

Inflammatory Biomarker of Peripheral CRP and Analyzing Serum Trace Elements like Zinc, Copper, and Cu to Zn Ratios in Stable Chronic Obstructive Pulmonary Disease: Tehran-Iran

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ABSTRACT

Background: Chronic Obstructive Pulmonary Disease (COPD) is characterized with incomplete airway reversibility by spirometric testing, chronic local airway and systemic inflammation. COPD is the most common lung disease related to smoking habit in general population. Oxidative stress (Os) is the key mechanism in inducing and progression of inflammatory productions in COPD pathogenesis. However, CRP is a peripheral biomarker to reflect systemic inflammation and tissue damage. Nutritional trace elements like Zinc (Zn) and Copper (Cu) are components of Os mechanism and as the anti-oxidant elements. Those changing serum levels influence on the reaction of recent process and production of inflammatory protein. Propose of the study was to evaluate status of CRP and Zn, Cu, Cu/Zn ratios among male patients with stable Chronic Obstructive Pulmonary Disease.

Method: Stable COPD patients substantially were enrolled from pulmonary chest medicine. Applying criteria followed an instruction of thoracic society.

Results: A total of 35 patients completed criteria of study. Mean age \pm SD was 52.8 \pm 5years (ranged 43-60). Hypozincemia and hypocupremia were found 11% and 14%, respectively.

Conclusion: Increased CRP levels reflected presence systemic and pulmonary inflammation among stable COPD patients. In addition, Zinc and copper deficiency detected among target population. The current trace elements are a part of oxidative stress, and their deficiencies may be effective in the induction of inflammation.

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► *Implication for health policy/practice/research/medical education:*
Inflammatory Biomarker of Peripheral CRP

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1. Introduction:

Chronic Obstructive Pulmonary Diseases (COPD) is characterized as an inflammatory, chronic disease with partial reversibility of airway obstruction. It will be the fifth leading cause of death globally at 2020. Prevalence of COPD joins with the growth of the cigarette smoking habit among general population (1). Early response of abnormal inflammation in COPD appears against to noxious particles or gases. Subsequently, It is conducted with oxidative stress as the main stem mechanism in the inducing, progression and continuation of the inflammation (2).

Biomarker of C-reactive protein (CRP) is a member of acute phase proteins and one of the systemic inflammatory markers. The increase in CRP levels observed in the early and chronic inflammation and also in tissue damage (3). Partial of a clinical feature of COPD characterization reflected as systemic inflammation (4). CRP level correlates with the degree of pulmonary inflammation during stable condition. It can be supported early disease, exacerbations and predict prognosis of COPD (5). On the other hand, it can be determined the risk of future events in COPD such as hospitalization, mortality and morbidity in chronic respiratory failure (6). CRP has a reciprocal link with decreasing of FEV1 (7) and association with cardiovascular disorders (8).

Zinc is an essential micronutrition, anti-inflammatory and anti-oxidative stress agents. Zinc deficiency has been found in numerous chronic inflammatory diseases (9).

Copper is the third abundant trace element in the body, and is essential for normal cell's biology. Excess copper can be induced oxidative stress and leads to chronic inflammation (10). Interaction

between zinc and copper levels are complex, and balance between them is an important key in the homeostasis. They effect on the superoxide dismutase (11, 12).

The aim of the study was to survey the status of peripheral CRP inflammatory biomarker, serum zinc, copper and Cu/Zn ratios among stable patients with COPD.

2. Materials and Methods:

The design of the study was cross-sectional. It conducted in Shahid Beheshti University of Medical Sciences (SBUMS)–Logman Hakim general teaching hospital, 2011 year. Logman hospital chest clinic located at the south of Tehran. Annual rate of outpatients visit estimated 1800 subjects. The hospital was covering health services to the poor income people. It is a territory center of respiratory diseases. The study designed at autumn season.

Subjects of the study population substantially enrolled from adult male with stable COPD. COPD and stable state defined based upon symptoms and pulmonary function testing. Stable condition consisted of a normal day-to-day variation of clinical features (symptoms and signs) in the last three months previous the study. They had not airway infection, admission at the hospital or emergency room and apparently fixed consumption of medications. All the patients examined in two steps. During the first visit, subjects selected based upon the history, physical examination, a standard chest x-ray, pulmonary function test and routine laboratory test. Next step, all the data reviewed and design criteria for the study applied.

