

How to Cite:

Kareem, M. J. K. (2022). Assessment of Cu-Zn, D-dimer, and superoxide dismutase in patients with pulmonary diseases. *International Journal of Health Sciences*, 6(S5), 9523–9531.
<https://doi.org/10.53730/ijhs.v6nS5.11194>

Assessment of Cu-Zn, D-dimer, and superoxide dismutase in patients with pulmonary diseases

Mohammed Jasim Kareem Kareem

Master of Science in Chemistry

Abstract---Lung disease is defined by increased airway responsiveness to environmental stimuli and reversible airflow restriction as a long-term inflammatory condition of the lungs. Using serum to measure antioxidant enzyme activity and erythrocytes (packed cells) to separate and purify the Cu-Zn superoxide dismutase enzyme, the researchers studied 50 lung patients treated with inhaled salbutamol and another 50 healthy people. This is what we found out in our study: It has been found that individuals who use inhaled salbutamol had a significant drop ($P < 0.001$) in total antioxidant capacity (TAC) and Cu-Zn superoxide dismutase activity in their serum, according to the study.

Significant decreases in SOD activity and TAC were seen in the sera of pulmonary patients, as well as significant differences in the levels of Zn, Cu, and D-dimer in the sera of the patients compared to the healthy group ($P < 0.001$). Sera of pulmonary patients who utilised inhaled salbutamol showed a substantial ($p < 0.001$) drop in (Copper and selenium concentration) and a significant ($p > 0.05$) increase of (Zinc concentration).

Keywords---Cu-Zn, Superoxide dismutase, D-Dimer, Pulmonary diseases.

1. Introduction

Airway hyperresponsiveness to environmental cues and reversible airflow limitation are the hallmarks of pulmonary disease, an ongoing inflammatory condition of the lungs. Wheezing, dyspnea, chest tightness, and coughing, especially late at night or early in the morning, are common symptoms of chronic inflammation, which makes the airways more sensitive. Airflow obstruction is common in these episodes, but it is usually reversible either naturally or with therapy. Asthma affects 300 million people worldwide, and another 100 million will be diagnosed with the disease by 2025, according to current predictions. Phenomena that have emerged to better understand how asthma develops and how likely it is that a child may get asthma during their first wheezing episodes have been developed. In early childhood, bronchial constriction is a common

occurrence, making the detection of pulmonary disease persistent rather than transient a challenge. Research into early life events that can improve lung growth in the injured lung and prevent damage to the possibly healthy lung early on and over the course of a person's life is clearly needed as well (Arandelovic *et al.* 2007). During childhood, boys are more prone to have pulmonary disease, but after puberty, girls are more likely to suffer from the same condition. Having to miss time from school or work, having to go to the hospital, or even dying prematurely are all consequences of untreated pulmonary disease. Asthma is diagnosed based on symptoms such as shortness of breath, wheezing, coughing, and chest tightness. Decades of research into various asthma measurement techniques (symptoms, peak flow, bronchial provocation and spirometry) have demonstrated the difficulty of investigating the normal and disturbed airways functionally. In asthma, significant bronchial variability is defined as a drop in forced expiratory volume in one second (FEV1) or a decrease in peak expiratory flow (PEF) after exercise of 15% or more of the pre-exercise value, or a rise in FEV1 of 11% and at least 200 ml after a bronchodilator or a drop in FEV1 by 15% after an elevated concentration of histamine. Not every patient has the same set of defining signs and symptoms of pulmonary disease, and even when they do appear, they can vary greatly and irregularly from one patient to the next. Pathophysiological features that are most frequently noticed may also influence the therapeutic response. There are numerous ways to manage patients with difficult-to-manage Pulmonary illness in clinical practise, and pulmonary disease severity classification is one of the most important tools in the toolbox. Measurement and prediction of lung disease progression risk can be improved by using novel statistical physics and fluctuation analysis approaches, but in order for them to be effective, research into the existing biological underpinnings of airway disease monitoring must also be prioritised. To further understand inflammatory airway disease (IAD), greater research is needed in numerous areas, including how IAD varies from COPD and in youngsters (Aas 1981). One-third of humanity is infected with tuberculosis, which is transmitted by numerous treatment resistant strains, among people with AIDS or other immunodeficiency disorders, and tuberculosis is the most common site of opportunistic infection in these patients. Numerous notable discoveries in the field of science have been made in the last few years. Modern advances in lung genetics and cell biology have opened the door to novel therapies like anti-inflammatory medicines and airway-delivered gene therapy for cystic fibrosis. Asthma and ARDS may soon be treated with rational, mechanism-based therapies thanks to advances in our understanding of the cellular and molecular causes (Arif *et al.* 2003). Aim of This Study that Researching the correlation between lung patients who were treated with inhaled salbutamol and pulmonary patients who were treated with medications containing montelukast in terms of the levels of oxidative stress that both groups experienced, Studying the association between antioxidants and levels of zinc and copper in the sera of control subjects and pulmonary patients, as well as evaluating the antioxidant enzymes (TAC and SOD). And Isolating and purifying the Cu-Zn superoxide dismutase from the erythrocytes of healthy subjects, pulmonary patients who had been treated with inhaled salbutamol, and asthmatic patients who had been treated with medicines containing montelukast.

