

# 1. Introduction, Rationale, Objectives

## 1.1 Introduction:

Asthma is a common chronic respiratory disease affecting 1-18% of the population in different countries. asthma is characterized by variable symptoms of wheeze, shortness of breath, chest tightness and/or cough, and by variable expiratory airflow limitation. Both symptoms and airflow limitation characteristically vary over time and in intensity. These variations are often triggered by factors such as exercise, allergen or irritant exposure, change in weather, or viral respiratory infections (Ginna.,2016). The prevalence among Sudanese adults is approaching 10% in many areas of the Sudan (Magzoubet *al.*, 2010).

In the recent studies, free oxygen radicals were accused for the pathogenesis of bronchial asthma (Jarjour and Calhoun.,1994). There are some defense mechanisms to escape from the effects of oxidant radicals. The most important antioxidant endogen systems are mitochondrial cytochrome oxidase, superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) systems. Also albumin, seruloplasmin, ferritin and hemoglobin which are found in the extracellular space have antioxidant properties(Halliwell, 1994). Chromium induced the protein expression of Mnsuperoxidedismutase,Cu/Zn-superoxide dismutase, catalase, glutathione peroxidase, and heme oxygenase-1 (HO-1) (Biomed., 2010).Magnesium is directly involved in the mechanisms of cellular antioxidant defense by increasing the activity of important glutathione enzyme glutathione peroxidase (GPx). Decreasing of these trace elements causes the effects of antioxidant systems to be lower and this leads tohyperactivity and inflammation in the respiratory tract (Pucheuet *al.*, 1995).

## **1.2. Rationale**

Asthma is a major problem globally leading to high morbidity and mortality in developing countries, Sudan being one of developing countries and asthma is widely distributed among children.

The occurrence of asthma has increased significantly since 1970s, in 2011, about 300 million people globally were diagnosed with asthma and its caused 250.000 deaths per year around the world, the metabolism of several trace elements has been reported to alter in bronchial asthma and these elements might have specific roles in the pathogenesis and progress of this disease.

To my knowledge there are few published studies about this in Sudan, so this study may help to provide the monitoring of magnesium and copper in asthmatic patients.

## **1.3.Objectives of the study:**

### **1.3.1.General objective:**

Assessment of plasma levels of the magnesium and copper in Sudanese patients with bronchial asthma.

### **1.3.2.Specific objectives:**

- 1- To measure plasma copper and magnesium levels in blood samples of patients with bronchial asthma, compared with non asthmatic individual.
- 2- To correlate plasma magnesium and copper level with age, sex, and duration of disease.

## **2. Literature review**

### **2.1. Bronchial asthma:**

Asthma is a common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasm. Common symptoms include wheezing, coughing, chest tightness, and shortness of breath (Burney. *etal.*,1990).

Asthma is thought to be caused by a combination of genetic and environmental factors. Its diagnosis is usually based on the pattern of symptoms, response to therapy over time and spirometry. it is clinically classified according to the frequency of symptoms, forced expiratory volume in one second (FEV1), and peak expiratory flow rate. asthma may also be classified as atopic (extrinsic) or non atopic (intrinsic) where atopy refers to a predisposition toward developing type 1 hypersensitivity reactions(Omran and Russell., 1996).

Treatment of acute symptoms is usually with an inhaled short-acting beta-2 agonist (such as salbutamol) and oral corticosteroids. in very severe cases, intravenous corticosteroids, magnesium sulfate, and hospitalization may be required. symptoms can be prevented by avoiding triggers, such as allergens and irritants, and by the use of inhaled corticosteroids. Long-acting beta agonists (LABA) or anti leukotriene agents may be used in addition to inhaled corticosteroids if asthma symptoms remain uncontrolled. The occurrence of asthma has increased significantly since the 1970s. In 2011, 235–300 million people globally were diagnosed with asthma, and it caused 250,000 deaths (Seatonet *al.*,1994).

#### **2.1.1. Anatomy of bronchiae:**

The bronchial tree begins when the trachea divides into the right and left main stem bronchi at the level of the T5 vertebra. the right main stem

bronchus is shorter, wider, and more vertical than the left main stem bronchus. the right and left main stem bronchi divide first into lobar bronchi and subsequently into segmental (or tertiary) bronchi. Arteries, veins, and lymphatics also enter the lungs at the hilum along with the bronchi. A bronchopulmonary segment is a portion of lung that is supplied by a segmental bronchus and its adjacent blood vessels. the bronchial wall is made up of mucosa, lamina propria, smooth muscle, and sub mucosa with interspersed cartilage. the initial generations of the bronchi are similar to each other in their histological structure, except for the amount of hyaline cartilage. In the trachea, the cartilage encircles the lumen, but in subsequent divisions of bronchi, it is replaced by diminishing quantities of cartilage plates. The bronchial mucosa is made of pseudo stratified ciliated columnar epithelium with goblet cells and basal cells. The rhythmic movement of the cilia promotes the flow of the superficial liquid lining of the epithelium, along with mucin and other particulate material (eg, cells and debris) from within the lung to the pharynx. Goblet cells are devoid of apical cilia and have mucus granules in the cytoplasm and are responsible for secretion of mucin. The density of goblet cells progressively decreases from the periphery and disappears at the level of terminal bronchioles(West., 2008).

The bronchial submucosa contains mixed compound tubule acinar glands, composed largely of mucin-secreting cells and some serous-secreting cells, that secrete mucin, water, and electrolytes into the bronchial lumen. Mucin is a complex glycoprotein that is responsible for trapping particulate material in the bronchi (Adams *et al.*, 2010).

### **2.1.2. Signs and symptoms of bronchial asthma:**

Asthma is characterized by recurrent episodes of wheezing, shortness of breath, chest tightness, and coughing. Sputum may be produced from the

lung by coughing but is often hard to bring up (Seaton *et al.*, 1996) During recovery from an attack, it may appear pus-like due to high levels of white blood cells called eosinophils. Symptoms are usually worse at night and in the early morning or in response to exercise or cold air. Some people with asthma rarely experience symptoms, usually in response to triggers, whereas others may have marked and persistent symptoms (Godden and Devereux, 1999).

### **2.1.3. Causes of bronchial asthma:**

Asthma is caused by a combination of complex and incompletely understood environmental and genetic interactions. These factors influence both its severity and its responsiveness to treatment. It is believed that the recent increased rates of asthma are due to changing epigenetics (heritable factors other than those related to the DNA sequence) and a changing living environment (Martinez, 2007).

### **2.1.4. Environmental factors of bronchial asthma:**

Many environmental factors have been associated with asthma's development and exacerbation including allergens, air pollution, and other environmental chemicals (Yawn, 2008).

Smoking during pregnancy and after delivery is associated with a greater risk of asthma-like symptoms. Low air quality from factors such as traffic pollution or high ozone levels has been associated with both asthma development and increased asthma severity (Yawn, 2008).

Exposure to indoor volatile organic compounds may be a trigger for asthma; formaldehyde exposure, for example, has a positive association. Also, phthalates in certain types of PVC are associated with asthma in children and adults. There is an association between acetaminophen (paracetamol)

use and asthma. The majority of the evidence does not, however, support a causal role. A 2014 review found that the association disappeared when respiratory infections were taken into account (Scott and Peters,2013).

Asthma is associated with exposure to indoor allergens. common indoor allergens include dust mites, cockroaches, animal dander, and mold (Jindal,,2011).

