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STUDY THE EFFECT OF PARAMETER CU-ZN, D-DIMER, AND SUPEROXIDE DISMUTASE IN PATIENTS WITH PULMONARAY DISEASES

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May 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

STUDY THE EFFECT OF PARAMETER CU-ZN, D-DIMER AND SUPEROXID DISMUTASE IN PATIENTS WITH PULMONARAY DISAEASES

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Master of Science in Chemistry

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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).

2022, 41 pages

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

ÖZET

PULMONER HASTALIĞI OLAN HASTALARDA CU-ZN, D-DIMER VE SÜPEROKSIT DISMUTAZ PARAMETRELERININ ETKISI ÇALIŞMASI

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Akciğer hastalığı, çevresel uyaranlara karşı artan hava yolu tepkisi ve akciğerlerin uzun süreli inflamatuar bir durumu olarak geri dönüşümlü hava akımı kısıtlaması ile tanımlanır. Araştırmacılar, antioksidan enzim aktivitesini ölçmek için serum ve Cu-Zn süperoksit dismutaz enzimini ayırmak ve saflaştırmak için eritrositleri (paketlenmiş hücreler) kullanarak, inhale salbutamol ile tedavi edilen 50 akciğer hastasını ve diğer 50 sağlıklı insanı inceledi. Bizim çalışmamızda şunu bulduk: İnhale salbutamol kullanan bireylerin serumlarında toplam antioksidan kapasitede (TAC) ve Cu-Zn süperoksit dismutaz aktivitesinde önemli bir düşüş ($P < 0,001$) olduğu tespit edildi. çalışmak. Akciğer hastalarının serumlarında SOD aktivitesi ve TAC'de önemli düşüşlerin yanı sıra hastaların serumlarında sağlıklı gruba kıyasla Zn, Cu ve D-dimer düzeylerinde önemli farklılıklar görüldü ($P < 0,001$). İnhale salbutamol kullanan akciğer hastalarının serumu (Bakır ve selenyum konsantrasyonu) değerinde önemli ($p<0,001$) bir düşüş ve (Çinko konsantrasyonu) değerinde önemli ($p>0,05$) bir artış gösterdiği gözlenmiştir.

2022, 41 sayfa

Anahtar Kelimeler: Süperoksit dismutaz, Cu-Zn, D-Dimer, Akciğer hastalıkları

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Mohammed Jasim Kareem KAREEM

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LIST OF SYMBOLS

dL	Deciliter
°C	Degrees Celsius
g	Gram
L	Liter
µL	Microliter
mg	Milligram
mL	Milliliters
mmol	Millimoles
ng	Nanogram
nm	Nanometer
nmol	Nanomoles
%	Percent
±	Plus-minus

LIST OF ABBREVIATIONS

ADIs	Acceptable daily intakes
AIDS	Acquired immunodeficiency syndrome
ARDS	Acute respiratory distress syndrome
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BMI	Body mass index
CAT	Catalase
NDGA	Nordihydroguaiaretic acid
OxS	Oxidative stress
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
SD	Standard deviation
SOD	Superoxide dismutase

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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).

1.1 Aim of This Study

1- 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.

2- 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).

3- 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. LITERATURE REVIEW

2.1 Severe Pulmonary Disease

Researchers showed that people with severe asthma who were given large doses of corticosteroids still had elevated levels of thymic-stromal lymphopoietin (TSLP) in their bloodstreams, even though they were receiving high doses of inhaled steroids. As this cytokine may be responsible for TH2-induced airway changes, anti-TSLP therapy in murine animal models could be helpful in reducing TSLP production and TH2 inflammation (Shikotra *et al.* 2012).

Anti-inflammatory medicines can help to reverse some of these processes to a great extent. However, a successful response to therapy can take weeks to obtain and may be incomplete in certain cases. There may be a link between chronic inflammation and airway remodelling, which is a term used to describe changes in the structure of the airways that are neither prevented nor entirely responsive to the treatments currently available. As a result, the pulmonary illness paradigm has moved in the last 10 years from bronchospasm as well as airway inflammation to include airway remodelling in some persons. More than 110 beats per minute is possible in cases of severe acute lung illness. In most cases, the diaphragm is lowered and the thoracic cavity is hyperexpanded. Sluggishness, exhaustion, cyanosis, disorientation, and other symptoms of low oxygen saturation tension can develop from rapid and shallow breathing (breathing rates >30 breaths per minute) (Al-Shawwa *et al.* 2006).

2.2 Pathophysiology and Pathogenesis of Pulmonary Disease

This is only one example of the many anomalies in the airway that may contribute to recurrent airflow restriction in a patient with a pulmonary illness.

2.2.1 Bronchoconstriction

Constriction of the airways and the resulting reduction in airflow are the most common physiological events that cause clinical symptoms in pulmonary illness. It is possible to develop bronchoconstriction when you have a severe asthma attack, and your airways are obstructed by irritants or allergens. Bronchoconstriction is caused by mast cells releasing histamine, trypsin, leukotrienes, and prostaglandins into the airways. When aspirin or other nonsteroidal anti-inflammatory medications cause acute airflow limitation, this non-IgE-dependent reaction may also entail the release of mediators from airway cells. This restriction might be caused by other factors, such as chilly air and allergies. However, the degree of airway inflammation appears to be correlated with the intensity of reaction, although no one knows exactly how these variables affect the airways. (Bakerly *et al*. 2008).

2.2.2 Airway edema

As the sickness worsens and the inflammation spreads, the airway becomes increasingly more clogged and restricted. The formation of massive airway mucus plugs and edoema has been linked to structural changes in the airways, such as airway smooth muscle hypertrophy and hyperplasia. In the long-term, they may be more resistant to treatment (Barnes 2008).