2. Materials and Methods

2.1 Subjects and samples of the study

A. Group 1: 50 healthy-looking subjects were recruited. They didn't smoke and didn't have any respiratory ailments. And B. Group 2: 50 lung patients were studied. A year of inhaled salbutamol therapy for nonsmokers. The trial ran from January to March 2022. Allergy Center in Heat City provided patient samples. The study was conducted at the Medical college, AL-Ramadia.

2.2 Collection of blood samples

Disposable syringes were used to collect venous blood samples from healthy individuals and asthmatic patients after a time of fasting. In order to avoid the use of a tourniquet, five millilitres of blood were extracted from each individual without the use of an anticoagulant. Sera were collected by centrifuging blood at 1500 g for roughly 10-15 minutes and then transferred into clean new disposable plain tubes after the blood had clotted for about 10-15 minutes.

2.3 Methods

Determination of plasma total antioxidant capacity (TAC)

Principle: Using a unique Protein Mask, the TAC Assay Kit created by Bio Vision can analyse small molecule antioxidant with proteins together or separately. Small molecules and proteins both participate in the conversion of Cu^{2+} ions to Cu^{+} ions. Small molecule antioxidant analysis can be performed because the Protein Mask blocks the reduction of Cu^{2+} by protein Cation-chelation of the reduced Cu^{2+} gives rise to an absorbance peak in the range of 570nm, which is directly proportional to the overall antioxidant capacity (Holgate and Polosa 2006).

Superoxide dismutase (Cu-Zn SOD) activity

(Cu-Zn) SOD activity is evaluated using a simple and rapid approach based on the enzyme's capacity to block the autoxidation of pyrogallol. Pyrogallol autoxidation in the presence of EDTA at pH 8.2 is 50%. Pyrogallol autoxidation by O_2 and radical dismutation by Cu-Zn SOD compete for each other in this method's premise. As a unit of (Cu-Zn) SOD activity, one unit is defined as the amount of enzyme necessary to block pyrogallol autoxidation by 50% (Frey and Suki 2008).

Determination of trace elements

Procedure for the Determination of Zinc, copper and Digestion of Samples The samples were digested by putting (0.5 mL) of serum into a glass test tube, adding (4mL) with (1:1) [con. HNO_3 and con. HClO_4], and placing the tube in an oil bath at 160 °C for one hour. After that, the tubes were taken out of the water bath and allowed to cool at room temperature; following that, the volume was brought up to 10 millilitres by adding 0.5 millimolar HCl (Spycher *et al.* 2010).