Efforts to decrease dust mites have been found to be ineffective. Certain viral respiratory infections, such as respiratory syncytial virus and rhinovirus, may increase the risk of developing asthma when acquired as young children. Certain other infections, however, may decrease the risk (George and Ronald,. 2005).

#### **2.1.5. Complications of bronchial asthma:**

Symptoms that interfere with sleep, work or recreational activities

Sick days from work or school during asthma flare-ups

Permanent narrowing of the bronchial tubes (airway remodeling) that affect how well you can breathe

Emergency room visits and hospitalizations for severe asthma attacks.

Side effects from long-term use of some medications used to stabilize severe asthma.

Proper treatment makes a big difference in preventing both short term and long-term complications caused by asthma ( Christine,,2013).

#### **2.1.6.Pathophysiology of bronchial asthma:**

The pathophysiology of asthma is a complex and involves the following component(Busse *et al.*,. 1993).

### **2.1.6.1. Airway inflammation:**

The mechanism of inflammation in asthma may be acute, sub acute, or chronic, and the presence of airway edema and mucus secretion also contributes to air flow obstruction and bronchial reactivity. Varying degrees of mononuclear cell and eosinophil infiltration, mucus hyper secretion, desquamation of the epithelium, smooth muscle hyperplasia, and airway remodeling are present. Some of the principal cells identified in airway inflammation include mast cells, eosinophils, epithelial cells, macrophages, and activated T lymphocytes. T lymphocytes play an important role in the regulation of airway inflammation through the release of numerous cytokines. Other constituent airway cells, such as fibroblasts, endothelial cells, and epithelial cells, contribute to the chronicity of the disease. Other factors, such as adhesion molecules (eg, selectins, integrins), are critical in directing the inflammatory changes in the airway. Finally, cell-derived mediators influence smooth muscle tone and produce structural changes and remodeling of the airway(Busset *al.*, 1993) .

The presence of airway hyper responsiveness or bronchial hyper reactivity in asthma is an exaggerated response to numerous exogenous and endogenous stimuli. The mechanisms involved include direct stimulation of airway smooth muscle and indirect stimulation by pharmacologically active substances from mediatorsecreting cells such as mast cells or non myelinated sensory neurons. The degree of airway hyper responsiveness generally correlates with the clinical severity of asthma(Gauvreaue *et al.*, 2011).

A study by Balzaret *al* reported changes in airway resident mast cell populations from a large group of subjects with asthma and normal control subjects(Gauvreaue *et al.*, 2011).A greater proportion of chymase-positive

mast cells in the airways and increased prostaglandin D2 levels were identified as important predictors of severe asthma as compared with other steroid-treated subjects with asthma (Gauvreau *et al.*, 2011).

Chronic inflammation of the airways is associated with increased bronchial hyper responsiveness, which leads to broncho spasm and typical symptoms of wheezing, shortness of breath, and coughing after exposure to allergens, environmental irritants, viruses, cold air, or exercise. In some patients with chronic asthma, airflow limitation may be only partially reversible because of airway remodeling (hypertrophy and hyperplasia of smooth muscle, angiogenesis, and sub epithelial fibrosis) that occurs with chronic untreated disease(Gauvreau *et al.*, 2011).

Airway inflammation in asthma may represent a loss of normal balance between two "opposing" populations of The lymphocytes. Two types of The lymphocytes have been characterized: Th1 and Th2. Th1 cells produce interleukin (IL)-2 and IFN- $\alpha$ , which are critical in cellular defense mechanisms in response to infection(Gauvreau *et al.*, 2011).

Th2, in contrast, generates a family of cytokines (IL-4, IL-5, IL-6, IL-9, and IL-13)that can mediate allergic inflammation. a study by Gauvreau et al found that IL-13has a role in allergen-induced airway responses (Gauvreau *et al.*,2011).

The current "hygiene hypothesis" of asthma illustrates how this cytokine imbalance may explain some of the dramatic increases in asthma prevalence in westernized countries. This hypothesis is based on the concept that the immune system of then newborn is skewed toward Th2 cytokine generation (mediators of allergic inflammation). Following birth, environmental stimuli such as infections activateTh1 responses and bring the Th1/Th2 relationship to an appropriate balance (Anderson and Watson,. 2001).



### **2.1.6.2. Airflow obstruction:**

Airflow obstruction can be caused by a variety of changes, including acute broncho constriction, airway edema, chronic mucous plug formation, and airway remodeling. Acute broncho constriction is the consequence of immunoglobulin independent mediator release upon exposure to aeroallergens and is the primary component of the early asthmatic response. airway edema occurs 6-24 hours following an allergen challenge and is referred to as the late asthmatic response( Sears,. 2000).

Chronic mucous plug formation consists of an exudate of serum proteins and cell debris that may take weeks to resolve. Airway remodeling is associated with structural changes due to long-standing inflammation and may profoundly affect the extent of reversibility of airway obstruction. Airway obstruction causes increased resistance to airflow and decreased expiratory flow rates. These changes lead to a decreased ability to expel air and may result in hyperinflation. The resulting over distention helps maintain airway patency, thereby improving expiratory flow; however, it also alters pulmonary mechanics and increases the work of breathing( Sears,. 2000).

### **2.1.7. Diagnosis of Bronchial Asthma:**

While asthma is a well recognized condition, there is not one universal agreed upon definition. It is defined by the Global Initiative for Asthma as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment. There is currently no precise test with the

diagnosis typically based on the pattern of symptoms and response to therapy over time ( Schiffman and George,. 2009). A diagnosis of asthma should be suspected if there is a history of recurrent wheezing, coughing or difficulty breathing and these symptoms occur or worsen due to exercise, viral infections, allergens or air pollution. Spirometry is then used to confirm the diagnosis. In children under the age of six the diagnosis is more difficult as they are too young for Spirometry(Shiber and Santana,. 2006).

#### **2.1.7.1.Spirometry diagnosis of asthma:**

Spirometry is recommended to aid in diagnosis and management. It is the single best test for asthma. If the FEV1 measured by this technique improves more than 12% following administration of a bronchodilator such as salbutamol, this is supportive of the diagnosis. It however may be normal in those with a history of mild asthma, not currently acting up. As caffeine is a bronchodilator in people with asthma, the use of caffeine before a lung function test may interfere with the results. Single-breath diffusing capacity can help differentiate asthma from COPD. It is reasonable to perform spirometry every one or two years to follow how well a person's asthma is controlled (Kindermann,. 2007).

#### **2.1.7.2.Other methods of diagnosis of asthma:**

The methacholine challenge involves the inhalation of increasing concentrations of a substance that causes airway narrowing in those predisposed. If negative it means that a person does not have asthma; if positive, however, it is not specific for the disease. Other supportive evidence includes: a  $\geq 20\%$  difference in peak expiratory flow rate on at least three days in a week for at least two weeks, a  $\geq 20\%$  improvement of peak flow following treatment with either salbutamol, inhaled

corticosteroids or prednisone, or a  $\geq 20\%$  decrease in peak flow following exposure to a trigger. Testing peak expiratory flow is more variable than spirometry, however, and thus not recommended for routine diagnosis. It may be useful for daily self-monitoring in those with moderate to severe disease and for checking the effectiveness of new medications. It may also be helpful in guiding treatment in those with acute exacerbations (Ripollet *al.*,2011).

### **2.1.8. Asthma classification:**

Mild intermittent :Mild symptoms up to two days a week and up to two nights a month.

Mild persistent :Symptoms more than twice a week, but no more than once in a single day.

