1.1 Introduction

Zinc and copper are key components necessary to maintain cellular homeostasis. The primary reason for this necessity is associated with the fact that hundreds of metaloenzymes require a metal element, as cofactors, to be functional. Zinc has many important roles in the body and its deficiency causes broad and nonspecific signs and symptoms, including suppressed immunity (Sommers E, 1974) decreased growth velocity, delayed sexual maturity and dysgusia (Tripathi R.M. et al, 1997) Zinc, copper are elements in the prevented of immune resistance. Zinc is known to be essential for all highly proliferating cells in the human body, especially the immune system. A variety of in vivo and in vitro effects of zinc on immune cells mainly depend on the zinc concentration. All kinds of immune cells show decreased function after zinc depletion. In monocytes, all functions are impaired, whereas in natural killer cells, cytotoxicity is decreased, and in neutrophil granulocytes, phagocytosis is reduced. The normal functions of T cells are impaired, but auto reactivity and all reactivity are increased. Iron metabolism is of crucial importance in the biology and pathophysiology of the lower respiratory as with many other factors involved in inflammation, it is very important that an appropriate iron balance is maintained. Local deficiency could impair growth and proliferation of cells responsible for the inflammatory response and tissue repair and the synthesis of mediators8.Because calcium is the major second messenger regulating ASM contraction, investigators hypothesized that abnormalities in calcium homeostasis, manifested by increases in the flux of calcium or alteration in calcium regulatory proteins, may play a critical role in inducing ASM hyper reactivity in asthma. Different genetic and environmental factors are involved in the pathogenesis of asthma. (Kietizamann.A, 2000) the rise in asthma and allergic disease among children is a matter of worldwide concern. Many authors have

argued that the changes in diet may have been an important determinant of increased susceptibility to asthma. (Amrani and Panettieri ,2002) We believe that a reduction in antioxidant intake, reflected in the diet of pregnant women, would increase the susceptibility of the new born baby to allergens. (Dominguez LJ, 1998) Chronic inflammation causes a characteristic decline in serum zinc levels in experimental studies. It is well known that zinc deficiency affects the regulation of T-cell lymphocytes, which may play some part in the nutritional status between asthmatic and healthy subjects. Asthmatic children, in particular, seem to be at a risk of zinc deficiency. The changes in trace element status may be the effect of chronic disease state and do not associate with the cause of disease. (Fraenkel and Holgate (eds).

1.2 Literature review

1.2.1 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 PGJ. et al., 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 antileukotriene 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. (Seaton A. et al, 1994)

1.2.1.1 Signs and symptoms

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 A. 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)

1.2.1.2 Causes

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 FD, 2007)

1.2.1.2.1 Environmental

Many environmental factors have been associated with asthma's development and exacerbation including allergens, air pollution, and other environmental chemicals. 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 BP, 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-Golden, 2013) Use by a mother during pregnancy is also associated with an increased risk. Asthma is associated with exposure to indoor allergens. Common indoor allergens include dust mites, cockroaches, animal dander, and mold. (Jindal and editor-in-chief SK, 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 B, 2005)

1.2.1.2.2 Hygiene hypothesis

The hygiene hypothesis attempts to explain the increased rates of asthma worldwide as a direct and unintended result of reduced exposure, during childhood, to non-pathogenic bacteria and viruses. It has been proposed that the reduced exposure to bacteria and viruses is due, in part, to increased cleanliness and

decreased family size in modern societies. Exposure to bacterial endotoxin in early childhood may prevent the development of asthma, but exposure at an older age may provoke bronchoconstriction. (Dietert, RR, 2011) Evidence supporting the hygiene hypothesis includes lower rates of asthma on farms and in households with pets. Use of antibiotics in early life has been linked to the development of asthma. Also, delivery via caesarean section is associated with an increased risk (estimated at 20-80%) of asthma this increased risk is attributed to the lack of healthy bacterial colonization that the newborn would have acquired from passage through the birth canal. There is a link between asthma and the degree of affluence. (Bornehag and Nanberg, April 2010)

1.2.1.2.3 Genetic

Table (1:1) Family risk factor for asthma

CD14-endotoxin interaction based on CD14 SNP C-159T		
Endotoxin levels	CC genotype	TT genotype
High exposure	Low risk	High risk
Low exposure	High risk	Low risk

Family history is a risk factor for asthma, with many different genes being implicated. If one identical twin is affected, the probability of the other having the disease is approximately 25%. By the end of 2005, 25 genes had been associated with asthma in six or more separate populations, including GSTM1, IL10, CTLA-4, SPINK5, LTC4S, IL4R and ADAM33, among others. Many of these genes are related to the immune system or modulating inflammation. Even among this list of genes supported by highly replicated studies, results have not been consistent among all populations tested (Covar RA.et al, 2005). In 2006 over 100 genes were

associated with asthma in one genetic association study alone; more continue to be found. Some genetic variants may only cause asthma when they are combined with specific environmental exposures an example is a specific single nucleotide polymorphism in the CD14 region and exposure to endotoxin (a bacterial product). Endotoxin exposure can come from several environmental sources including tobacco smoke, dogs, and farms. Risk for asthma, then, is determined by both a person's genetics and the level of endotoxin exposure. (Pinnock and Shah, 2007)