Determenation of D-dimer

The levels of D-dimer in these patients were measured using Biomerieux's mini-VIDAS system (France) uses on the table is an ELFA (enzyme-linked fluorescence

assay) kit. On the first day of their stay, they were taken to the hospital's emergency room. Tubes were used to collect blood samples. trisodium citrate (3.8%) was added to a 3000 rpm centrifuge Plasma samples (weighing roughly 1500g) were rapidly collected and deposited in single-use bags in the laboratory. Each sample that was taken from the kit and brought to room temperature was analysed using DD2 rods and DD2 SPR. The pouch is then filled with VIDAS.

Statistical Analysis

Statistical analysis was carried out using SPSS 18.0, and all findings were given as that of the mean standard deviation. SPSS is used to examine the data once it has been entered into a database. The t-test was used to examine the statistical significance of the differences between the two groups investigated.

3. Results and Discussion

3.1 Duration of Pulmonary Diseases

More than half of the patients had asthma for less than ten years, while the percentage of those with asthma for ten to 19 years was 33%, and those with asthma for 20 years more than was 22%. They all had a history of shortness of breath, chest tightness, and occasional wheezing, and were takes various asthma drugs.

3.2 Distribution by Gender and Age

Table (1) shows the distribution of participants by gender and age.

Table (1) Age and sex distribution

Percentage	Total	Sex		Age (years)
Female/ Male				
50.22	50	25	25	20–29
52.99	50	25	25	30–39
100.00	100	50	50	Total

3.3 There were significant differences between the TAC levels of the patient and control groups

A total antioxidant capacity (TAC) test was performed on the serum of each of the two groups, and the results are shown in Table.2.

Table. 1 Mean total antioxidant capacity (TAC) level in the studied groups

Groups	Number.	Tac (mean $\pm$ sd nmol)	P value
G1	50	4.00 $\pm$ 0.32	P<0.001
G2	50	2.38 $\pm$ 0.55	

The total antioxidant capacity TAC values for the control group with the mean $\pm$ SD (4.00 $\pm$ 0.32). Also, the TAC values of the pulmonary patients treated with inhaled salbutamol therapy with the mean $\pm$ SD (2.38 $\pm$ 0.55). When compared to

the control group, the inhaled salbutamol-treated individuals have considerably lower TACs than the latter ($P < 0.001$). A significant decrease in the mean TAC in patients ($P < 0.001$) has also been observed from the control group, the study agreement with (Shikotra *et al.* 2012).

3.4 Cu-Zn SOD activity levels in patients and controls, respectively, were compared in this study

Blood samples from all two groups were tested for the presence of the antioxidant enzyme superoxide dismutase (Cu-Zn SOD), whose activity was quantified in Table 43.

Table. 2 Mean superoxide dismutase (Cu-Zn SOD) activity level in the studied groups

Groups	Number	CU-ZN SOD MEAN $\pm$ SD IU/ mL	P-Value
G1	50	23.04 $\pm$ 2.05	P<0.001
G2	50	16.87 $\pm$ 0.74	

The mean SD (23.04 $\pm$ 2.05) IU/mL of superoxide dismutase SOD activity in the control group. Salbutamol inhalation patients had Cu-Zn SOD activity values of 16.87 $\pm$ 0.74) IU/mL, which is a mean SD. Inhaled salbutamol-treated patients have considerably lower Cu-Zn SOD activity than the control group ($P < 0.001$). In addition, patients on oral Montelukast had significantly lower mean Cu-Zn SOD Activity than those in the control group ($P < 0.001$). Cu-Zn SOD activity levels were not substantially different between the groups that received inhaled salbutamol and those that received oral Montelukast for the study. Epithelial lining fluid and airway epithelial cells had considerably lower Cu-Zn SOD activity than healthy controls. When an individual with atopic asthma experiences an acute asthmatic reaction to a segmental antigen infusion, Cu-Zn SOD activity decreases within minutes. After antigens are introduced into the airways of atopic individuals, Cu-Zn SOD activity rapidly decreases, which is linked to a twofold rise in O₂-generation (Smith 2003). This linkage between Cu-Zn SOD activity and airway reactivity was first observed in Smith (2003). It was later found that Cu-Zn SOD activity has an inverse correlation with airway reactivity (Shaheen *et al.* 2000). All of these findings point to a connection between Cu-Zn SOD activity and asthma-related physiologic parameters.