Moderate persistent: Symptoms once a day and more than one night a week.

Severe persistent: Symptoms throughout the day on most days and frequently at night( NHLBI ,.2007).

### **2.1.9. Medications of asthma:**

Medications used to treat asthma are divided into two general classes: quick-relief medications used to treat acute symptoms; and long-term control medications used to prevent further exacerbation. Salbutamol metered dose inhaler commonly used to treat asthma attacks. Short-acting beta<sub>2</sub>-adrenoceptor agonists (SABA), such as salbutamol are the first line treatment for asthma symptoms. They are recommended before exercise in those with exercise induced symptoms (Fanta, .2009).

Anti cholinergic medications, such as ipratropium bromide, provide additional benefit when used in combination with SABA in those with moderate or severe symptoms. Anti cholinergic bronchodilators can also be used if a person cannot tolerate a SABA. If a child requires admission to

hospital additional ipratropium does not appear to help over a SABA. Older, less selective adrenergic agonists, such as inhaled epinephrine, have similar efficacy to SABAs. They are however not recommended due to concerns regarding excessive cardiac stimulation (Cates and Cates, 2008).

Corticosteroids are generally considered the most effective treatment available for long-term control. Inhaled forms such as beclomethasone are usually used except in the case of severe persistent disease, in which oral corticosteroids may be needed. It is usually recommended that inhaled formulations be used once or twice daily, depending on the severity of symptoms (Vos *et al.*, 2012).

Long-acting beta-adrenoreceptor agonists (LABA) such as salmeterol and formoterol can improve asthma control, at least in adults, when given in combination with inhaled corticosteroids. In children this benefit is uncertain. When used without steroids they increase the risk of severe side-effects and even with corticosteroids they may slightly increase the risk (Lozano R, 2012).

Leukotriene receptor antagonists (such as montelukast and zafirlukast) may be used in addition to inhaled corticosteroids, typically also in conjunction with a LABA. Evidence is insufficient to support use in acute exacerbations. In children they appear to be of little benefit when added to inhaled steroids. In those under five years of age, they were the preferred add-on therapy after inhaled corticosteroids by the British Thoracic Society in 2009. A similar class of drugs, 5-LOX inhibitors, may be used as an alternative in the chronic treatment of mild to moderate asthma among older children and adults. As of 2013 there is one medication in this family known as zileuton. Mast cell stabilizers (such as cromolyn sodium) are another non-preferred alternative to corticosteroids (Grant, *et al.*, 1999).

## **2.2. Trace elements:**

The term trace element was originally used to describe the - residual amount of inorganic analyte quantitatively determined in a sample. More sensitive analytical methods now provide more accurate determination of most inorganic micronutrients present at very low concentrations in body fluids and tissue. Those present in body fluids (pg/dL) and in tissue (mg/kg) are, however, still widely referred to as "trace elements" and those found at ng/dL or pg/kg as the "ultra trace elements." The corresponding dietary requirements are quoted in mg/day or pg/day, respectively. An element is considered essential when the signs and symptoms induced by a deficient diet are uniquely reversed by an adequate supply of the particular trace element (Burtis *et al.*, 2008).

### **2.2.1. copper:**

Is a chemical element with symbol Cu (from Latin: cuprum) and atomic number 29. It is a ductile metal with very high thermal and electrical conductivity. Pure copper is soft and malleable; a freshly exposed surface has a reddish-orange color. It is used as a conductor of heat and electricity, a building material, and a constituent of various metal alloys. The metal and its alloys have been used for thousands of years. In the Roman era, copper was principally mined on Cyprus, hence the origin of the name of the metal as cyprium (metal of Cyprus), later shortened to cuprum. Its compounds are commonly encountered as copper (II) salts, which often impart blue or green colors to minerals such as azurite and turquoise and have been widely used historically as pigments. Architectural structures built with copper corrode to give green verdigris (or patina). Decorative art prominently features copper, both by itself and as part of pigments. copper is essential to all living

organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome c oxidase. In mollusks and crustacea copper is a constituent of the blood pigment hemocyanin, which is replaced by the iron-complexed hemoglobin in fish and other vertebrates. The main areas where copper is found in humans are liver, muscle and bone. copper compounds are used as bacteriostatic substances, fungicides, and wood preservatives (Lippard and Berg,. 1994).

### **2.2.1.1. Biological role of copper:**

Copper proteins have diverse roles in biological electron transport and oxygen transportation, processes that exploit the easy inter conversion of Cu (I) and Cu (II). The biological role for copper commenced with the appearance of oxygen in earth's atmosphere. The protein hemocyanin is the oxygen carrier in most mollusks and some arthropods such as the horseshoe crab (*Limulus polyphemus*) (Decker and Terwilliger,. 2000).

Because hemocyanin is blue, these organisms have blue blood, not the red blood found in organisms that rely on hemoglobin for this purpose. Structurally related to hemocyanin are the laccases and tyrosinases. Instead of reversibly binding oxygen, these proteins hydroxylate substrates, illustrated by their role in the formation of lacquers. copper is also a component of other proteins associated with the processing of oxygen. in cytochrome c oxidase, which is required for aerobic respiration, copper and iron cooperate in the reduction of oxygen. copper is also found in many superoxide dismutases, proteins that catalyze the decomposition of superoxides, by converting it (by disproportionation) to oxygen and hydrogen peroxide:  $2 \text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$

Several copper proteins, such as the "blue copper proteins", do not interact directly with substrates, hence they are not enzymes. These proteins relay electrons by the process called electron transfer (M C Linder,. 1998).

#### **2.2.1.2. Dietary needs of copper:**

Copper is an essential trace element in plants and animals, but not some microorganisms. The human body contains copper at a level of about 1.4 to 2.1 mg per kg of body mass. Stated differently, the RDA for copper in normal healthy adults is quoted as 0.97 mg/day and as 3.0 mg/day (Bonhamet *al*, 2002). Copper is absorbed in the gut, then transported to the liver bound to albumin. After processing in the liver, copper is distributed to other tissues in a second phase. copper transport here involves the protein ceruloplasmin, which carries the majority of copper in blood. Ceruloplasmin also carries copper that is excreted in milk, and is particularly well-absorbed as a copper source (Gordonet *al*,. 1986). Copper in the body normally undergoes enterohepatic circulation (about 5 mg a day, vs. about 1 mg per day absorbed in the diet and excreted from the body), and the body is able to excrete some excess copper, if needed, via bile, which carries some copper out of the liver that is not then reabsorbed by the intestine (Bonhamet *al*,. 2002).

#### **2.2.1.3. Copper-based disorders:**

Because of its role in facilitating iron uptake, copper deficiency can produce anemia-like symptoms, neutropenia, bone abnormalities, hypopigmentation, impaired growth, increased incidence of infections, osteoporosis, hyperthyroidism, and abnormalities in glucose and cholesterol metabolism. Conversely, Wilson's disease causes an accumulation of copper in body tissues. Severe deficiency can be found by testing for low plasma or serum

copper levels, low ceruloplasmin, and low red blood cell superoxide dismutase levels; these are not sensitive to marginal copper status. The "cytochrome c oxidase activity of leucocytes and platelets" has been stated as another factor in deficiency, but the results have not been confirmed by replication (Gordon *et al.*, 1986).