1.2.1.3 Pathophysiology

Asthma is the result of chronic inflammation of the airways which subsequently results in increased contractability of the surrounding smooth muscles. This among other factors leads to bouts of narrowing of the airway and the classic symptoms of wheezing. The narrowing is typically reversible with or without treatment. Occasionally the airways themselves change. Typical changes in the airways include an increase in eosinophils and thickening of the lamina reticularis. Chronically the airways' smooth muscle may increase in size along with an increase in the numbers of mucous glands. Other cell types involved include: T lymphocytes, macrophages, and neutrophils. There may also be involvement of other components of the immune system including: cytokines, chemokines, histamine, and leukotrienes among others. (Moore and Pascual, 2010)

1.2.1.4 Diagnosis

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 hyper-responsiveness 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)

1.2.1.4.1 Spirometry

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. (KindermannW, 2007)

1.2.1.4.2 Other methods

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 ≥20% difference in peak expiratory

flow rate on at least three days in a week for at least two weeks, a ≥20% improvement of peak flow following treatment with either salbutamol, inhaled corticosteroids or prednisone, or a ≥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. (Ripoll. et al, 2011)

1.2.1.5 Classification

Table (1:2)

Clinical classification (≥ 12 years old)

Severity	Symptom frequency	Night time symptoms	%FEV_1 of predicted	FEV_1 Variability	SABA use
Intermittent	≤2/week	≤2/month	≥80%	<20%	≤2 days/week
Mild persistent	>2/week	3–4/month	≥80%	20–30%	>2 days/week
Moderate persistent	Daily	>1/week	60–80%	>30%	Daily
Severe persistent	Continuously	Frequent (7×/week)	<60%	>30%	≥twice/day

Asthma is clinically classified according to the frequency of symptoms, forced expiratory volume in one second (FEV_1), and peak expiratory flow rate. Asthma may also be classified as atopic (extrinsic) or non-atopic (intrinsic), based on whether symptoms are precipitated by allergens (atopic) or not (non-atopic). While

asthma is classified based on severity, at the moment there is no clear method for classifying different subgroups of asthma beyond this system. Finding ways to identify subgroups that respond well to different types of treatments is a current critical goal of asthma research. (Been and Jasper, 2014)

Although asthma is a chronic obstructive condition, it is not considered as a part of chronic obstructive pulmonary disease as this term refers specifically to combinations of disease that are irreversible such as bronchiectasis, chronic bronchitis, and emphysema. Unlike these diseases, the airway obstruction in asthma is usually reversible; however, if left untreated, the chronic inflammation from asthma can lead the lungs to become irreversibly obstructed due to airway remodeling. In contrast to emphysema, asthma affects the bronchi, not the alveoli. (Ducharme FM. et al, 2010)

1.2.1.6 Medications

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$_2$-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 CH, 2009)

- Anticholinergic medications, such as ipratropium bromide, provide additional benefit when used in combination with SABA in those with moderate or severe symptoms. Anticholinergic 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 T. et al, 2012)
- Long-acting beta-adrenoceptor 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 EN, et al, 1999)

1.2.1.6.1 Delivery methods

Medications are typically provided as metered-dose inhalers (MDIs) in combination with an asthma spacer or as a dry powder inhaler. The spacer is a plastic cylinder that mixes the medication with air, making it easier to receive a full dose of the drug. A nebulizer may also be used. Nebulizers and spacers are equally effective in those with mild to moderate symptoms. However, insufficient evidence is available to determine whether a difference exists in those with severe disease. (Bousquet . et al, 2005)

1.2.1.6.2 adverse effects

Long-term use of inhaled corticosteroids at conventional doses carries a minor risk of adverse effects. Risks include the development of cataracts and a mild regression in stature. (Anderson. et al, 2007)

1.2.1.6.3 Other medications

When asthma is unresponsive to usual medications, other options are available for both emergency management and prevention of flareups. For emergency management other options include:

- Oxygen to alleviate hypoxia if saturations fall below 92%.
- Oral corticosteroid are recommended with five days of prednisone being the same 2 days of dexamethasone.
- Magnesium sulfate intravenous treatment has been shown to provide a bronchodilating effect when used in addition to other treatment in severe acute asthma attacks. In adults it results in a reduction of hospital admissions.
- Heliox, a mixture of helium and oxygen, may also be considered in severe unresponsive cases.