3.5 Levels of Serum Zinc Concentrations in the Patient and Control Groups

In each of the two groups, zinc levels were checked, and the findings are shown in Table .4.

Table. 3 Mean serum Zinc level in the studied groups

Groups	Number	ZN (MEAN $\pm$ SD mg/dL)	P-Value
G1	50	0.94 $\pm$ 0.11	P<0.001
G2	50	1.08 $\pm$ 0.14	

The serum Zinc values for the control group with the mean $\pm$ SD (0.94 ± 0.11). Also, the serum Zinc values of the pulmonary patients treated with inhaled salbutamol therapy with the mean $\pm$ SD (1.08 ± 0.14) mg/dL. Compared to the control group and those using salbutamol, the mean serum Zinc level in patients taking oral Montelukast is significantly greater (P value < 0.001). A rise in the body's zinc storage could explain some of the negative effects of long-term Montelukast medication. (Sigurs *et al.* 2000).

In this study, the mean serum Zinc level in the salbutamol-treated patients is significantly higher than in the control group, and this minor non-significant rise can be attributable to salbutamol treatment. Oxidant release and the development of DNA damage and cancer may be linked to a drop in zinc concentration in the bloodstream because of its role in antioxidant defence, electron transport, DNA repair and protein expression. Metal-regulatory activator (MTF)-1 can induce thionein production when Zinc ions are bound to Zinc finger structures. When reactive oxygen species (ROS) or nitrogen species (RNS) undergo oxidation of thiols, the resultant oxidised protein toxin (Tox) and the subsequent zinc release occur (Kusel *et al.* 2007).

3.6 Patient vs Control Groups' Serum Copper Concentrations

Table 5 shows the results of copper tests on the sera of a two groups.

Table.4 Mean serum Copper level in the studied groups

Groups	Number	CU (MEAN $\pm$ SD mg/L)	P-Value
G1	50	1.62 ± 0.22	P<0.001
G2	50	0.94 ± 0.13	

The serum Copper values for the control group with the mean $\pm$ SD (1.62 ± 0.22) mg/dL. Also, the serum Copper values of the pulmonary patients treated with inhaled salbutamol therapy with the mean $\pm$ SD (0.94 ± 0.13) mg/dL. Patients who were treated with inhaled salbutamol had significantly lower mean Copper levels than those who were not treated with the medication. Oral montelukast-treated patients exhibited significantly lower mean Copper levels than placebo-treated patients. As previously noted, there is no significant difference in the mean Copper level between the inhaled salbutamol and oral montelukast groups. According to our findings, copper levels in the BA group were significantly lower than in the healthy group (Salam *et al.* 2005). The respiratory system's lower ability to remove free radicals may be connected to decreased Cu-Zn SOD activity. The enzymes involved in iron transport and use, as well as the formation of heme, cannot operate effectively if Copper is deficient. To be transported, iron must be converted from ferrous form to ferric form by the action of a ferroxidase like ceruloplasmin, which binds copper and converts it to ferric iron. Additionally, copper is essential for the oxidase Cytochrome-c to incorporate ferric iron reduction into the heme molecule (Stein *et al.* 1997). As a result of copper deficiency, RBC membrane Cu-Zn SOD is lowered by roughly 85 percent, which reduces the lifespan of RBCs. (Salvesen *et al.* 2017).