2.2.3 Airway hyper-responsiveness

Airway hyper-responsiveness, or an increased broncho-constrictor response to a variety of stimuli, is a hallmark of pulmonary illness. It's not always the case, though. Contractile responses to methacholine stimuli are an excellent predictor of airway hyper-responsiveness for assessing the severity of asthma. inflammation, poor neuroregulation, and structural alterations are all thought to have an effect on airway hyper-responsiveness; inflammation appears to be an important factor in airway hyper-

responsiveness. Anti-inflammatory medication can help regulate asthma symptoms and reduce the body's sensitivity to stimuli in the airways (Barnes 2008).

2.2.4 Airway remodeling

It is possible that just a portion of the airflow limitation can be reversed in patients with lung diseases. The loss of lung function that cannot be halted or reversed by current treatment is associated with long-term structural alterations in the airway. The activation of numerous structural cells during the re-modeling of the airway causes an increase in airflow obstruction and responsiveness to therapy. Sub-epithelial fibrosis, thickening of the basement membrane, airway smooth muscle hyperplasia, blood vessel proliferation and dilatation, and increased mucous gland production are examples of structural alterations. Both repair and remodelling processes are likely to have a role in the disease's persistence and therapeutic limitations, even if we don't completely understand the mechanics of repair and remodelling (Bernard *et al.* 1994).

2.3 Risk Factors of Pulmonary Disease

2.3.1 Family history of pulmonary disease

Tobacco smoking consumption and hispanic ethnicity were found to be the three most significant risk factors for developing bronchial pulmonary disease.

2.3.2 Passive smoking

Because children born to pregnant smokers have lower lung expansion, it suggests that passive smoking has an impact on the prognosis of bronchospasm in young children.

2.3.3 Prematurity

Prematurity and respiratory disease, like as respiratory distress syndrome, can cause long-term lung problems (bronchopulmonary dysplasia) (RDS).

2.3.4 Viral respiratory infection

Up to the age of seven years, severe respiratory syncytial virus (RSV) infections that necessitate hospitalization can cause persistent immunoglobulin E (IgE) mediated hypersensitivity reactions (Kalantar *et al.* 2016).

2.3.5 Gender

Although boys have a higher risk of developing allergies as youngsters compared to girls, this trend is reversed by the time people reach adulthood.

2.3.6 Early lung function abnormalities

Prior research suggests that children's poor airway function just after birth is a health risk for airflow blockage.

2.3.7 Food allergy

Childhood food allergies have been linked to wheezing that lasts through elementary school. To raise the risk of developing asthma in children, pregnant women should avoid eating processed battered fish that includes trans-fatty acids (TFAs). mechanism of increased sensitivity and airways inflammation caused by omega-6 fatty acid and margarine/vegetable oil derivative ingestion (Carlsen *et al.* 2000).

2.3.8 Race

Certain racial groups may be more susceptible to asthma than others, Mexican Americans reported asthma at a lower rate than non-Hispanic whites.

2.3.9 Occupation

Occupational asthma is induced by particular sensitization to a workplace agent in the vast majority of cases (> 90%). Car painting (isocyanates), hair dressing (cleaning solutions), and baking (flour dust) are all common occupations and exposures.

2.3.10 Drugs

According to Shaheen and his team, the more persons who use paracetamol, the higher the incidence and severity of asthma. Glutathione (an antioxidant) in the lungs and immunological cells is depleted by paracetamol (Croff 2014, Shaheen *et al.* 2000).

2.3.11 Thunderstorms

Thunderstorms with high activity (high sphere density) asthma admissions among children and adults, however the association was less with lower sphere density than without spheres.

2.3.12 Absence of breastfeeding

Asthma, celiac disease, and obesity are just a few of the chronic disorders in children that have been associated to breastfeeding.

2.3.13 Hypercholesterolemia, obesity, and body mass index

Asthma risk is elevated in people with high cholesterol and obesity. In a sample of 1000 people, (Schroeder *et al*. 2009) discovered an association between increased BMI and asthma in women and not in men (Schroeder *et al.* 2009).

2.3.14 Antibiotic use

Certain studies have identified a correlation between the number of courses of antibiotics taken by a kid and the likelihood of wheezing or pulmonary disease, as well as a dose-response relationship (Devereux and Seaton 2005).

2.3.15 Exposure to animals

In a study conducted by (Ramsdell 2010) it was discovered that exposure to cats was linked to a higher incidence of allergic sensitization.

2.3.16 Socio-economic status

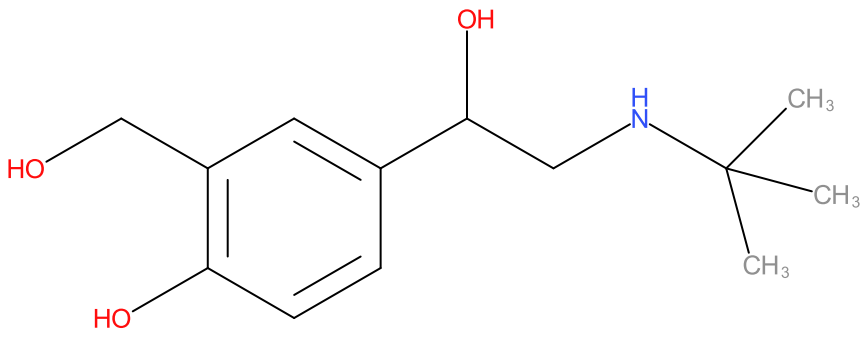
Parental stress has been connected to wheeze in children, and family problems have been linked to pulmonary disease.

