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**Title: Evaluation of serum resistin, visfatin and chemerin levels in patients with lung cancer and chronic obstructive pulmonary disease**

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## **Abstract**

**Objective:** Cachexia is an important problem in lung cancer and COPD. Some studies report an association between adipokines and cachexia. Our study aimed to investigate the association of three recently described new adipokines, namely resistin, visfatin, and chemerin with lung cancer and COPD.

**Methods:** Thirty patients with non-small-cell lung cancer (NSCLC), 30 patients with COPD and 30 healthy volunteers were included in the study. A statistically significant weight loss was

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found in COPD and lung cancer patients compared to the control group ( $p < 0.001$ ). Among the bio-markers, only resistin levels were significantly higher in cachectic patients compared to the patients without weight loss in all groups ( $p = 0.006$ ). Resistin level was significantly higher in patients with COPD ( $p = 0.002$ ). Visfatin level, however, was significantly higher in the control group ( $p = 0.001$ ). We found that a higher biomass exposure resulted in a significant increase in the resistin level ( $p = 0.007$ ) and a significant decrease in the visfatin level ( $p = 0.001$ ) in the patient groups. In addition, high asbestos exposure levels were associated with lower visfatin levels ( $p = 0.001$ ). For all groups, no statistically significant relationship was found between chemerin levels and weight loss or other variables. Furthermore, the type and stage of lung cancer were not associated with the bio-markers.

Results: No significant relationship was found between the bio-markers and lung cancer type (adenocarcinoma or squamous cell lung cancer), tumor stage, lymph node stage, and metastasis stage. There was no relationship between the bio-markers by TNM and GOLD stages ( $p > 0.05$ ). We observed no findings strong enough to support the use of these molecules as markers of disease stage or cachexia.

Conclusion: In conclusion, resistin, visfatin and chemerin cannot be used as potential bio-markers in patients with lung cancer or COPD nor as markers of disease stage or cachexia.

**Key Words:** Adipokines, cachexia, lung cancer, COPD

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**Key points:**

- Significant findings of the study: Resistin, visfatin and chemerin cannot be used as potential bio-markers in patients with lung cancer or COPD nor as markers of disease stage or cachexia.
- What this study adds: Relationship between indoor air pollutants (biomass, asbestos) and adipokines.

**INTRODUCTION**

Respiratory tract diseases are the leading cause of death worldwide. Among these, lung cancer and COPD are the leading diseases with regard to mortality [1]. Lung cancer is usually already in an advanced stage at the time of diagnosis and most patients have no chance of successful treatment. COPD is a chronic, progressive disease which severely affects the patients' quality of life. Weight loss is observed in certain disease stages in lung cancer and COPD, impairing quality of life and making the treatment more challenging. Cachexia is a clinical syndrome characterized by significant weight loss together with anemia and decrease in appetite [2]. In the literature, there are studies investigating the association between malignancies and adipokines [3,4]. However, there are few studies investigating molecules other than leptin in COPD [5]. While some studies report an association between adipokines and cachexia, there are also some studies indicating otherwise.

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Adipokines are protein bio molecules which are released from the adipose tissue. As recently described, adipose tissue is a highly active organ of the endocrine and immune systems. In vitro studies demonstrated that some adipokines trigger the growth and proliferation of cancer cells. In addition, many clinical studies reported that adipokines are significantly related to the tendency for tumor development, pathogenesis and prognosis [6,7]. Resistin, visfatin and chemerin are newly described adipokines and in our study, we investigated the role of these adipokines in lung cancer and COPD and their relationship with cachexia.