3.7 Comparison of Copper/Zinc Ratio in the Treatment and Control Groups

Serum copper/zinc ratios from two groups were analysed, and the results are shown in Table .6.

Table.5 Mean Copper/ Zinc ratio in the studied groups

Groups	Number	CU/ZN (MEAN $\pm$ SD)	P-Value
G1	50	1.74 $\pm$ 0.32	P<0.001
G2	50	0.87 $\pm$ 0.14	

The Copper/ Zinc ratio for the control group with the mean $\pm$ SD (1.74 $\pm$ 0.32). Also, the Copper/ Zinc ratio of the pulmonary patients treated with inhaled salbutamol therapy with the mean $\pm$ SD (0.87 $\pm$ 0.14). In G1 and G2, the zinc/copper ratio dropped considerably, possibly as a result of a drop in copper concentration. Even if elevated levels of serum Zinc have been linked to an increased risk of allergy illnesses like pulmonary (Otterbein *et al.* 1995). Our findings are in line with those of earlier research, which identified higher levels of serum Zinc in their asthmatic subjects (Steinsvg 2009). As a result, evidence on the serum concentrations of Copper and Zinc in asthmatic patients is contentious. If you look at Zinc and Copper levels separately, it seems like a lower ratio of copper to zinc is more essential than an increase or decrease.

D-Dimer Concentrations in Patients and Controls

Mean D-dimer concentrations of 4899 4753 ng/mL and 2168 2031 ng/mL, respectively, were found in the Pulmonary patient. Table .7 shows that Ddimer values in the pulmonary group were significantly higher than those in the control group (P 0.001).

Table. 6 Data on D-dimer levels in the pulmonary and non-pulmonary patient populations

Groups	MEAN D-DIMER $\pm$ SD ng/mL	P-value
Pateints	4899 $\pm$ 4753	<0.001
Control	2168 $\pm$ 2031	

Pulmonary patients had a D-dimer level that was linked to the severity of their condition in the past, but a new study has found that the D-dimer level increases with the severity of the pulmonary condition, as characterised by the pulmonary severity index and the Physiology as well as chronic health evaluation (APACHE II) score (Purokivi *et al.* 2008). A variety of grading methodologies can be used to determine the severity of lung disease (Whu *et al.* 2007). For the purposes of this investigation, the ATS score was used to categorise the pulmonary patients. When it came to the severity of the disease, there was no correlation between D-dimer levels and it. It has been demonstrated in a study that D-dimer levels can be used to determine the severity of community-acquired pneumonia. D-dimer levels may be affected by multiple organ failure in these patients as the disease progresses, according to the presentation. The pulmonary patient outcomes research team

(PORT) severity score was used to categorise ambulatory care pulmonary groups into I, II, and III, respectively. D-dimer was not appropriately linked to pulmonary severity in this analysis because we only included patients with ATS Groups III or IV who were receiving treatment throughout their stay.

Grau and his friends looked on the relationship between pulmonary and D-dimer elevation. A patient with a D-dimer level of more than 5k ng/mL was shown to have a 2.9-fold greater risk of death according to the researchers (Wiszniewska *et al.* 2021). Increased D-dimer levels were also observed in this study as a result of the embolus's size. Massive embolus patients had significantly higher D-dimer levels than submassive embolus patients.

4. Conclusions and Recommendation

In the light of the findings of this study, the following conclusions are drawn that Oral Montelukast has some undesirable effects on some trace elements., Compared to healthy individuals, the BA group had a considerably reduced Copper concentration, which may be linked to a decreased Cu-Zn SOD activity, which removes free radicals from the respiratory system., Oral Montelukast possesses an effect on Cu-Zn SODs activity, TAC due to decrease Cu and Se levels., Inhaled salbutamol has little influence on all the measured parameters. It is found that oral montelukast and inhaled salbutamol have no significant effect on the total serum protein and Zinc levels in the patient groups.