2.4 Anti-Pulmonary Drugs

2.4.1 Salbutamol

Only in the maintenance management of asthma, not in the treatment of acute attacks, is salbutamol used as a selective 2-adrenergic agonist. Inducing bronchodilation is one of the effects of cAMP. PDE inhibitors, such as drug, can slow down the breakdown of cAMP, while agonists of the adrenal noradrenergic receptor can speed up the synthesis

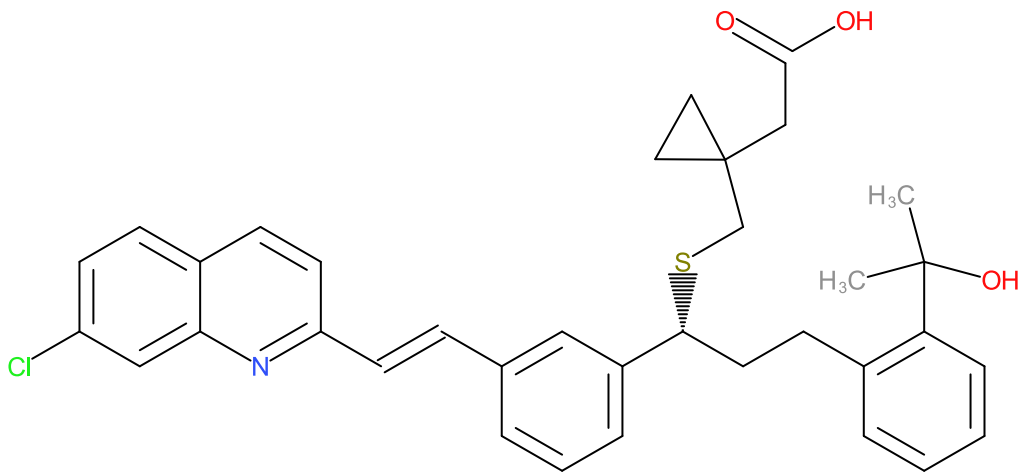
of adenylyl cyclase (AC). Muscarinic antagonists and potentially adenosine antagonists can prevent bronchoconstriction (Hostrup *et al.* 2018).



salbutamol structure

2.4.2 Montelukast

As a prototype for these medications, the cysteinyl leukotriene receptor antagonist montelukast was developed as an anti-type 1 cysteinyl leukotriene receptor medicine (CysLT1Rs). Cellular structures (epithelial, fibroblasts/myoblasts, smooth muscle) and inflammatory cells (neoplasia, mast cells, dendritic cells, lymphocytes) express LTD4 and LTC4/LTE4 on the plasma membrane. Proinflammatory cytokines and chemokines are regulated by these CysLTs. are recognized by these G protein-coupled receptors (Holloway and Koppelman 2008).



2.5 Oxidative Stress (OxS)

OXS is defined as an ailment in which the rate at which reactive oxygen species (ROS) are created exceeds our capacity to combat them, resulting in an increase in biomolecule oxidative damage. Reactive oxygen species (ROS) are produced by immune system cells in response to a challenge. OxS is a prevalent component of inflammatory diseases. Atherosclerosis and systemic lupus erythematosus (SLE) are two examples of conditions in which OxS can be localised in joints or the arterial wall. Oxidative and antioxidative imbalances are hallmarks of pulmonary disease, which is a long-term inflammatory sickness. Oxidative stress processes are more likely to occur in the pulmonary environment because of its high quantities of oxygen. There are biomarkers for disease development that are created in this setting, including reactive oxidant and reactive nitrogen species (ROS and RNS). When the inflammatory response occurs, the synthesis of reactive oxygen and nitrogen species (ROS and RNS) is regulated by the production of inflammatory mediators. ROS and RNS are generated by inflammatory cells such as eosinophils, neutrophils, and macrophages. Asthma-related inflammation is exacerbated when these oxidants are released. A combination of these observations, including an increase in ROS and RNS, oxidative stress, and nonenzymatic production of inflammatory mediators, highlights the importance of oxidative imbalance in asthma. In metabolic reactions, such as electron transport in the mitochondria during respiration, ROS can be created. Ozone and air particles, which include pollutants like cigarette smoke, are another source of ROS production that affects the airways. Airway inflammation conditions including asthma and chronic obstructive lung disease increase the production of reactive oxygen species (ROS) (Hogg *et al.* 2004).

2.5.1 Antioxidant defense

Another pulmonary feature is a lack of antioxidant defence. These anti-cancer antioxidants, which are found in the lungs and blood and also include carotenoids, vitamins E and A and C, and the enzymes glutathione peroxidase (GPx), superoxide

dismutase (SOD) and catalase (CAT), protect the body against oxidant damage, which can lead to cancer. ROS and RNS can produce structural and functional protein modifications that lead to inflammation, which can then persist. Decreased activity has been observed in several enzymes, including SOD, CAT, and GPx. Consequently, the lungs of asthmatic patients produce high levels of ROS and RNS, which may be too much for the antioxidant system to handle. New reactive species are formed since the body is unable to manage this process, which is significantly more harmful to it. In the absence of antioxidant defences, cell lesions such as DNA and lipid membrane damage can develop. Enzyme-activated antioxidants and non-enzyme-activated oxidants are the two most common types of antioxidants found in humans (Figure 2.1). Primary and secondary enzymatic antioxidants are the two types available (Holloway and Koppelman 2008).