## **MATERIALS AND METHODS**

This was a randomized controlled study conducted between July 2014 and March 2016 after the ethics approval (Approval no: 2014/85) was granted by the Medical Faculty Ethics Committee. Thirty patients with newly diagnosed, untreated and histopathologically confirmed non-small-cell lung cancer ( 14 adenocancer, 16 squamous cell lung cancer) and 30 patients with mostly moderate to severe COPD, who admitted to the chest diseases outpatient clinic, were included in the study. Thirty healthy volunteers without other diseases were included in the study as the control group. Informed consent was obtained from all enrolled participants and the demographic data of the participants were recorded. Patients with lung cancer were staged using TNM 7 [8] staging system. The results of pulmonary function tests and arterial blood gas analyses of COPD patients were recorded from the hospital information system. The patients with COPD were categorized by the 2014 updated GOLD [9] classification. The patients with COPD underwent lung CT scan to exclude any underlying pulmonary malignancy. For all participants, current body weight was compared to the body weight value measured 3

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months ago. The subjects with a weight loss of over 5% over the previous 3 months were considered to have cachexia. Asbestos and biomass exposures were questioned. Exposure was confirmed in subjects with an asbestos or biomass exposure of 10 years or more. Blood samples were taken from all participants between 07:00 and 09:00 in the morning after overnight fasting. Lipid profiles and HgA1c levels were analyzed in the collected blood samples. In addition, blood samples of 5 ml were centrifuged at 5000 rpm for 5 minutes and the obtained serum samples were transferred into 1.5-ml Eppendorf tubes and stored at -80 °C until the analysis. Following collection, all samples were randomly numbered and submitted to the biochemistry laboratory for analysis. Samples were analyzed in the laboratory with enzyme-linked immunosorbent assay (ELISA) method by using Human Resistin Platinum ELISA kit (Bender MedSystems GmbH, Vienna, Austria), human visfatin (VF) ELISA kit (Sunred Biological Technology Co., Ltd., Shanghai) and human chemerin ELISA kit (Boster Biological Technology Co., Ltd., Pleasanton, CA, USA). The obtained data were recorded. SPSS 19.0 software was used for the analysis of data. Parametric group comparison tests were used to analyze the variables conforming to normal distribution. Student t-test was used for the two independent groups and one-way analysis of variance was used for multiple groups. Tukey's HSD pairwise comparison tests were used for significant results. The groups showing differences were indicated with superscripts. Mann-Whitney U-test for two independent groups was used to compare the variables not conforming to normal distribution and Kruskal-Wallis test was used for multiple group comparisons. Paired comparisons of Kruskal-Wallis test were performed in multiple groups with general significance. Chi-square test with Monte Carlo correction was used to determine the relationship between qualitatively measured variables. Spearman's Rho correlation analysis was used to assess the correlation between the

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proportional scale variables. Significant results were shown with graphics. In all analyses, a p value of <0.05 was considered statistically significant at a type I error level of 5%.

## RESULTS

The subjects' median age was 60 years (range 28-84 years) in all groups. The mean body weight was  $73.88 \pm 16.08$  kg. In all groups, 84.4% of the participants were male. The proportion of patients with a history of smoking was 76% (median, 50 pack-year) among the lung cancer and COPD patients. The mean length of asbestos and biomass exposure was over 20 years in the lung cancer and COPD patients (Table 1). Approximately half of the patients with lung cancer were stage 4 (43.3%), with one-third at stage 3b (33.3%). The rest of the patients were stage 3a (13.3%), stage 2b (6.7%) and stage 2a (1.1%; n=1). More than half of the patients in the COPD group were GOLD D (%57.1; n=20).

Weight loss was higher in lung cancer and COPD patients compared to the control group ( $p < 0.001$ ), while no significant difference was found between COPD and lung cancer groups in terms of weight loss (Figure 1). Gender had no significant effect on the measured markers.

The bio-markers resistin and chemerin were higher in patients with COPD, while visfatin was significantly higher in the control group (Figure 2).

## STATISTICAL ANALYSIS

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Biomass exposure had a significant effect on resistin and visfatin levels. Resistin was higher while visfatin was lower in subjects with biomass exposure. Approximately one-third of patients had weight loss. Only resistin had a significant effect ( $p=0.002$ ) in patients with weight loss.

No significant relationship was found between the bio-markers and lung cancer type (adenocarcinoma or squamous cell lung cancer), tumor stage, lymph node stage, and metastasis stage. There was no relationship between the bio-markers by TNM and GOLD stages ( $p>0.05$ ) (Figure 3). There was no significant correlation between the resistin, visfatin and chemerin levels in the lung cancer, COPD and control groups. A positive and significant correlation was found only between age and chemerin levels ( $R=0.426$ ;  $p=0.019$ ). No significant correlation was found between the markers among COPD patients. Resistin, visfatin and chemerin levels were not different in lung cancer patients with cachexia. It was observed that cachexia was effective on visfatin levels in COPD patients ( $p=0.031$ ). The mean visfatin level was as high as  $9,82\pm 7,29$  ng/mL in four patients with cachexia, while as low as  $3,87\pm 5,80$  ng/mL in patients without cachexia.