### **2.2.2. Magnesium:**

Is a chemical element with symbol Mg and atomic number 12. It is a shiny gray solid which bears a close physical resemblance to the other five elements in the second column (Group 2, or alkaline earth metals) of the periodic table: they each have the same electron configuration in their outer electron shell producing a similar crystal structure (Housecroft and Sharpe, 2008).

#### **2.2.2.1. Dietary sources:**

The common nutritional sources of magnesium are green leafy vegetables, legumes, nuts, and animal protein (Whang *et al.*, 1994).

#### **2.2.2.2. Magnesium Physiology:**

Magnesium ( $Mg^{2+}$ ) is the fourth most abundant cation in the body and second most abundant intracellular ion. The average human body (70 kg) contains 1 mole (24 g) of  $Mg^{2+}$ . Approximately 53% of  $Mg^{2+}$  in the body is found in bone, 46% in muscle and other organs and soft tissue, and less than 1% is present in serum and red blood cells. Of the  $Mg^{2+}$  present in serum, about one third is bound to protein, primarily albumin. Of the remaining two thirds, 61% exists in the free or ionized state and about 5% is complexed with other ions, such as  $PO_4$  and citrate. Similar to  $Ca^{2+}$ , it is the free ion that is physiologically active in the body. The role of  $Mg^{2+}$  in the body is widespread. It is an essential cofactor of more than 300 enzymes, including



those important in glycolysis, transcellular ion transport, neuromuscular transmission, synthesis of carbohydrates, proteins, lipids, and nucleic acids, and release of and response to certain hormones. The clinical usefulness of serum Mg<sup>2+</sup> levels has greatly increased in the past 10 years as more information about the analyte has been discovered. The most significant findings are the relationship between abnormal serum Mg<sup>2+</sup> levels and cardiovascular, metabolic, and neuromuscular disorders. Although serum levels may not reflect total body stores of Mg<sup>2+</sup>, serum levels are useful in determining acute changes in the ion (Polancic, 1991).

### **2.2.2.3. Absorption, Transport, and Excretion of Magnesium:**

Rich sources of Mg<sup>2+</sup> in the diet include raw nuts, dry cereal, and “hard” drinking water; other sources include vegetables, meats, fish, and fruit. Processed foods, an ever-increasing part of the average U.S. diet, have low levels of Mg<sup>2+</sup> that may cause an inadequate intake. This in turn may increase the likelihood of Mg<sup>2+</sup> deficiency. The small intestine may absorb 20%–65% of the dietary Mg<sup>2+</sup>, depending on the need and intake. The overall regulation of body Mg<sup>2+</sup> is controlled largely by the kidney, which can reabsorb Mg<sup>2+</sup> in deficiency states or readily excrete excess Mg<sup>2+</sup> in overload states. Of the non protein-bound Mg<sup>2+</sup> that gets filtered by the glomerulus, 25%–30% is reabsorbed by the proximal convoluted tubule (PCT), unlike Na, in which 60%–75% is absorbed in the PCT. Henle’s loop is the major renal regulatory site, where 50%–60% of filtered Mg<sup>2+</sup> is reabsorbed in the ascending limb. In addition, 2%–5% is reabsorbed in the distal convoluted tubule. The renal threshold for Mg<sup>2+</sup> is approximately 0.60–0.85 mmol/L (1.46–2.07 mg/dL). Because this is close to normal serum concentration, slight excesses of Mg<sup>2+</sup> in serum are rapidly excreted by the kidneys. Normally, only about 6% of filtered Mg<sup>2+</sup> is excreted in the urine per day. Mg<sup>2+</sup>

regulation appears to be related to that of  $\text{Ca}^{2+}$  and  $\text{Na}^+$ . Parathyroid hormone (PTH) increases the renal reabsorption of  $\text{Mg}^{2+}$  and enhances the absorption of  $\text{Mg}^{2+}$  in the intestine. However, changes in ionized  $\text{Ca}^{2+}$  have a far greater effect on PTH secretion. Aldosterone and thyroxine apparently have the opposite effect of PTH in the kidney, increasing the renal excretion of  $\text{Mg}^{2+}$ . Normal range: 0.7–1 mmol/L (1.5–2 mEq/L; 1.7–2.4 mg/dL) (Bringham *et al.*, 2012).

#### **2.2.2.4. Hypomagnesemia:**

Hypomagnesemia is most frequently observed in hospitalized individuals in intensive care units or those receiving diuretic therapy or digitalis therapy. These patients most likely have an overall tissue depletion of  $\text{Mg}^{2+}$  as a result of severe illness or loss, which leads to low serum levels. Hypomagnesemia is rare in non-hospitalized individuals. There are many causes of hypomagnesemia; however, it can be grouped into general categories. Reduced intake is least likely to cause severe deficiencies in the United States. An  $\text{Mg}^{2+}$ -deficient diet as a result of starvation, chronic alcoholism, or  $\text{Mg}^{2+}$ -deficient IV therapy can cause a loss of the ion. Various GI disorders may cause decreased absorption by the intestine, which can result in an excess loss of  $\text{Mg}^{2+}$  via the feces. Malabsorption syndromes; intestinal resection or bypass surgery; nasogastric suction; pancreatitis; and prolonged vomiting, diarrhea, or laxative use may lead to an  $\text{Mg}^{2+}$  deficiency (Schlingman *et al.*, 2002).

Neonatal hypomagnesemia has been reported as a result of various surgical procedures. A primary deficiency has also been reported in infants as a result of a selective malabsorption of the ion. A chronic congenital hypomagnesemia with secondary hypocalcemia (autosomal

recessive disorder) has also been reported; molecular studies have revealed a specific transport protein defect in the intestine (Schlingman *et al.*, 2002).

Mg<sup>2+</sup> loss due to increased excretion by way of the urine can occur as a result of various renal and endocrine disorders or the effects of certain drugs on the kidneys (Polancic, 1991).

Renal tubular disorders and other select renal disorders may result in excess amounts of Mg<sup>2+</sup> being lost through the urine because of decreased tubular reabsorption. Several endocrine disorders can cause a loss of Mg<sup>2+</sup>. Hyperparathyroidism and hypercalcemia may cause increased renal excretion of Mg<sup>2+</sup> as a result of excess Ca<sup>2+</sup> ions. Excess serum Na levels caused by hyperaldosteronism may also cause increased renal excretion of Mg<sup>2+</sup>. A pseudo hypomagnesaemia may also be the result of hyperaldosteronism caused by increased water reabsorption. Hyperthyroidism may result in an increased renal excretion of Mg<sup>2+</sup> and may also cause an intracellular shift of the ion. In persons with diabetes, excess urinary loss of Mg<sup>2+</sup> is associated with glycosuria. Hypomagnesemia can aggravate the neuromuscular and vascular complications commonly found in this disease. Some studies have shown a relationship between Mg<sup>2+</sup> deficiency and insulin resistance; however, Mg<sup>2+</sup> is not thought to play a role in the pathophysiology of diabetes mellitus. The American Diabetes Association has issued a statement regarding dietary intake of Mg<sup>2+</sup> and measurement of serum Mg<sup>2+</sup> in patients with diabetes. Several drugs, including diuretics, gentamicin, cisplatin, and cyclosporine, increase renal loss of Mg<sup>2+</sup> and frequently result in hypomagnesemia. The loop diuretics, such as furosemide, are especially effective in increasing renal loss of Mg<sup>2+</sup> (Polancic, 1991).