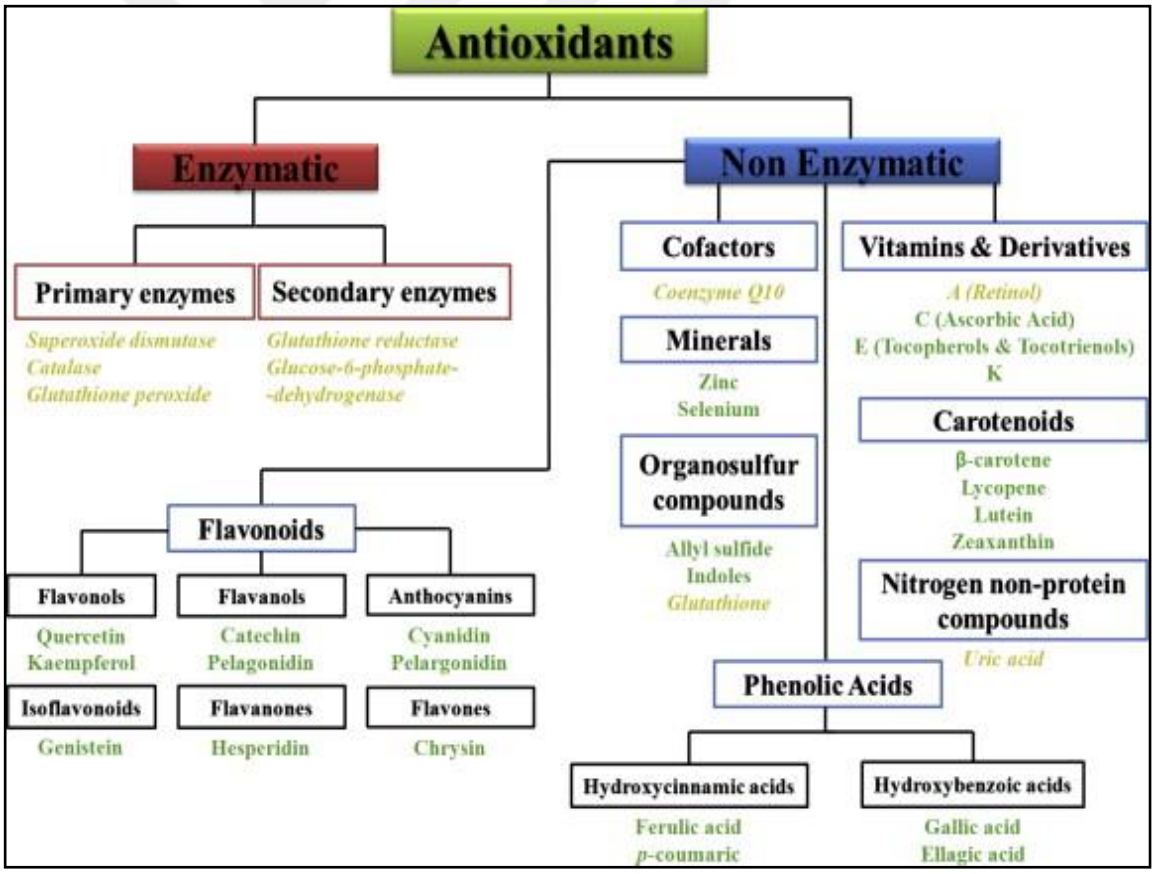


Figure 2.1 Antioxidant defense adapted form (Holloway and Koppelman 2008)

2.5.2 Superoxide dismutase enzymes (SODs)

The four metal cofactor SODs are MnSODs, Cu-ZnSODs, FeSODs, and NiSODs. It is found in eukaryotic cells, while the FeSOD and MnSOD are found in prokaryotes, chloroplast, and mitochondria, respectively, in the cytoplasm of eukaryotes. Metarhizium anisopliae, an entomopathogenic fungus, contains three SODs. One of these enzymes was the Cu-Zn SOD enzyme, which was isolated and reported in part. Superoxide dismutation is catalysed by Cu-Zn SOD (EC.1.15.1.1), or erythrocuprein, which is also known as erythrocuprein. Two identical subunits, each containing one Cu2+ and one Zn2+, are found in the active site of Cu-Zn SOD, which is found in the human erythrocytes. Because they tolerate organic solvents and maintain activity in concentrations as low as 8.0 mol/L urea or 2 percent SDS, Cu-ZnSOD enzymes are frequently very stable. Some sources of Cu-Zn SOD are unstable in the presence of these denaturants. SOD enzymes from plants, fungi, birds, and mammals all resemble one another, proving that it is extraordinarily resistant to evolutionary changes in nature. Research has shown that Cu-ZnSOD may be effective in lowering the deleterious effects of superoxide radicals, which can have a variety of physiological impacts. For a number of conditions, antioxidant supplementation is necessary to counteract the harmful effects of reactive oxygen species. (Illi *et al.* 2004).

2.6 Zinc (Zn)

Zinc is one of the most commonly used biometals in biology; it can be found in all of the body's organs, albumin transports it to these sites from the body's secretions, fluids, and tissues. It can be found everywhere over the human body. Muscle (60%), bone (30%), and skin (4-6%) are the most common locations, with the liver, kidney, and plasma accounting for the rest. Although zinc is liberated from muscle and made available to the plasma in catabolic phases, there is no easily identifiable storage of zinc. The liver is in charge of fine-tuning the zinc levels in the blood, which are strictly regulated. It is taken up by the liver during infection, and plasma levels drop. In the case of inadequate zinc intakes, zinc levels in other tissues such as bone, brain, lung, and heart stay generally steady. When it comes to participating in biological processes, zinc

excels due to the presence of two distinct properties. The homeostatic mechanism that regulates zinc is so effective that it is nearly non-toxic. It can also interact with a wide variety of enzymes and protein involved in cellular metabolism, including those that regulate gene transcription. Metalloenzymes bind to the non-exchangeable pool of intracellular zinc, which can't be affected by zinc deficiency or supplementation, but the more labile and dynamic pool can (Luzak *et al*. 2017).

2.6.1 Zinc (Zn) and pulmonary diseases

Several metalloenzymes and transposable elements are cofactors for zinc, a key antioxidant and anti-inflammatory chemical that may play a function in the biology of the respiratory epithelium. Toxins and inflammatory mediators in respiratory epithelial tissue may be protected from the effects of intracellular labile zinc, according to the theory.

2.6.2 Zinc functions

Depriving lymphocytes and myeloid cells of zinc enhances DNA breakage and mortality in the internucleosomal region in vitro and in vivo. In a 1993 study, Luzak and his team discovered an inverse correlation between the level of intracellular zinc labile and the sensitivity of lymphocytes to DNA fragmentation. We hypothesise that zinc concentrations below a certain level increase the rate of DNA breakage in this pool. Dermis and the gastrointestinal tract's epithelial tissue depend on zinc for their health and development. Taste and smell, healing and glucose metabolism are all affected by insulin. It has been established during the past 30 years that zinc is essential for a wide range of physiological activities, including growth and development, the maintenance and priming of the body's immune system, and the repair and regeneration of tissue. It's a mineral found in food that's essential to the body's optimal operation (Luzak *et al*. 2017).