When the marker levels were compared by GOLD grades in COPD patients and by TNM stages in lung cancer patients, marker levels were not significantly different by GOLD grades or lung cancer stages. It was found that visfatin level increased with the disease stage, while there was no increase or decrease in resistin and chemerin levels.

Among the bio-markers, only resistin levels were significantly higher in cachectic patients compared to the patients without weight loss in all groups ( $p=0.006$ ). Among the three groups, resistin level was significantly higher in patients with COPD ( $p=0.002$ ). The visfatin level, however, was significantly higher in the control group ( $p=0.001$ ) (Figure 2). We

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found that a higher biomass exposure resulted in a significant increase in the resistin level ( $p=0.007$ ) and a significant decrease in the visfatin level ( $p=0.001$ ) in the patient groups. Again, high asbestos exposure levels were associated with lower visfatin levels ( $p=0.001$ ). For all groups, no statistically significant relationship was found between chemerin levels and weight loss or other variables.

## DISCUSSION

Cachexia is a clinical syndrome characterized by significant weight loss together with disorders such as decrease in appetite and anemia and it is mostly seen in cancer patients although can also be seen with other systemic diseases. Cachexia is an important problem in lung cancer and COPD. Cachexia has a significant effect on treatment success and quality of life of patients. Many hormones and mediators play roles in the pathogenesis of cachexia. A significant proportion of these are released from the adipose tissue. As recently described, adipose tissue is a highly active organ of the endocrine and immune systems. Firstly, leptin was described in 1994 [10] and its association with many diseases was investigated in the following years. Today, it is known that the adipose tissue secretes more than 20 hormones and signal molecules and that these play roles in many biological events related to the autocrine and paracrine systems, vascular system through blood circulation, energy and glucose metabolism, reproduction, bone metabolism and immunity [11]. Resistin, visfatin and chemerin are newly described bio molecules and they have not been investigated in many studies. Many clinical studies reported that adipokines are significantly associated with the tendency for tumor development, pathogenesis and prognosis[11,12]. In a study investigating serum leptin levels in cachectic and non-cachectic lung cancer patients, serum leptin concentration was

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significantly lower in cachectic lung cancer patients compared to non-cachectic patients and the control group [13].

In a review of critical patients and adipokine levels, resistin and visfatin levels were reported to increase irrespective of disease etiology in critical patients. This increase was reported to be related to organ failure and tissue inflammation[14]. In a study investigating the association between resistin, leptin and lung cancer, resistin level was higher and leptin level was lower in patients with lung cancer compared to the control group [15]. Also in our study resistin level was higher in the lung cancer group compared to the control group, while the difference was not statistically significant. Furthermore, visfatin level was higher in healthy volunteers than in the patients in our study, which was in contrast with the results from the available studies. In a study conducted in 2009, the relation between chemerin and lung cancer was investigated in 42 lung cancer patients and 32 healthy volunteers and it was demonstrated that chemerin was not related to lymph node metastasis, stage and pathological type, while chemerin levels were significantly higher in lung cancer patients than in healthy volunteers and the authors proposed that chemerin could be a diagnostic marker [16]. In a new study conducted by CH Xu et al. in 2017, chemerin levels were significantly higher in patients with non-small cell lung cancer compared to healthy volunteers and the higher chemerin level was reported to be related to the TNM stage, lymph node metastasis and distant metastasis [17]. In our study, however, chemerin level was higher in both lung cancer patients and COPD patients compared to healthy volunteers but this increase was not statistically significant. Moreover, no relationship was noted between the increase in chemerin levels and TNM stage or tumor type. Approximately 25% of patients with chronic obstructive pulmonary disease (COPD) develop cachexia and this is associated with a severe decrease in patient survival. Only leptin was