Thiazide diuretics require a longer period of use to cause hypomagnesemia. Cisplatin has a nephrotoxic effect that inhibits the ability of the renal tubule to conserve Mg<sup>2+</sup>. Cyclosporine, an immunosuppressant, severely inhibits the renal tubular reabsorption of Mg<sup>2+</sup> and has many adverse effects, including nephrotoxicity, hypertension, hepatotoxicity, and neurologic symptoms such as seizures and tremors. Cardiac glycosides, such as digoxin and digitalis, can interfere with Mg<sup>2+</sup> reabsorption. The resulting hypomagnesemia is a significant finding because the decreased level of Mg<sup>2+</sup> can amplify the symptoms of digitalis toxicity. Excess lactation has been associated with hypomagnesemia as a result of increased use and loss through milk production. Mild deficiencies have been reported in pregnancy, which may cause a hyperexcitable uterus, anxiety, and insomnia (Polancic, 1991).

#### **2.2.2.5. Symptoms of hypomagnesemia:**

Hypomagnesemia may be asymptomatic until serum levels fall below 0.5 mmol/L. A variety of symptoms can occur. The most frequent involve cardiovascular, neuromuscular, psychiatric, and metabolic abnormalities. The cardiovascular and neuromuscular symptoms result primarily from the ATPase enzyme's requirement for Mg<sup>2+</sup>. Mg<sup>2+</sup> loss leads to decreased intracellular K levels because of a faulty Na-K pump (ATPase). This change in cellular RMP causes increased excitability that may lead to cardiac arrhythmias. This condition may also lead to digitalis toxicity. Muscle contraction also requires Mg<sup>2+</sup> and ATPase for normal Ca<sup>2+</sup> uptake following contraction. Normal nerve and muscle cell stimulation requires Mg<sup>2+</sup> to assist with the regulation of acetylcholine, a potent neurotransmitter. Hypomagnesemia can cause a variety of symptoms from weakness to tremors, tetany, paralysis, or coma. The CNS can also be affected, resulting in psychiatric disorders that range from subtle changes to depression or

psychosis. Metabolic disorders are associated with hypomagnesemia. Studies have indicated that approximately 40% of hospitalized patients with hypokalemia are also hypomagnesemic. In addition, 20%–30% of patients with hyponatremia, hypocalcemia, or hypophosphatemia are also hypomagnesemic. Mg<sup>2+</sup> deficiency can impair PTH release and target tissue response, resulting in hypocalcemia. Replenishing any of these deficient ions alone, often does not remedy the disorder unless Mg<sup>2+</sup> therapy is provided. Mg<sup>2+</sup> therapy alone may restore both ion levels to normal; serum levels of the ions must be monitored during treatment (Polancic, 1991).

#### **2.2.2.6. Hypermagnesemia:**

Hypermagnesemia is observed less frequently than hypomagnesemia (Polancic 1991). Causes for elevated serum Mg<sup>2+</sup> levels the most common is renal failure (GFR, <30 mL/min). The most severe elevations are usually a result of the combined effects of decreased renal function and increased intake of commonly prescribed Mg<sup>2+</sup>-containing medications, such as antacids, enemas, or cathartics. Nursing home patients are at greatest risk for this occurrence. Hypermagnesemia has been associated with several endocrine disorders (Elin, 1994).

Thyroxine and growth hormone cause a decrease in tubular reabsorption of Mg<sup>2+</sup>, and a deficiency of either hormone may cause a moderate elevation in serum Mg<sup>2+</sup>. Adrenal insufficiency may cause a mild elevation as a result of decreased renal excretion of Mg<sup>2+</sup>. MgSO<sub>4</sub> may be used therapeutically with preeclampsia, cardiac arrhythmia, or myocardial infarction. Mg<sup>2+</sup> is a vasodilator, and can decrease uterine hyperactivity in eclamptic states and increase uterine blood flow. This therapy can lead to maternal hypermagnesemia, as well as neonatal hypermagnesemia due to the immature kidney of the newborn. Premature infants are at greater risk to develop

actual symptoms. Studies have shown that IV $Mg^{2+}$  therapy in myocardial infarction patients may reduce early mortality(Elin,. 1994).

Dehydration can cause a pseudohypermagnesemia, which can be corrected with rehydration. Because of increased bone loss, mild serum  $Mg^{2+}$  elevations can occur in individuals with multiple myeloma or bone metastases(Elin,. 1994).

#### **2.2.2.6.1. Symptoms of hypermagnesaemia:**

Symptoms of hypermagnesemia typically do not occur until the serum level exceeds 1.5mmol/L. The most frequent symptoms involve cardiovascular, dermatologic, GI, neurologic, neuromuscular, metabolic, and homeostatic abnormalities (Polancic, 1991). Mild to moderate symptoms, such as hypotension, bradycardia, skin flushing, increased skin temperature, nausea ,vomiting, and lethargy may occur when serum levels are 1.5–2.5mmol/L. Life-threatening symptoms ,such as electro cardiogram changes ,heart block, a systole, sedation ,coma, respiratory depression or arrest, and paralysis, can occur when serum levels reach 5.0 mmol/L. Elevated  $Mg^{2+}$  levels may inhibit PTH release and target tissue response. This may lead to hypocalcemia and hypercalcuria. Normal hemostasis is a  $Ca^{2+}$ -dependent process that may be inhibited as a result of competition between increased levels of  $Mg^{2+}$  and  $Ca^{2+}$  ions.Thrombin generation and platelet adhesion are two processes in which interference may occur (Polancic,.1991).

#### **2.2.3. Trace elements and bronchial asthma:**

Trace elements are essential micronutrients that exist in very low concentrations in the body, forming 0.01% of the total body weight( Laker ,2007).They play an important role in various physiological processes,

and are crucial for proper functioning of the immune system (Lukac and Massanji, 2007).

Deficiency of trace elements and infectious diseases are often concomitantly observed and result in complex interactions. The major trace elements have immunomodulatory effects and thus influence susceptibility and the course of a variety of infections. This is mainly due to the fact that these elements are part of the structure of antioxidant enzymes. These enzymes act as antioxidant defense and are able to regulate the host immune system, and alter viral genome (Lukac and Massanji, 2007).

Magnesium is directly involved in the mechanisms of cellular antioxidant defense by increasing the activity of important glutathione enzyme glutathione peroxidase (GPx). This enzyme speeds up the reaction between glutathione and free radicals, particularly toxic hydrogen peroxide. Magnesium also increased the activity of other important antioxidants – superoxide dismutase (SOD) and catalase (Yavuz and Mollaoglu, 2013). There is increasing evidence that reactive oxygen species can be of particular importance in the pathophysiology of several lung diseases. Changes in the level of these trace elements decrease the efficiency of antioxidant systems and this leads to hyper-reactivity and inflammation in the respiratory tract (Pucheuet *al.*, 1995).

### **3. Materials and Methods**

#### **3.1. Study design:**

This was a cross sectional case-control study conducted to measure plasma magnesium and copper levels in bronchial asthmatic patients.

#### **3.2. Study area:**

Blood sample were collected from Alshaab Teaching Hospital, asthma room patient, in Khartoum state during period from 15 July to 15 September 2017.

#### **1.3. Study population:**

A total of 100 persons were included to perform this study (50 persons were included from diagnosed bronchial asthmatic patients as case group, and 50 persons were included from normal individuals as control group).

#### **3.4. Inclusion criteria:**

Patients with bronchial asthma.

#### **3.5. Exclusion criteria:**

Patients with diabetes mellitus, hypertension, liver disease, kidney disease, congenital disease, gout.

