decline in lung function and the increased overall disease severity and mortality. In both groups, CRP was negatively correlated with 6MWT results denoting its relation to a worsening functional status of the COPD patients.

BODE scores showed a positive association with severity assessment by GOLD staging based on FEV1% and the combined COPD assessment staging in both studied groups ($p<0.001$ for both). BODE index also showed a positive correlation with CRP level during exacerbation ($p=0.025$). Results reflected its ability as multidimensional composite score to reflect the complexity and heterogeneity of the disease, including systemic inflammation, functional status and overall disease severity.

CONCLUSIONS

- The adipokine leptin is a multifunctional hormone, beside its role in energy balance; it plays an important role in systemic inflammation characterizing COPD.
- Leptin is related to the low grade systemic inflammation in patients with stable COPD as well as during exacerbations.
- During COPD exacerbations, leptin levels are much higher than stable state owing to the upgraded systemic inflammatory response.
- The higher leptin levels during COPD exacerbations are related to lung function decline and also to a worsening overall morbidity and mortality.
- In patients with stable COPD, maintaining normal correlation between leptin and BMI as a nutritional parameter denotes a maintained physiological leptin signaling pathway in contrast to cachexia state.
- In patients with stable COPD, maintaining normal leptin metabolism is in favor of better functional state and better overall morbidity and mortality
- The acute phase protein CRP is related to the decline in lung function in both stable COPD and during exacerbation, CRP is related also to higher morbidity and mortality.
- The multidimensional BODE index is a valuable method to express the complex nature of COPD disease including the inflammatory aspect, functional status plus its role as a predictor of mortality.