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investigated in the studies assessing the association of adipokines with COPD and weight loss. Takabatake et al. in their study found that the serum leptin levels were low in patients with weight loss and increased with weight gain. This finding was not completely clear since it applied to all COPD patients except for cachectic patients [18]. In their study Schols et al. found that leptin levels were significantly lower in emphysematous COPD patients compared to COPD patients predominantly with chronic bronchitis [19]. However, lean body mass was the same in both groups and it was observed that the decrease was in the adipose tissue mass. In addition, it was reported that leptin stimulates inflammatory cytokines [20,21]. In the light of all these studies, it was emphasized that leptin cannot be a marker for cachexia.

Anna Kumor-Kisielewska et al. in their study found that leptin and resistin levels were higher in COPD patients than in healthy subjects and they reported that this could be related to the increased systemic inflammation in COPD [22]. Also in our study we found that resistin level was high in the COPD patients and was related to weight loss.

The limitations of the study and the factors that may have affected the study results included the low number of patients in the groups, the fact that patients with advanced stage were included in the lung cancer and COPD groups and the lower mean age in the control group compared to the other two groups.

In conclusion, this study investigated the relationship between cachexia and the bio-markers released from the adipose tissue in lung cancer and COPD. It was concluded that resistin, visfatin and chemerin cannot be used as potential bio-markers in patients with lung cancer and COPD. We observed no findings strong enough to support the use of these molecules as markers of disease stage or cachexia. However, the higher levels of visfatin in the control

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group and in subjects with low biomass and asbestos exposure were considered as a different result compared to the literature.

**Disclosure:** No authors report any conflict of interest.

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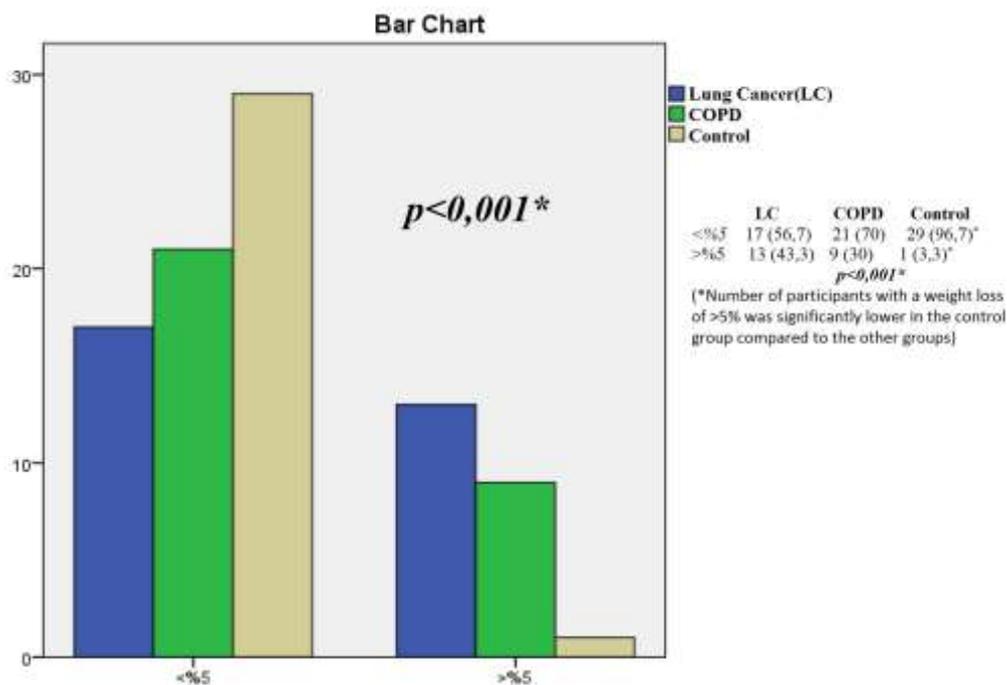
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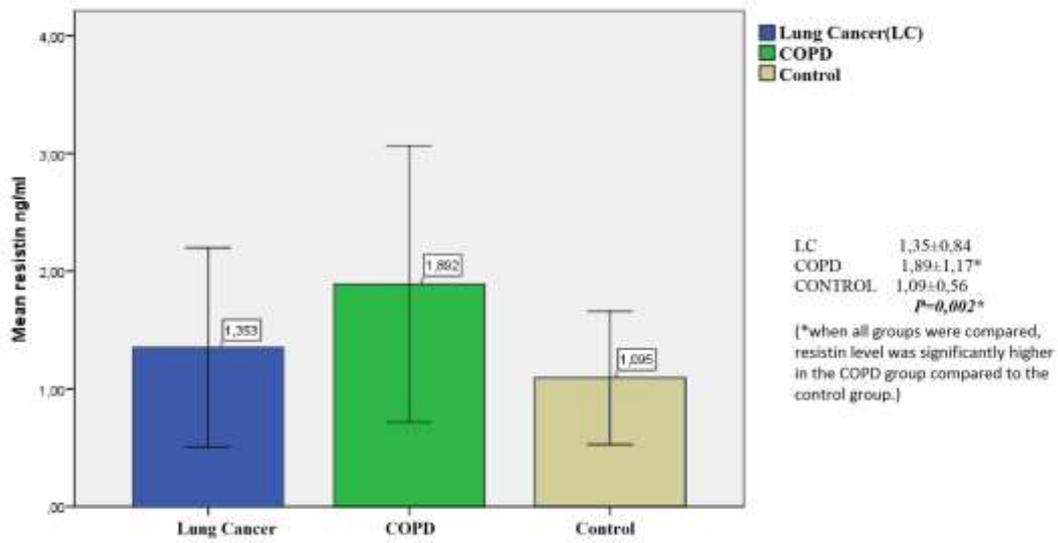
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### Figure legends