Criteria of established COPD are included sputum production, dyspnea and cough. It

is at least in the last two years ago for most days in a consecutive 3-months period; particularly, in autumn and winter seasons. It should be associated with standard pulmonary function parameters, such as FEV1<70%, FEV1/FVC<80% predicted. The airway reversibility is less than 12% from baseline after inhaling 400 microgram bronchodilator (Salbutamol via a metered- dose inhaler) (13).

Inclusion criterion included adult, male sex, stable, COPD, smoking history, consensus of participations. Onset diagnosis of COPD should be over than three years and should be spent at least a period of hospitalization. Exclusion criteria consisted of female sex (due to affect of hormonal changes on the outcome of laboratory data), cardiovascular diseases, those who use statin compound (14) or oral corticosteroid (15), and multivitamin containing zinc and copper compounds.

Blood samples were taken in the morning fasting state. Cut of points for zinc, copper, ceruloplasmin, albumin and CRP levels were 110.1-195 µg/dl, 70-140 µg/dl, 187-322 mg/l and >6 mg and 3.5-5.3g/dl, respectively. The serum zinc was analyzed by direct colorimetric methods using kit (Greiner diagnostic GmbH-Bahlingen-Germany). Copper level was measured with colorimetric test with Dibrom-PAESA (Greiner diagnostic GmbH-Bahlingen-Germany). Ceruloplasmin serum was detected by radial immunodiffusion kit (Binding site group, Birmingham-UK). CRP was assayed by microplate immunoenzymometric (Monobind Inc-USA).

The data were analyzed by SPSS software version 18. The variable summarized by the frequency. The study's means compared with independent-samples t-test and the variables correlated with Pearson's test. A P-value <0.05 was set for all statistical test results.

3. Results:

A total of 35 stable COPD followed the study criteria. Mean age±SD was 52.8±5.0

years. It ranged 43-60 years (median,

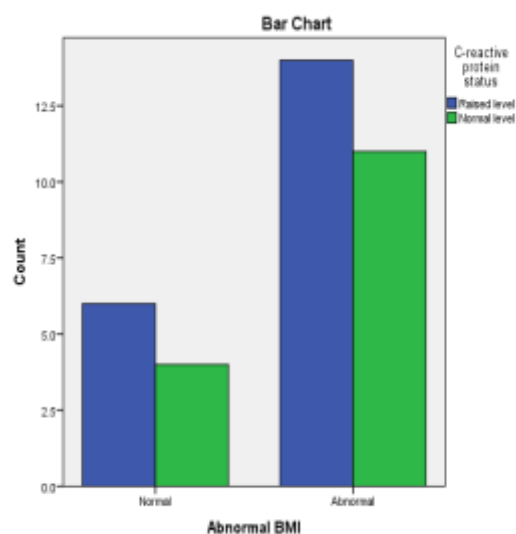


Fig. 1. It reveals distribution of CRP value between BMI ranges.

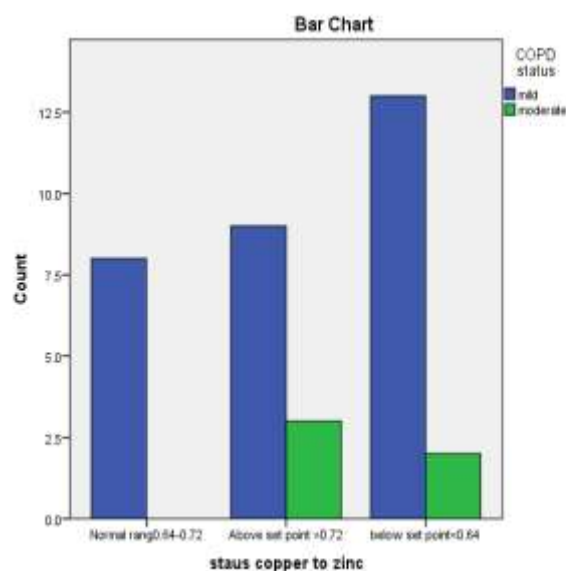


Fig. 2. It displays status of COPD pulmonary function stages and distributed ranges of Cu/ Zn ratio.

mode 55 year).