#### **3.6. Ethical consideration:**

The study approved from clinical chemistry department in sudan university verbal Informed consent were obtained from all individuals participating in this study.

#### **3.7. Data collection:**

The clinical data were obtained and recorded on questionnaire (appendix I).

#### **3.8. Sample collection:**

Three ml of venous blood was drawn from each volunteer in this study using a disposable plastic syringe under aseptic conditon. The blood was poured



ina lithium heparin containers and then centrifuged. The plasma was kept at -20°C in eppendorff tube till used.

### 3.9. Coper estimation:

The estimation of plasma copper were performed by using Mindary BA 88A.

#### 3.9.1. Principle of the method:

Copper is released from ceruloplasmine complex and forms with the specific complexant 3-5 Di Br-PAESA a stable coloured complex. The color intensity is proportional to the concentration of copper in the sample (Burtis C,2008) (appendix II).

#### 3.9.2.Procedure:

	Blank	Standard	Sample
WR (ml)	1.0 ml	1.0 ml	1.0 ml
Distilled water	0.05 ml	-	-
Standard (ml)	-	0.05 ml	-
Sample	-	-	0.05 ml
Reagent 2(ml)	0.05 ml	0.05 ml	0.05 ml

Mixed and incubated for 5 min at 37°C and the results were read.

### 3.10. Magnesiummeasurment:

The estimation of plasma Magnesium were performed in International Hospital inKhartoum by using Mindary BA 88A. (appendix3).

### 3.10.1. Principle of the method:

Magnesium forms a purple coloured complex in alkaline solution. in the presence of EGTA ,the reaction is specific. The intensity of the purple colour is proportional to the magnesium concentration (Burtis,2008) (appendix III).

### 3.10.2.Procdure :

	Blank	Standard	Sample
WR (ml)	1.0 ml	1.0 ml	1.0 ml
Standard (ml)	-	0.01 ml	-
Sample (ml)	-	-	0.01 ml

Mixed thoroughly and let stand the tubes for 2 minutes at room temperature.

The results were read.

Calculations automatically done by Mindary BA 88A.

### 3.11. Quality control:

Normal and abnormal control sera were used to insure the accuracy of results.

### 3.12. Statistical analysis:

All data were analyzed by SPSS software version 11.5. Descriptivestatistics were used to analyze study variables such as age, sex, and duration of disease.t test was used for comparison of means concentration ofstudy

parameters. Correlation analysis between variables was used. Statistical significant was accepted at level  $P.value \leq 0.05$ .

## 4. Results

The results of the biochemical parameters of plasma trace elements (magnesium and copper) were given in tables.

**Table (4-1):** Illustrates mean concentrations of magnesium and copper in patients and control groups. The level of magnesium was insignificantly different among asthmatic patients compared to control group: magnesium (mean  $\pm$  SD:  $1.92 \pm 0.26$  mg/dL versus  $2.01 \pm 0.20$  mg/dL, P.value (0.072). but the levels of copper was significantly increased compared to control group (mean  $\pm$ SD:  $23.82 \pm 5.05$   $\mu$ mol/L versus  $20.20 \pm 3.15$   $\mu$ mol/L, P.value 0.000).

**Table (4-2):** Shows the mean concentrations of magnesium and copper in male and female groups. magnesium:( mean  $\pm$  SD :  $1.98 \pm 0.29$  mg/dL versus  $1.85 \pm 0.22$  mg/dL ,P.value = 0.08). copper: (mean  $\pm$ SD:  $22.58 \pm 5.01$   $\mu$ mol/L versus  $25.41 \pm 4.77$   $\mu$ mol/L, P.value =0.154).

**Figure (4-1):** Shows correlation between the level of plasma magnesium and age among asthmatic patients, There was insignificant correlation ( $r = -0.167$ , p-value= 0.246).

**Figure (4-2):** Shows correlation between the level of plasma copper and age among asthmatic patients, There was insignificant correlation ( $r = 0.038$ , p-value= 0.793).

**Figure (4-3):** Shows correlation between the level of plasma magnesium and duration of disease , There was insignificant correlation ( $r = -0.206$ , p-value= 0.151).

**Figure (4-4):** Shows correlation between the level of plasma copper and duration of disease, insignificant correlation ( $r = 0.061$ , p-value= 0.675).

**Table (4-1):** Comparison between means concentration of plasma magnesium and plasma copper in case and control groups.

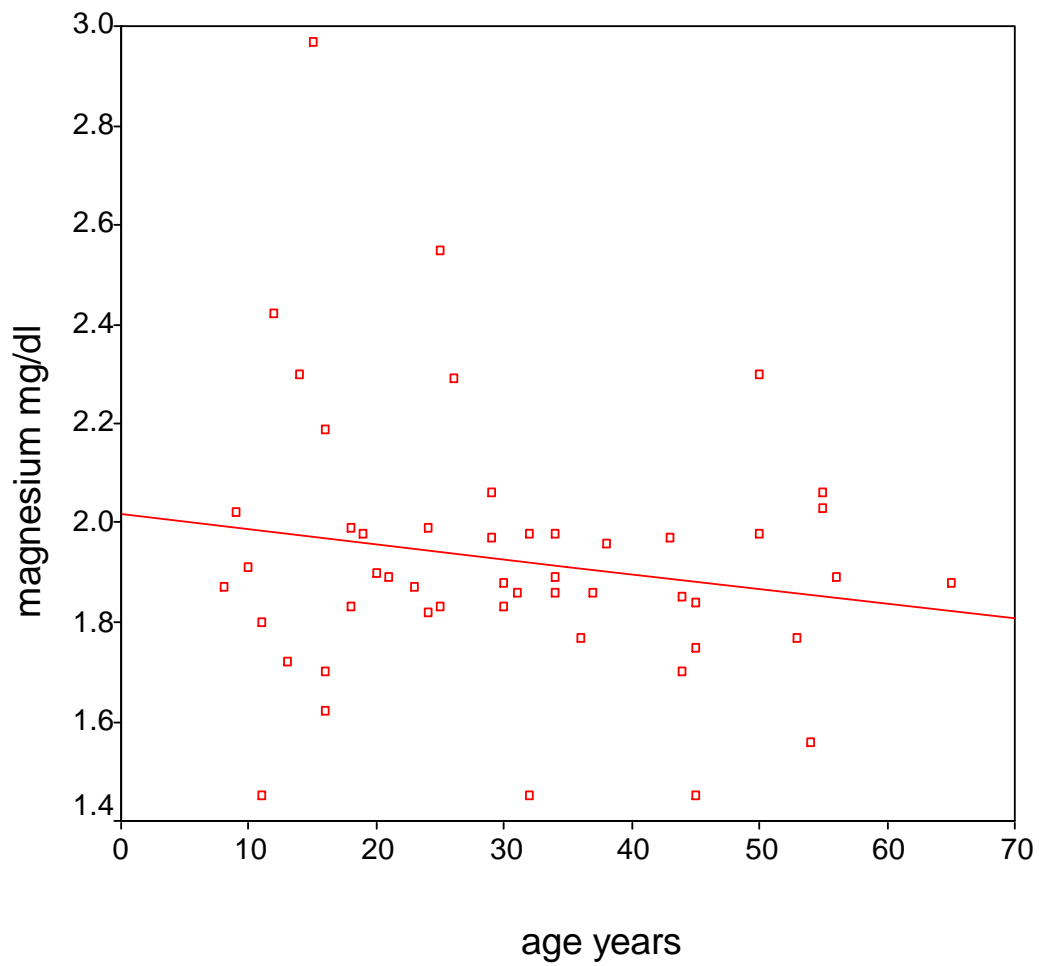
Variable	Case N=50 Mean±SD	Control N=50 Mean±SD	p.value
Magnesium mg/dL	1.92± 0.26	2.01 ± 0.20	0.072
Copper $\mu$ mol/L	23.82± 5.05	20.20± 3.15	0.000*

Result given in mean  $\pm$  SD, P.value  $\leq 0.05$  considered significant. Independent sample T test was used for comparison.