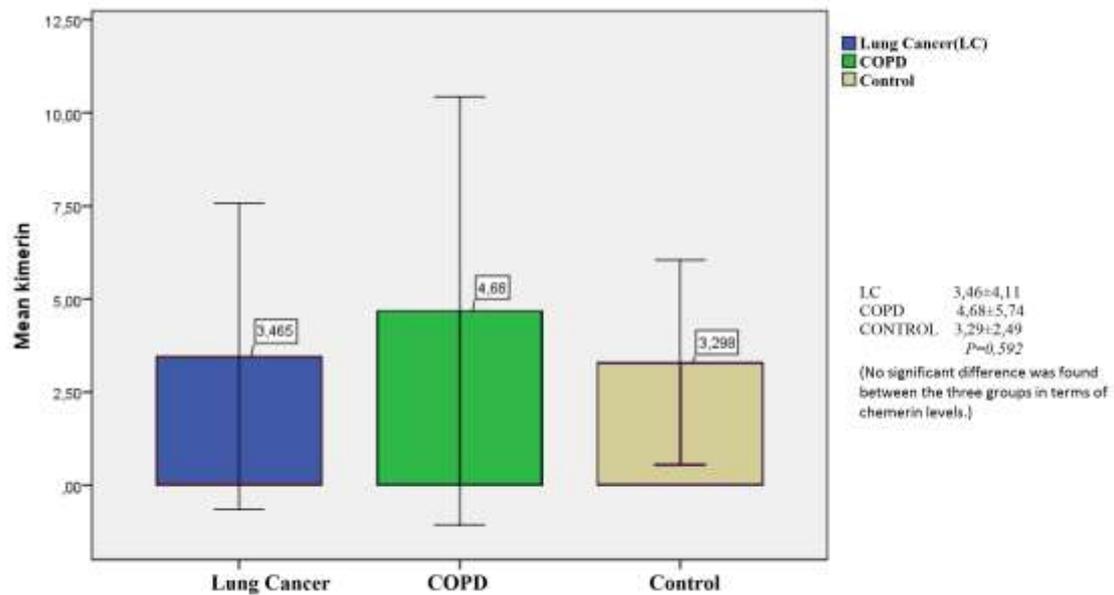
**Figure 1.** Participants with a weight loss of over 5% over 3 months.