Figure 1 reveals distribution of BMI and CRP value. Status of COPD included 86% in mild and 14% in the severe stages. Raised CRP class consisted of 63% mild stage of COPD. BMI status recorded as 66% higher than a set point, 29% normal value and 6% in lower set point. 56% of abnormal BMI was seen in raised CRP class. Distribution of COPD pulmonary

function test stages indicated that Status of copper to zinc ratio arranged as 43% in the below set point in mild stage and 60% above cut of point in moderate stage. Figure 2 displays status of COPD pulmonary function stages and distributed ranges of Cu/Zn ratio. Zinc and copper deficiencies were distributed in mild stage of COPD 100% and 80%, respectively.

The total mean of serum zinc level was 111.03 ± 35.97 SD $\mu\text{g/dl}$. The mean of zinc was recorded in the raised CRP subset 170.50 ± 43.1 SD. It was lower than normal CRP level subgroup 162.7 ± 33.05 SD. However, zinc deficiency was observed in 11% of the study sample. 50% of them were in the elevated CRP subgroup. Figure 3 shows mean distribution of zinc level between subsets of normal and raised CRP classes.

Figure 4 displays serum copper value in different CRP classes. The total mean of copper level noted 167.14 ± 38.78 SD $\mu\text{g/dl}$. The means of serum ceruloplasmin and albumin were 290.17 ± 88.30 SD mg/l and 4.80 ± 0.43 SD g/dl , respectively. The mean of serum copper recorded in elevated CRP subset 127.3 ± 31.03 SD. It was noticeably higher than the serum copper mean in a normal set point level of CRP 86.33 ± 3.87 SD. In addition, copper deficiency was disclosed in 14% of the target population. However, 20% of them were observed in the raised CRP group. Excess copper level from normal set point was found in the 17% of samples. 83% of them were detected in the standard range of zinc concentration. Relevant correlation was found between zinc with copper deficiency ($r=0.36$, $p=0.03$).

The mean of CRP level was 35 ± 35.62 SD mg/dl . It ranged between 2 to 98 (median=24, mode 4). CRP value up to the normal set point was observed in 57% of focus population. In addition, there were meaningful differences between serum copper and CRP level ($P=0.001$, confidence intervals of the difference $L=16.45$, $U=59.48$).

Mean of Cu/ Zn ratio was 0.67 ± 0.24 SD. It ranged between 0.3 -1.24. Distribution of

the ratio levels arranged 23% in the normal range, 34% in above and 43% below than cut of point. Mean value of the ratio was

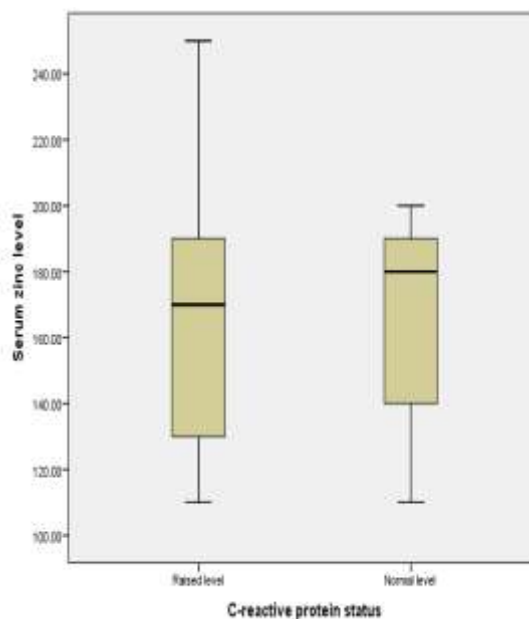


Fig. 3. It shows serum zinc concentration in different C-reactive protein classes.