References

- Aas, K. 1981. Heterogeneity of Bronchial Asthma: Sub-populations or different stages of the disease. *Allergy*, 36(1): 3-14.
- Akbarov, A. N., & Xabilov, D. N. U. (2021). The condition of the oral cavity in patients who have had a viral infection COVID-19. *International Journal of Health & Medical Sciences*, 4(4), 381-383. <https://doi.org/10.21744/ijhms.v4n4.1796>
- Al-Shawwa, B., Al-Huniti, N., Titus, G. and Abu-Hasan, M. 2006. Hypercholesterolemia is a potential risk factor for asthma. *Journal of Asthma*, 43: 231-233.
- Arandelovic, M., Stankovic, I. and Nikolic, M. 2007. Swimming and persons with mild persistent asthma. *The Scientific World Journal*, 7: 1182-1188.
- Arif, A. A., Delclos, G. L., Lee, E. S., Tortolero, S. R. and Whitehead, L. W. 2003. Prevalence and risk factors of asthma and wheezing among US adults: an analysis of the NHANES III data. *European Respiratory Journal*, 21: 827-833.
- Frey, U. and Suki, B. 2008. Complexity of chronic asthma and chronic obstructive pulmonary disease: implications for risk assessment, and disease progression and control. *The Lancet*, 372: 1088-1099.
- Holgate, S. T. and Polosa, R. 2006. The mechanisms, diagnosis, and management of severe asthma in adults. *The Lancet*, 368: 780-793.
- Schroeder, A., Kumar, R., Pongracic, J. A., Sullivan, C. L., Caruso, D. M., Costello, J. and Wang, X. 2009. Food allergy is associated with an increased risk of asthma. *Clinical & Experimental Allergy*, 39: 261-270.
- Shikotra, A., Choy, D. F., Ohri, C. M., Doran, E., Butler, C., Hargadon, B. and Bradding, P. 2012. Increased expression of immunoreactive thymic stromal

- lymphopoietin in patients with severe asthma. *Journal of Allergy and Clinical Immunology*, 129: 104-111.
- Sigurs, N. E., Bjarnason, R., Sigurbergsson, F. and Kjellman, B. 2000. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *American Journal Of Respiratory and Critical Care Medicine*, 161: 1501-1507.
- Smith, J. 2003. An update on bronchopulmonary dysplasia: is there a relationship to the development of childhood asthma. *Medical Hypotheses*, 61: 495-502.
- Sovijärvi, A. R., Malmberg, L. P., Reinikainen, K., Ryttilä, P. and Poppius, H. 1993. A rapid dosimetric method with controlled tidal breathing for histamine challenge: repeatability and distribution of bronchial reactivity in a clinical material. *Chest*, 104: 164-170.
- Spycher, B. D., Silverman, M. and Kuehni, C. E. 2010. Phenotypes of childhood asthma: are they real. *Clinical & Experimental Allergy*, 40: 1130-1141.
- Stein, R. T., Holberg, C. J., Morgan, W. J., Wright, A. L., Lombardi, E., Taussig, L. and Martinez, F. D. 1997. Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. *Thorax*, 52: 946-952.
- Steinsvåg, S. K. 2009. Nose and lungs--two of a kind. *Tidsskrift for den Norske Laegeforening: Tidsskrift for Praktisk Medicin, ny Raekke*, 129: 1982-1984.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>
- Whu, R., Cirilo, G., Wong, J., Finkel, M. L., Mendez, H. A. and Leggiadro, R. J. 2007. Risk factors for pediatric asthma in the South Bronx. *Journal of Asthma*, 44: 855-859.
- Wiszniewska, M., Dellis, P., van Kampen, V., Suojalehto, H., Munoz, X., Walusiak-Skorupa, J. and Hoyle, J. 2021. Characterization of occupational eosinophilic bronchitis in a multicenter cohort of subjects with work-related asthma symptoms. *The Journal of Allergy and Clinical Immunology: In Practice*, 9: 937-944.