2.6.3 Absorption of zinc

Red foods, sea food, fresh fruit and veg, and dairy products are all good sources of zinc. Zinc intake should be no more than 12 mg daily. In most cases, the exocrine pancreas releases a zinc-binding ligand that aids in absorption (Martinez *et al*. 1995).

2.6.4 The effect of zinc salt inhalation on health

Zinc salts such as zinc oxide inhaled excessively have previously been linked to toxic metal fume fever, an influenza-like sickness caused by acute or subacute inflammation of the respiratory system (mild interstitial pneumonia). To avoid occupational asthma, it is important to treat or identify fume fever as soon as possible. Zinc sulphate aerosols have been shown to protect guinea pigs from allergies by preventing mast cells from producing histamine.

2.6.5 Role of zinc in common cold

Zinc has also been demonstrated to directly reduce the prevalence of respiratory illnesses in young children from underdeveloped nations. There was a 45 percent reduction in the occurrence and prevalence of acute lower respiratory infections in children aged 6 to 35 months who were given daily supplements of 10 mg of zinc. Zinc, on the other hand, may help alleviate the symptoms of a common cold by lowering inflammation. Controversial evidence about Zinc lozenges for colds has been circulating. It has been shown that Zinc can shorten the duration of cold symptoms, including cough (3.1 versus 6.8 days), nasal discharge (4.1 versus 5.8 days), and sneezing (4.5 versus 8.1 days). By inhibiting viral docking, capsid and replication in respiratory epithelium Zinc has been suggested to be a possible mechanism of action (Hemilä 2017).

2.6.6 Zinc toxicity

Inappropriate supplementation with zinc can cause toxic effects on the digestive system, including nausea, vomiting, diarrhoea, and even fever and tiredness. The signs and symptoms resemble those of the flu. The risk of cardiovascular disease increases as a result of long-term supplementation with iron and copper, which lowers HDL (high density lipoprotein).

2.7 Copper (Cu)

Copper is required by more than 30 enzymes in order to function properly. Cuproenzymes use copper's ability to cycle between an oxidised state, Cu(II), and a reduced one, Cu(I), in redox reactions (I). Toxic to the human body, Copper is prone to the production of superoxide radicals and hydroxyl radicals due to its oxidation state. You need it in your diet as a cofactor for numerous enzymes and cellular functions, so make sure you're getting enough! Most of the body's copper is contained in bones, liver, kidney, and muscle. Copper is a trace mineral that is essential to human health. During digestion, copper is absorbed in the stomach and upper small intestines, and the remainder is expelled in the faeces. 2-4 mg is the average daily intake. Radially labelled Copper was taken up by more over half of those who consumed the recommended daily intake of 0.900 milligrammes of Copper (0.785 milligrammes, 1.68 milligrammes, and 7.53 milligrammes). Copper's absorption is regulated in mysterious ways. A saturable copper absorption into intestinal cells has been seen in vitro (Johansson *et al*. 2001).

If defined previously Copper transporters (hCtr1 and hCtr2) are involved, they may be regulating Copper uptake. An ATPase called Menkes (MNK) may be involved in the basolateral transport of copper across intestinal cells' basolateral membrane. Metal transport to Copper-dependent enzymes and metal efflux from cells are both facilitated by MNK protein. Copper export via the MNK protein appears to be controlled by intracellular copper content. When the MNK protein is exposed to copper, it undergoes a conformational change that leads to the development of a Copper cluster, which then provides access to the phosphorylation site, which then triggers Copper translocation.

Toxic copper is transported by albumin, which binds to liver cells, where it is metabolised, and expelled in the bile. Ceruloplasmin also carries copper out of the liver (Sethi *et al*. 2006).

CERULOPLASMIN IS A 2-GLOBULINAL MEMBRANE. The hepatocytes are the primary producers of plasma ceruloplasmin, however gene expression has been found in the brain, lung, spleen, and testis. One of the most important carriers of Copper in plasma, Ceruloplasmin, is found in the liver and transports 90% of this ion. The remaining 10% of plasma Copper is transported via albumin. Research into the interactions between Copper and various other metals has yielded numerous results. Dietary Zinc has a significant impact on Copper absorption. Diets high in zinc are linked to copper deficiency, and vice versa. Copper deficiency has been identified in trials of women taking Zinc supplements (50 mg Zn /day) for 10 weeks and males taking Zinc supplements (50 mg Zn /day) for 6 weeks **(**Lötvall *et al*. 2011).

The specific method by which Zinc and Copper are linked is unaffected by deficiency anaemia while eating fortified foods. It has been suggested that taking iron supplements at least two hours before or two hours after a meal may help reduce the impact of iron on zinc absorption. However, high doses of supplemental zinc taken for long periods of time could result in decreased intestinal absorption of copper and a copper deficiency with associated anaemia. When it comes to bind Copper in the gut, Zinc has a larger capacity than Zinc to induce the production of metallothionein. Copper accumulates in the intestinal mucosal cell and is excreted as faeces when the cell is shed after consumption of Cu. Copper absorption is inhibited in Wilson's illness by high Zinc consumption. Toxic levels of copper can oxidise proteins and fats, attach to nucleic acids, and enhance the production of free radicals. In order to keep copper levels in the body within normal limits, precautions must be taken. (Sovijärvi *et al*. 1993).

2.7.1 Copper functions

Production of energy: Cobalt and iron are included in the multi-subunit complex of cytochrome C oxidase, located on the mitochondrial membranes. The enzyme catalyzes a four-electron reduction of molecular oxygen, which is necessary for ATP production.

Hephaestin, a copper-containing enzyme found in the enterocyte, is responsible for oxidising iron to the ferric ion that can be used in the body. This allows incorporation of ferric ion into transferrin and eventually into hemoglobin (Morice 2004).