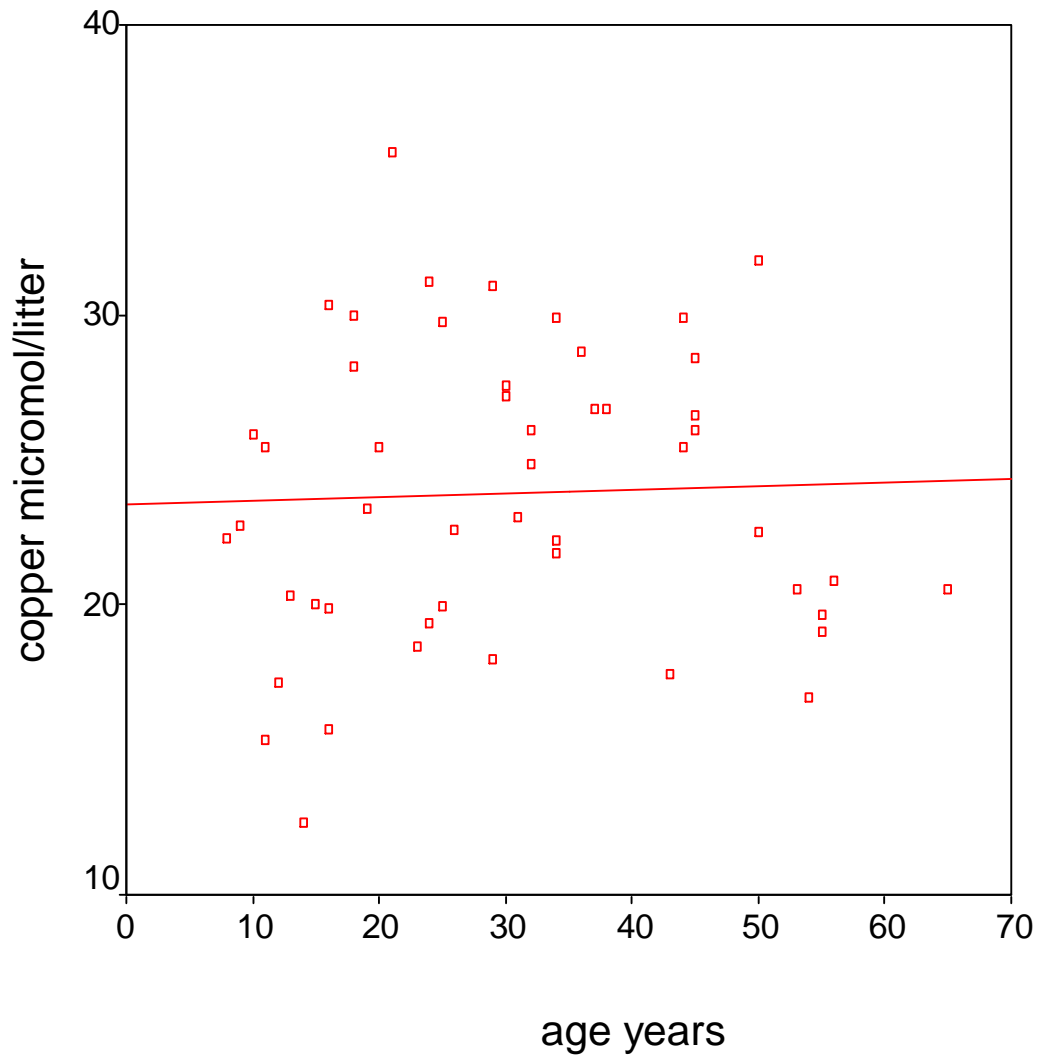
**Table (4-2):** Comparison between means concentration of plasma magnesium and plasma copper in male and female among asthmatic group.

Variable	Male N=28 Mean±SD	female N=22 Mean±SD	p.value
Magnesium mg/L	1.98± 0.29	1.85 ± 0.22	0.08
Copper $\mu$ mol/L	22.58± 5.01	25.41± 4.77	0.04*

Result given in mean  $\pm$  SD, P.value  $\leq$ 0.05 considered significant.  
Independent sample T test was used for comparison.



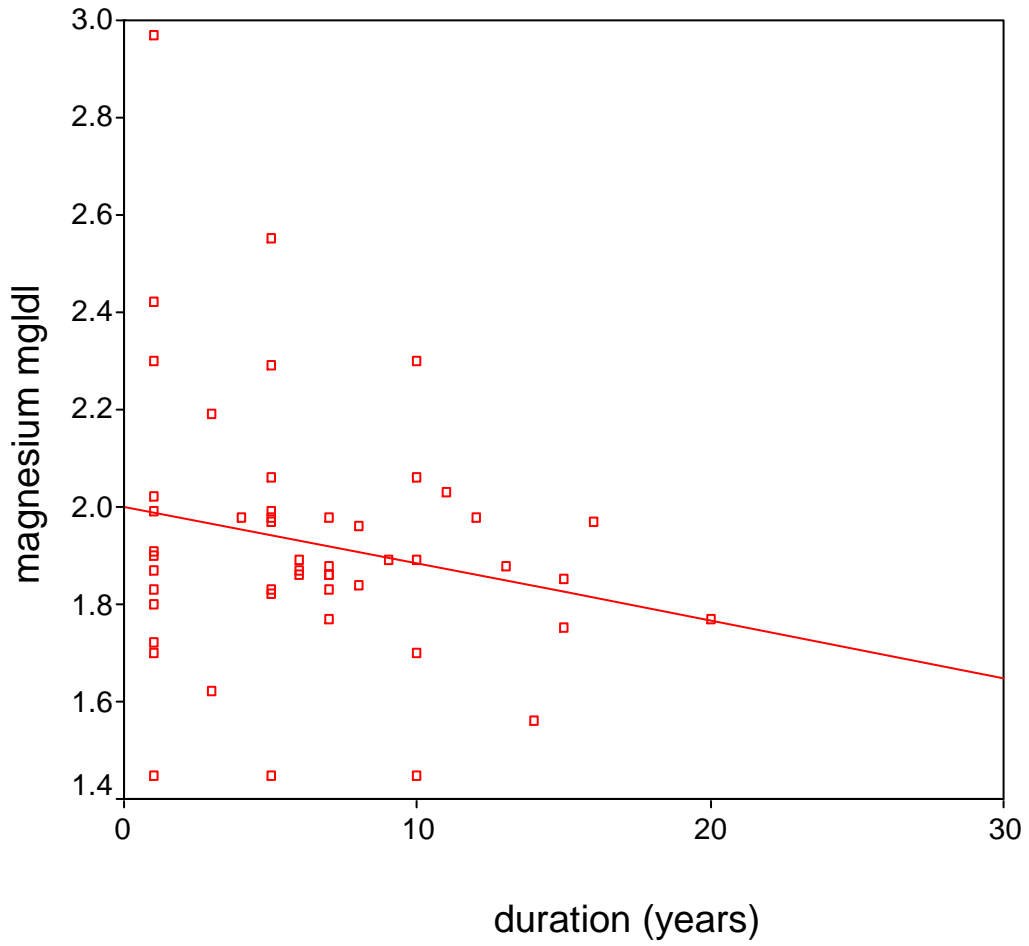
**Figure (4-1):** Correlation between plasma magnesium level and age in case group ( $r = -0.167$ ,  $p\text{-value} = 0.246$ ). There was insignificant weak negative correlation.