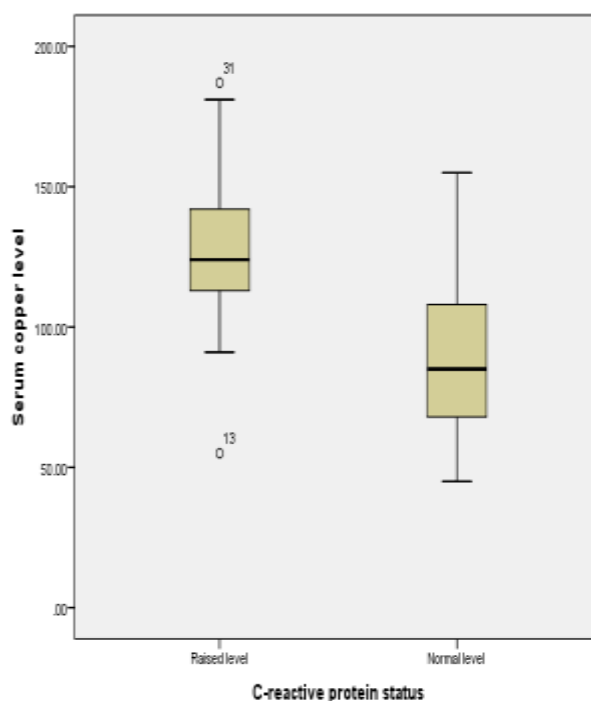


Fig. 4. It demonstrates serum copper concentration between different C-reactive protein classes.

increasing in raised CRP subset (0.76 ± 0.25)

SD) than a normal value (0.55 ± 0.15 SD). 45% of the rising value of CRP class was seen in the abnormal ratio of Cu/Zn ratio. Graph 5 shows the distribution of CRP and

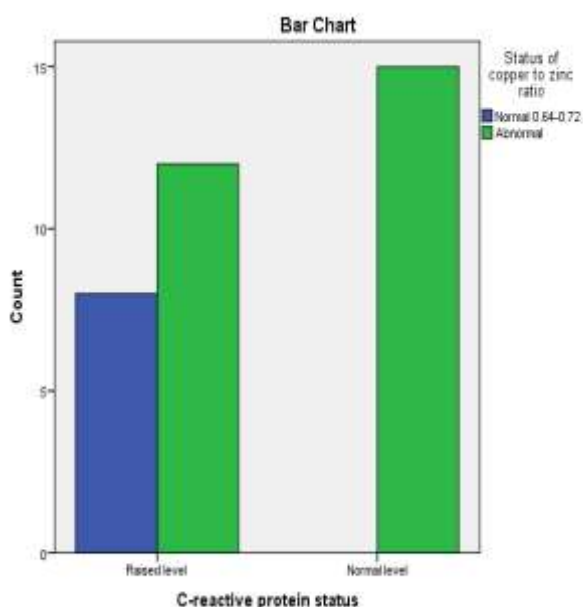


Fig. 5. It shows distribution of CRP and copper to zinc ratio value.

ratio value. Serum CRP had noticeable differences with Cu/Zn ratio ($P=0.007$, df: 33, 95% CI 0.6- 0.36). Mean of CRP level was higher in the normal range of Cu/Zn ratio than abnormal range.

4. Discussion:

CRP is a part of the acute phase responses. Upraised serum level of CRP may be indicated infection or inflammatory process. Elevation also reported in chronic diseases (16). It measures as monitoring or diagnostic inflammatory systemic disease activity. COPD is the most prevalent of respiratory diseases. Chronic systemic inflammation is considered a hallmark of COPD. Raising two to fourfold of circulating pro-inflammatory, anti-inflammatory cytokines and acute phase protein reported in COPD (17). Moreover, a few of circulating inflammatory protein detected even in stable chronic condition, such as CRP, interleukin 6, 8 and tumor necrosis factor-alpha. It is believed that the origin of inflammatory protein is a

consequence of spillover of pneumo-proteins. They transferred from vasculature permeability of the lung to the circulation. CRP level was notably elevated in 57% of subjects. It was represented systemic inflammation between the samples of study with stable COPD. Recent reports indicate that there is a correlation between CRP levels and pulmonary inflammation (18). Oxidative stress is known cardinal mechanism in the pathogenesis of COPD and respiratory inflammation. It can increase inflammation, and vice versa. Oxidative stress defines as imbalance between the amount of load oxidants and anti-oxidant capacity for scavenge produced radicals. The origin of oxidants may be result from inflammatory cells or inhalation of oxidant's conditions, as cigarette smoking or air pollution. However, level of activity of anti-oxidant capacity may be under influence of nutritional status and dysfunction of antioxidant enzymes (19).