Melanin synthesis: Myelination is catalysed by copper-containing tyrosinases, which are found in melanoma cells.

Enzymes that convert O2•- to H2O2 are found in both intracellular and extracellular SODs, which are capable of removing it from the body **(**Karvala *et al*. 2010).

Central nervous system: Mono amine oxidase is a Copper containing enzymes that catalyze the degradation of serotonin in the brain.

Connective tissue formation: Copper, a lysyl oxidase cofactor, cross-links collagen and elastine (Louhelainen *et al*. 2008).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Table 3.1 lists the chemicals studied and their sources.

Table 3.1 Chemicals used in this study

CHEMICAL	SYMBOL	PURITY%	ORIGIN
Acetylene gas	C_2H_2	99.97	KSA(Jazan industrial gases)
Ammonium sulfate	$(NH_4)_2SO_4$	96	UK(BDH)
Acrylamide	C_3H_5NO	96	Switzerland(Fluka)
Ammonium persulfate	$(NH_4)_2S_2O_8$	98	UK(BDH)
Aceteic acid	CH_3COOH	96	Germany Fisher chemical
Bis acrylamide	$C_7H_{10}N_2O_2$	96	Switzerland(Fluka)
Bovine serum lbumin (BSA)	-	95	Sigma-Aldrich chemical
Copper stok solution (1000 ppm)	Cu	99	Switzerland(Fluka)
Coomassie brilliant blue R 250	$C_{47}H_{50}N_3NaO_7S_2$	99	UK(BDH)
Dipotassium hydrogen phosphate	K_2HPO_4	96	Germany(Merk – Darmstadt)
DEAE Sepharose CL6B		20	(Pharmacia)USA
Disodium hydrogen phosphate	$Na_2HPO_4.2H_2O$	96	Germany(Merk-Darmstadt)
Ethylene diamine tetra acetic acid disodium	EDTA Na_2	87	Switzerland(Fluka)
Ethanol	C_2H_5 OH	99 and 70	Switzerland (Fluka)
Glacial acetic acid	CH_3COOH	99	Germany Fisher chemical
Glycine	NH_2CH_2COOH	99	UK(BDH)
Hypochloric acid	$HClO_4$	99.99	Switzerland (Fluka)
Hydrochloric acid	HCl	99.99	UK(BDH)
Methanol	CH_3OH	99	UK(BDH)
N,N,N-,N- Tetrmethylenediamine	TEMED	95	INDIA (HIMEDIA)
Nitric acid	HNO_3	99.99	Germany (Fischer)
Prestained Protein Ladder V	-	-	USA(Geneaid)
Pyrogallol	$C_6H_6O_3$	96	Switzerland(Fluka)
Sodium acetate	$C_2H_3NaO_2$	99	UK(BDH)
Sodium Boro Hydride	$NaBH_4$	95	UK(RDH)
Sulfuric acid	H_2SO_4	96	UK(BDH)
Selenium stok solution (1000 ppm)	Se	99	Switzerland(Fluka)
Sodium chloride	NaCl	96	Germany(Merk Darmstadt)
Sodium hydroxide	NaOH	-	UK(BDH)
Sodium dodecyl sulfate(SDS)	$CH_3(CH_2)_{11}OSO_3Na$.	99	UK(BDH)
Sodium carbonate	Na_2CO_3	99	UK(BDH)
Total protein kit	-		France(Bio LABOSA)
Zinc stok solution (1000 ppm)	Zn	99	Switzerland(Fluka)
Tris bace	-	99	UK(BDH)

3.1.2 Instruments

Table 3.2 shows study instruments and sources.

Table 3.2 Instruments used in this study

INSTRUMENTS	ORIGIN COMPANY
Autoclave	Japan (Stermite)
Balance	Germany(Sartorius)
Centrifuge	Germany(Hettich)
Centrifuge	EBA 20, Germany
Digital camera	Sony/Japan
Distillator	(England) Bibby Science products limited
Deep Freeze	GFL / Germany
Flame atomic absorption spectrometer with hydride generation system(HG-FAAS)	Japan(Shimadzu) AA-630-12
Flame atomic absorption spectrometer (PG AA500)	England
Gel Electrophoresis	Cleaver Scientific/ UK
Mixer (Electronic)	ib rofix, VF-1 Janke and Kunkle (Germany)
Micropipettes-automatic 5-50,100-1000	Slamed Germany
Micropipette (100-1000) µL	Germany (Slamed)
Micropipette (100) µL	Germany (Slamed)
Micropipette (2-20) µL	Germany (Slamed)
Micropipette (20-200) µL	Germany (Slamed)
Micropipette (5-50) µL	Germany (Slamed)
PH paper	Germany (Jenway)
pH meter	Hanna / China
Photo documentation	Cleaver Scientific/ UK
Power supply	Cleaver Scientific/ UK
Plane tube	Jordan (Afma. Dispo)
SP- 300Spectrophotometer	Japan (Optima)
SP- 3000 nano	Japan (Optima)
Spectro photometer (PD-303)	Japan (APEL)
Sensitive balance	Sartorius/ Germany
Vortex mixer	Germany (Karlkole)
UV-VIS-spectrophotometer	AREL PD-303 UV Japan
UV source	Cleaver Scientific/ UK
Waterbath	Yamato, Japan
Water path	Germany(Karikde)

3.1.3 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.

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.

A questionnaire was submitted to the patients and controls as explained in Table 3.3.

Table 3.3 Questionnaire

Number:		
Name:		
Sex:	Male	Female
Age:		
Drug used:		
Dose:		
Duration of treatment:		
Duration of disease:		
Other disease:		
Other drug used:		
Weight:		
Height:		
Family history:		
Smoking:		
Passive smoking:		
Residency:		
Recent hospitalization:		

3.1.4 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.