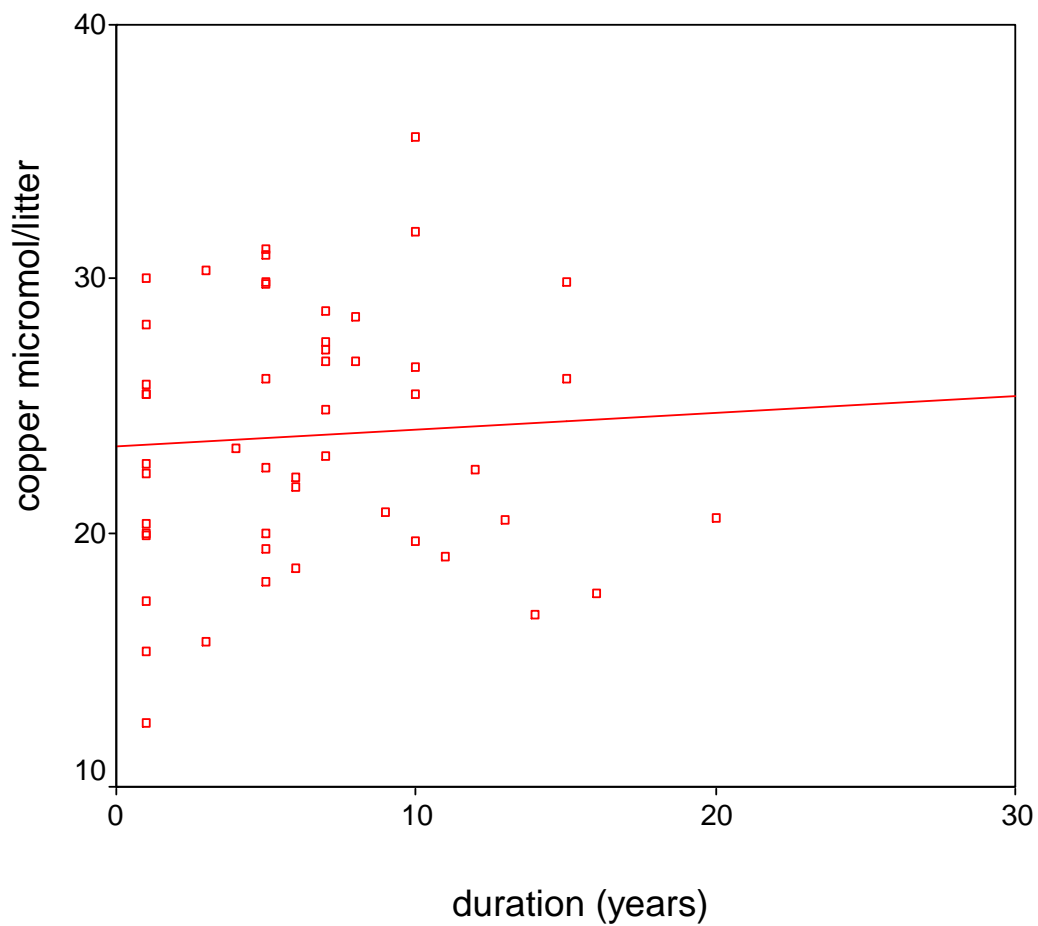
c

**Figure (4-2):** Correlation between plasma copper level and age in case group ( $r= 0.038$ ,  $p\text{-value}= 0.793$ ). There was insignificant week positive correlation.





**Figure (4-3):** Correlation between plasma magnesium level and duration of disease in case group ( $r = -0.206$ ,  $p\text{-value} = 0.151$ ). There was insignificant weak negative correlation.



**Figure (4-4):** Correlation between plasma copper level and duration of disease in case group ( $r= 0.061$ ,  $p\text{-value}= 0.675$ ). There was insignificant week positive correlation.

## 5. Discussion, Conclusion, Recommendation

### 5.1. Discussion:

One of the factors contributed to more severe asthma may be an increased susceptibility to the effects of reactive oxygen species (ROS) generated by inflammatory cells recruited into the lungs. The potentially damaging effects of oxidative stress are normally limited by antioxidants that scavenge ROS in the respiratory tract lining fluid (Wood *et al.*, 2003; Cross 2003). Due to the role of free oxygen radicals in pathogenesis of asthma, most studies have recently paid attention to the role of antioxidant defense systems. The antioxidant mechanisms that protected the lung against these oxidants include: the superoxide dismutases (SODs) and Glutathione peroxidase (GSH-Px) (Vuokko *et al.*, 2003).

The results of present study revealed that, there was insignificant difference in mean concentration of plasma magnesium among bronchial asthmatic patients when compared with control group ( $p$ .value 0.072). This result was similar to some reports in the literature (Ermis *et al.*, 2004; Vural, 2000; Picado *et al.*, 2001) and differ from other ones. (Anetore *et al.*, 2003). Magnesium significantly increased the activity of antioxidants enzymes. (Luo *et al.*, 2002; Sheirli *et al.*, 2003; Howard, 1999). Increased dietary magnesium has been shown to be associated with an independent beneficial effect on lung function, airway responsiveness, and wheezing in the United Kingdom population (Britton *et al.*, 1994). Another study showed that a low intake of magnesium, which is involved in the relaxation of smooth muscle, is associated with reduced lung function, bronchial hyper-reactivity and self reported wheezing (Baker *et al.*, 1999). When given

intravenously magnesium can lead to broncho- dilatation in acute severe asthma (Hill *et al.*, 1996).

The current study revealed that; mean of plasma levels of Mg was insignificantly differ among asthmatic patients compared to control group Mg (P.value (0.072, mean  $\pm$  SD:  $1.92 \pm 0.26$  mg/dL versus  $2.01 \pm 0.20$  mg/dL). but the levels of Cu was significantly increased compared to control group (P.value 0.000 mean  $\pm$ SD:  $23.82 \pm 5.05$   $\mu$ mol/L versus  $20.20 \pm 3.15$   $\mu$ mol/L). In asthmatic patients plasma levels of Mg and Cu not influenced by age (r= - 0.167, p-value= 0.246 for Mg) (r= 0.038, p-value= 0.793 for Cu ) and duration of disease (r= - 0.206, p-value= 0.151 for Mg) (r= 0.061, p-value= 0.675 for Cu).

## **5.2. Conclusion:**

Plasma concentration of magnesium was insignificantly differ in asthmatic patients but plasma concentration of copper was significantly higher.

According to this study there is no effect of age, sex, and duration of disease on the plasma trace elements levels.

### **5.3. Recommendations:**

1- Measurement of copper level is recommended in asthmatic patients to monitoring antioxidants enzymes system .

2- More studies include estimation for more trace elements in asthmatic patients is recommended to be done.

3- Dietary deficiencies in zinc, and magnesium should be avoided with proper supplementations in the management of airway inflammation due to free oxygen radicals in asthmatic patients to increase the effect of antioxidant defense system.

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