**Figure 2a-c.** Comparison of biomarkers by groups.

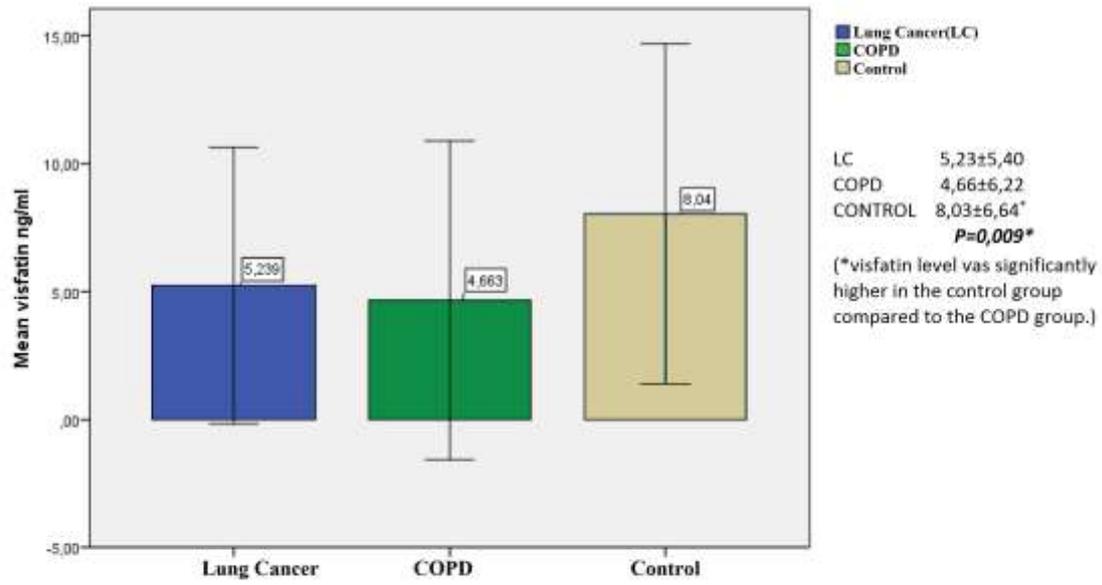


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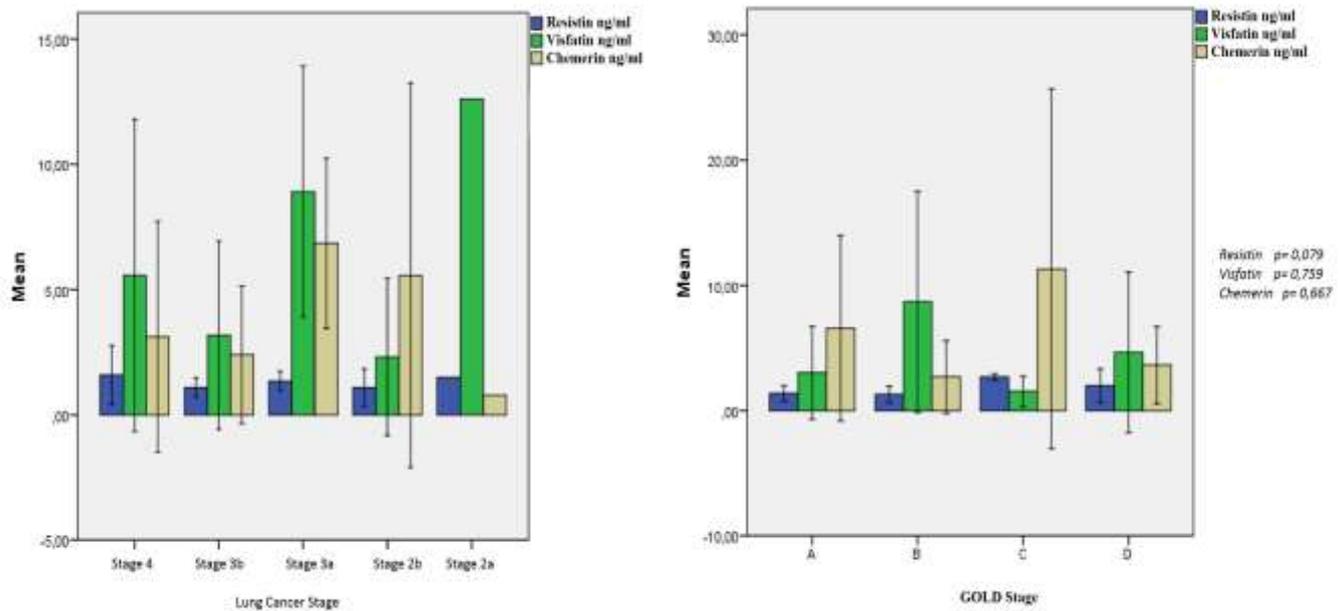
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**Figure 3.** Marker levels by disease stages in lung cancer patients and COPD patients.