3.2 Methods

3.2.1 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 Cu2+ ions to Cu2+ ions. Small molecule antioxidant analysis can be performed because the Protein Mask blocks the reduction of Cu2+ by protein Cation-chelation of the reduced Cu2+ gives rise to an absorbance peak in the range of 570nm (Table 3.4), which is directly proportional to the overall antioxidant capacity (Holgate and Polosa 2006).

Table 3.4 Components of the total antioxidant capacity kit

VOLUME	DESCRIPTION
0.2 mL	Cu^{2+} Reagent
10 mL	Assay Diluent
10 mL	Protein Mask
1 vial	Trolox Standard (1 μmol)

Putting the Reagents Back Together:

1. The Cu2+Reagent, the Assay Diluent, and the Protein Mask can be used immediately after being given and can be stored at room temperature.

2. Trolox Standard: To make a 1 mM solution, first dissolve a lyophilized Total phenolic standard in 20 L of pure DMSO by vertxing it, then add 980 L of distilled water and mix it well. This should result in a solution with a concentration of 1 mM.

3. After the reconstituted solution has been aliquoted, it should be stored at -20 degrees Celsius. When kept at a temperature of -20 degrees Celsius, the reconstituted standard is stable for a period of four months.

Antioxidant testing:

1. To create a curve for the trolox standard, add 0, 4, 8, 12, or 16 μL of the trolox standard to each individual well. Using ddH2O, bring the total volume up to 100 L so that you can obtain 0, 4, 8, 12, or 16 nmol of the Trolox standard.

2. The preparation of the sample The kit has been evaluated using serum, urine, culture medium, as well as different types of food and drinks. There is no need for the sample to be purified if it comes from these sources. Samples should be diluted 1:1 with a protein mask if just the small molecule TAC is desired in the end result. Each well may accommodate sample volumes ranging from 0-100 L, and all measurements should be made in duplicate. When analysing serum samples, we recommend using an amount of 0.01 - 0.1 μL without a Protein Mask and anywhere from 1 - 10 μL with a Protein Mask. It is recommended that the volumes of all wells be adjusted to 100 L using ddH2O.

3. The absorbance of the samples needs to fall within the linear range of the standard curve, which goes from 0 to 20 nmol per well. If they are found to be outside of this

range, they ought to be diluted further and run again. The amount of trolox that can be detected using this assay is roughly 0.1 nmol per well, which is equal to 1 μM

4. To prepare the working solutions, dilute one part of the Cu+2 reagent with forty-nine parts of the Assay diluent. Dilute the working solution to an appropriate level for the number of tests. The amount of Cu2+working solution required for each well is 100 μL.

Procedure for testing:

1. Ahead of time, I poured one hundred microliters of Cu2+ working solution into each of the standard and sample wells.

2. The cover plate was incubated for an additional hour and a half at room temperature.

3. Using the plate reader, the absorbance was measured at a wavelength of 570 nm.

Calculations: Trolox concentration (mmolar) should be plotted as illustrated in Figure 3.1 to create the standard curve.

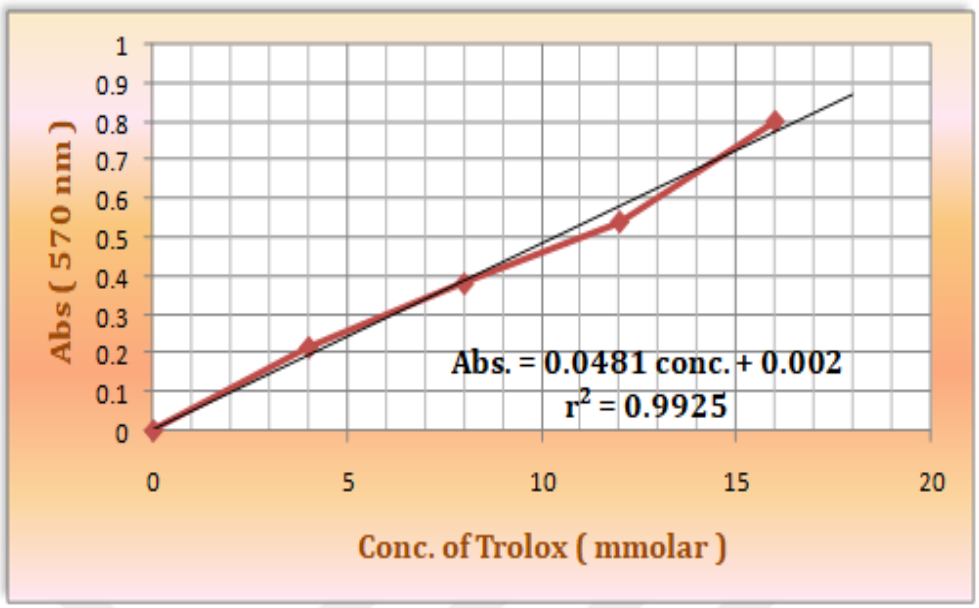


Figure 3.1 TAC standard curve

3.2.2 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 O2 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).

Preparation of the reagents:

buffer containing tris-EDTA In 1 litre of DW, we dissolve 8.2 g of Tris and 1.11 g of EDTA-Na 2 to obtain a PH of 8.2. 1L of D.W. is used to dissolve 0.25 grammes of Pyrogallol in the pyrogallol solution (0.2 mg/L), which contains 0.6 mg of concentrated HCl and 1 mg of pyrogallol per mL.

Procedure: Pyrogallol Tris-EDTA buffer is used to calibrate the spectrophotometer, which is subsequently used to make control and samples tests tubes, as shown in Table 3.5).

Table 3.5 control and sample test tubes

REAGENTS	**TEST (µL)**	**CONTROL (µL)**
Serum	50	
Tris buffer D.W	1000	1000
Pyrogallol	-1000	50 1000

The amount of pyrogallol that has been absorbed is measured at a wavelength of nm wavelength against the Tris-EDTA buffer both at zero time and after one minute of addition.

3.2.3 Determination of trace elements

Procedure for the Determination of Zinc 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. HNO3 and con. HClO4], 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).