Trace elements are the component of oxidative stress, and had the role in pathophysiology of COPD (20) Oxidative stress (Os) is the known mechanism. It contributes in human chronic diseases, such as COPD, aging process, immunologic disorders, atherosclerosis, mutagenesis, and cancer. Reactive oxygen species includes O_2^- , $\cdot OH$ and H_2O_2 . They are catalyzed by the NADPH oxidases, super oxide dismutase (SOD) and metallothionein (MT), respectively. The role of Zn is the inhibitor in the NADPH oxidase, induced production of MT and associated with copper in the SOD (21). The effect of Zn on the respiratory system evaluated, and Current report indicated that Zn intake can be lowered risk of COPD associated with cigarette smoking (22). It may be resulted from an anti-inflammatory and anti-oxidant effect of Zn-dependent enzymes and additionally through decreasing production of inflammatory-cytokines. Moreover, there was a correlation between systemic activity of super oxide dismutase (SOD)

with airway limitation and developing bronchial hyper-responsiveness (23).

Marginal Zinc deficiency observed among chronic stable COPD 11%. The first report of Zn deficiency was coming from Iranian males since 1961, year and Iran encounters as low risk countries. Zn deficiency detected among Iranian male in Tehran (24). Zn deficiency has not severed impact on the health but induced widespread problems (25). The effect of Zn on the health consists affecting on the multiple aspect of the immune system, antioxidant, modulating oxidative stress and stabilize membranes. Zn deficiency can occur either acquires or inherited. Total body store of Zn is estimated 2-3 g that 90% of them are found in the muscle and bone. The recommended dietary allowance (RDA) is 11 mg/day for men. Zinc is entered into the human body through tree ways: inhalation, skin root and ingestion. Nutritional deficiency is the major route of Zn deficiency. Cereal protein intake, rich in phytate is the causal factor in developing Zn deficiency. However, lung is the organs that contain noticeable to zinc. Metallothionein/thionein-system has a key role in the homeostasis of Zn. COPD patients are susceptible to zinc deficiency. It can develop due to the possibility of infection (26) and as a chronic disease. However, the role of zinc is established in the pharmacotherapy of asthma, upper airway viral infection, and cystic fibrosis. In addition, Zn deficiency induced hypoxic lung injury (27).

Our study indicated that noticeable Cu deficiency detected among chronic stable COPD. True Cu deficiency is rare but mild deficiency may be seen in several conditions such as pregnancy, growth and using high intake of Zn, molybdenum and iron. Cu deficiency has not reported among COPD patients. Copper is nutritional trace element and essential for normal metabolism. The main source of Cu for human is planted and human breast milk. Cu is contributed in two keys important enzymatic reaction as cofactors; cytochrome- c oxidase and Cu, Zn-SOD.

They are effective role in induction of inflammation in COPD, and beside it mediate in the oxygen transport, oxygen reaction, electron transfer, and tumor progration and tissue development (28). Absorption of Zn and Copper is competitive at the enterocytes, mediated by MT. Outcome of data disclosed positive and significant correlation between Zn with Cu deficiencies. Copper deficiency causes hypocupremia, decreased level of superoxide dismutase, cytochrome-c oxidase, impaired iron metabolism and anemia. Aside, it can increase the level of plasma cholesterol and developing abnormal cardiac function.

Cu toxicity has been seen more than cu deficiency. Our finding showed excess than a normal level of Cu among COPD patients 17%. Elevated Cu concomitants with low Zn were reported in the pregnancy, acute infection, malignancy, cardiovascular, renal and endocrine diseases (29). We did not find recent relationship in our study. Aside, raising level of Cu than set point can be concomitant to normal level of Zn. It found in 83% subjects in the study.

Estimation of Cu to Zn ratios is clinically important than separately. They are reciprocal effect on each other in serum concentrations. It indicates nutritional status, immune system function, oxidative stress damage and inflammation 24 (30). However, it may be reflected unusual function of the current topics and pulmonary function (31). Recent concept indicated that noticeably abnormal Cu/Zn ratio ranges detected between moderate and mild stages of COPD patients. In addition, unusual ratios indicated extraordinary distribution of early trace elements in the body.

In conclusion; the elevated of CRP inflammatory biomarker and zinc, copper deficiencies detected noticeably between stable COPD patients. Raised CRP class included 50% zinc, 20% copper deficiencies and 45% abnormal range of Cu/Zn ratio. The current trace elements are a part of oxidative stress, and their

deficiencies may be effective in induced inflammation.

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