(No statistically significant difference was observed in biomarker levels by lung cancer stages and GOLD stages).

**Table 1.** Demographic data and bio-marker levels

|                 |                   | LC                               | COPD                         | Control                      | Overall          |                   |
|-----------------|-------------------|----------------------------------|------------------------------|------------------------------|------------------|-------------------|
|                 |                   | Mean $\pm$ SD (median, min, max) |                              |                              |                  | p                 |
| <b>Age</b>      | years             | 63; 41; 83 <sup>a</sup>          | 65; 46; 81 <sup>a</sup>      | 39; 28; 84 <sup>b</sup>      | 60; 28; 84       | <b>&lt;0,001*</b> |
| <b>BMI</b>      | kg/m <sup>2</sup> | 24,71 $\pm$ 3,89                 | 25,61 $\pm$ 6,16             | 27,55 $\pm$ 4,16             | 25,96 $\pm$ 4,93 | 0,073             |
| <b>Smoking</b>  | pack-years        | 50; 30;100 <sup>a</sup>          | 50; 10; 125 <sup>a</sup>     | 13; 5; 27 <sup>b</sup>       | 45; 5; 125       | <b>&lt;0,001*</b> |
| <b>Asbestos</b> | years             | 30; 6; 50 <sup>a</sup>           | 22; 9; 80                    | 15; 5; 50 <sup>b</sup>       | 25; 5; 80        | <b>0,022*</b>     |
| <b>Resistin</b> | ng/ml             | 1,35 $\pm$ 0,84                  | 1,89 $\pm$ 1,17 <sup>a</sup> | 1,09 $\pm$ 0,56 <sup>b</sup> | 1,44 $\pm$ 0,94  | <b>0,002*</b>     |
| <b>Visfatin</b> | ng/ml             | 5,23 $\pm$ 5,40                  | 4,66 $\pm$ 6,22 <sup>a</sup> | 8,03 $\pm$ 6,64 <sup>b</sup> | 5,98 $\pm$ 6,22  | <b>0,009*</b>     |
| <b>Chemerin</b> | ng/ml             | 3,46 $\pm$ 4,11                  | 4,68 $\pm$ 5,74              | 3,29 $\pm$ 2,49              | 3,81 $\pm$ 4,37  | 0,592             |

|                         |     | LC                     | COPD                   | Control                |                   |
|-------------------------|-----|------------------------|------------------------|------------------------|-------------------|
|                         |     | n (%)                  | n (%)                  | n (%)                  | p                 |
| <b>Biomass exposure</b> | Yes | 22 (73,3) <sup>a</sup> | 22 (73,3) <sup>a</sup> | 8 (26,7) <sup>b</sup>  | <b>&lt;0,001*</b> |
|                         | No  | 8 (26,7) <sup>a</sup>  | 8 (26,7) <sup>a</sup>  | 22 (73,3) <sup>b</sup> |                   |

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|                          |     |                      |                        |                        |                   |
|--------------------------|-----|----------------------|------------------------|------------------------|-------------------|
| <b>Asbestos exposure</b> | Yes | 27 (90) <sup>a</sup> | 26 (86,7) <sup>a</sup> | 11 (36,7) <sup>b</sup> | <b>&lt;0,001*</b> |
|                          | No  | 3 (10) <sup>a</sup>  | 4 (13,3) <sup>a</sup>  | 19 (63,3) <sup>b</sup> |                   |

**LC:**Lung cancer; **COPD:** Chronic obstructive pulmonary disease

<sup>a, b</sup> : p values are significant for comparison of groups marked with "a" and "b"