Sample solution of Zn (1000 g/mL) was made by diluting (1mL) of 1000 g/mL Zn to (100 mL) by 6 M HCl and storing it in the refrigerator. A 10 g/ mL standard was created and then utilised to construct stock standards of (0.1 - 1.6) g/ mL. Deionized water was used as a diluent in the preparation of all stock standards, which is shown in Table 3.6.

Table 3.6 Optimal circumstances for the measurement of zinc.

VARIABLE	IDEAL CONDITION
Lamp current (mA)	5
Wavelength (nm)	213.9
Slit width (nm)	0.5
Slit height	Normal
Read time (s)	3
Replicates	3

3.2.4 Determination of Copper

0.5 mL of serum was added to each spyrex test tube, along with 4 mL of [con. HNO3 and con. H2SO4] (1:1), and the tubes were heated in an oil bath at 130°C for one hour before being removed from the heat and allowed to cool at room temperature before being refilled with 0.5 M HCl.

Stock solution of Copper was prepared by diluting the stock solution with 6 millilitres (6 mL) of hydrochloric acid for the standard solution (1000 g/mL) (HCl). It was then used to make stock standards of (0.1 - 1.6) g/mL, which were used in future tests. Deionized water was employed as the diluent in the preparation of all stock standards (Table 3.7).

Table 3.7 Ideal conditions for determining the amount of copper.

VARIABLE	IDEAL CONDITION
Lamp current (mA)	3
Wavelength (nm)	324.7
Slit width (nm)	0.4
Slit height	Normal
Read time (s)	3
Replicates	3

3.2.5 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. Taking a tray for loading and processing plasma samples Each and every hole in a pipette was placed into a sample. The device completed all processes on its own in 35 minutes after the initial step. Calibration curves on a computer linked to the equipment were used to determine the results. For results larger than 10 4 /mL, the plasma sample was diluted at a ratio of 1/5. In this study, positive values were classified as above 500 ng/mL.

3.3 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.

RESULTS AND DISCUSSION

4.1 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.

4.2 Distribution by Gender and Age

Table 4.1 shows the distribution of participants by gender and age.

Table 4.1 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

4.3 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 4.2.

Table 4.2 Mean total antioxidant capacity (TAC) level in the studied groups

GROUPS	**NUMBER**	**TAC (MEAN ± SD nmol)**	**P VALUE**
G1	50	4.00 ± 0.32	P<0.001
G2	50	2.38 ± 0.55	

The total antioxidant capacity TAC values for the control group with the mean ± SD (4.00 ± 0.32). Also, the TAC values of the pulmonary patients treated with inhaled salbutamol therapy with the mean ± SD (2.38 ± 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).

4.4 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 4.3.

Table 4.3 Mean superoxide dismutase (Cu-Zn SOD) activity level in the studied groups

GROUPS	NUMBER	CU-ZN SOD MEAN ±SD IU/ mL	P-VALUE
G1	50	23.04 ± 2.05	P<0.001
G2	50	16.87 ± 0.74	

The mean SD (23.04 ± 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± 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.

4.5 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 4.4.

Table 4.4 Mean serum Zinc level in the studied groups

GROUPS	NUMBER	ZN (MEAN ±SD mg/dL)	P-VALUE
G1	50	0.94 ± 0.11	P<0.001
G2	50	1.08 ± 0.14	

The serum Zinc values for the control group with the mean ± SD (0.94 ± 0.11). Also, the serum Zinc values of the pulmonary patients treated with inhaled salbutamol therapy with the mean ± 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).

4.6 Patient vs Control Groups' Serum Copper Concentrations

Table 4.5 shows the results of copper tests on the sera of a two groups.

Table 4.5 Mean serum Copper level in the studied groups

GROUPS	NUMBER	CU (MEAN ± 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 ± SD (1.62 ± 0.22) mg/dL. Also, the serum Copper values of the pulmonary patients treated with inhaled salbutamol therapy with the mean ± 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).

4.7 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 4.6.

Table 4.6 Mean Copper/ Zinc ratio in the studied groups

GROUPS	NUMBER	CU/ZN (MEAN ± SD)	P-VALUE
G1	50	1.74 ± 0.32	P<0.001
G2	50	0.87±0.14	

The Copper/ Zinc ratio for the control group with the mean ± SD (1.74 ± 0.32). Also, the Copper/ Zinc ratio of the pulmonary patients treated with inhaled salbutamol therapy with the mean ± SD (0.87 ± 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.

4.8 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 4.7 shows that Ddimer values in the pulmonary group were significantly higher than those in the control group (P 0.001).

Table 4.7 Data on D-dimer levels in the pulmonary and non-pulmonary patient populations .

GROUPS	MEAN D-DIMER ± SD ng/mL	P-VALUE
Pateints	4899 ± 4753	<0.001
Control	2168 ± 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 elevationA 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.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

In the light of the findings of this study, the following conclusions are drawn:

1- Oral Montelukast has some undesirable effects on some trace elements.

2- 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.

3- Oral Montelukast possesses an effect on Cu-Zn SODs activity, TAC due to decrease Cu and Se levels.

4- 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.

5.2 Recommendations

In the light of the results, the researcher recommends the followings:

1. Inhaled salbutamol is generally safer than oral montelukast, therefore, it is better to use inhaled salbutamol whenever possible and at the maximum acceptable dose when needed.
2. Medical professionals should, first and foremost, avoid the use of corticosteroids when they are not essential and should make all attempts to stop fractures in people who are beginning or continuing to receive therapy with corticosteroids.
3. Montelukast seems to be an appropriate alternative for the treatment of asthma that is only mildly persistent or only occurs occasionally. Salbutamol is best for treating moderate chronic pulmonary diseases unresponsive to Montelucast.

4. Measurements of blood levels of thyroid hormone, C-reactive protein and γ-globulin which affect Immune response and some trace elements.

5. Measurements of interleukin-8 and tumour necrosis factor- α on the genotype and used as clinical significance in asthma after treatment.

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