- Intravenous salbutamol is not supported by available evidence and is thus used only in extreme cases.
- Methylxanthines (such as theophylline) were once widely used, but do not add significantly to the effects of inhaled beta-agonists. Their use in acute exacerbations is controversial.
- The dissociative anesthetic ketamine is theoretically useful if intubation and mechanical ventilation is needed in people who are approaching respiratory arrest; however, there is no evidence from clinical trials to support this. (Masoli and Matthew, 2004)

  For those with severe persistent asthma not controlled by inhaled corticosteroids and LABAs, bronchial thermoplasty may be an option. It involves the delivery of controlled thermal energy to the airway wall during a series of bronchoscopies. While it may increase exacerbation frequency in the first few months it appears to decrease the subsequent rate. Effects beyond one year are unknown. Evidence suggests that sublingual immunotherapy in those with both allergic rhinitis and asthma improves outcomes. (Manniche L, 1999)

1.2.1.6.4 Alternative medicine

Many people with asthma, like those with other chronic disorders, use alternative treatments; surveys show that roughly 50% use some form of unconventional therapy. There is little data to support the effectiveness of most of these therapies. Evidence is insufficient to support the usage of Vitamin C. There is tentative support for its use in exercise induced brochospasm.

Acupuncture is not recommended for the treatment as there is insufficient evidence to support its use. Air ionisers show no evidence that they improve asthma symptoms or benefit lung function; this applied equally to positive and negative ion generators. (Thorowgood, 1873)

Manual therapies, including osteopathic, chiropractic, physiotherapeutic and respiratory therapeutic maneuvers, have insufficient evidence to support their use in treating asthma. The Buteyko breathing technique for controlling hyperventilation may result in a reduction in medication use; however, the technique does not have any effect on lung function. Thus an expert panel felt that evidence was insufficient to support its use. (Gaskoin G, 1872)

1.2.1.7 Prognosis

The prognosis for asthma is generally good, especially for children with mild disease. Mortality has decreased over the last few decades due to better recognition and improvement in care. Globally it causes moderate or severe disability in 19.4 million people as of 2004 (16 million of which are in low and middle income countries). Of asthma diagnosed during childhood, half of cases will no longer carry the diagnosis after a decade. Airway remodeling is observed, but it is unknown whether these represent harmful or beneficial changes. Early treatment with corticosteroids seems to prevent or ameliorates a decline in lung function. (Johnson MD, 2008)

1.2.2 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 molluscs 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. (Lippard and Berg, 1994) Copper compounds are used as bacteriostatic substances, fungicides, and wood preservatives.

1.2.2.1 Biological role

Copper proteins have diverse roles in biological electron transport and oxygen transportation, processes that exploit the easy interconversion 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:

$2\ HO_2 \rightarrow H_2O_2 + O_2$

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)

1.2.2.2 Dietary needs

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. (Bonham.et 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. (Gordon.et 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. (Maret and Wolfgang, 2013)

1.2.2.3 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)

1.2.3 Zinc

In commerce also spelter, is a chemical element with symbol Zn and atomic number 30. It is the first element of group 12 of the periodic table. In some respects zinc is chemically similar to magnesium: its ion is of similar size and its only common oxidation state is +2. Zinc is the 24th most abundant element in Earth's crust and has five stable isotopes. The most common zinc ore is sphalerite (zinc blende), a zinc sulfide mineral. The largest mineable amounts are found in Australia, Asia, and the United States. Zinc production includes froth flotation of the ore, roasting, and final extraction using electricity (electro winning).

1.2.3.1 Biological role

Zinc is an essential trace element for humans [and other animals, for plants and for microorganisms. Zinc is found in nearly 100 specific enzymes (other sources say 300), serves as structural ions in transcription factors and is stored and transferred in metallothioneins. It is "typically the second most abundant transition metal in organisms" after iron and it is the only metal which appears in all enzyme classes.

In proteins, Zn ions are often coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine. The theoretical and computational

description of this zinc binding in proteins (as well as that of other transition metals) is difficult. (Sugarman B, 1983)

There are 2-4 grams of zinc distributed throughout the human body. Most zinc is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye. Semen is particularly rich in zinc, which is a key factor in prostate gland function and reproductive organ growth.

In humans, zinc plays "ubiquitous biological roles". It interacts with "a wide range of organic ligands" and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression. It also regulates apoptosis. A 2006 study estimated that about 10% of human proteins (2800) potentially bind zinc, in addition to hundreds which transport and traffic zinc; a similar in silico study in the plant Arabidopsis thaliana found 2367 zinc-related proteins. (Plum.et al, 2010)

In the brain, zinc is stored in specific synaptic vesicles by glutamatrgic neurons and can "modulate brain excitability". It plays a key role in synaptic plasticity and so in learning. However, it has been called "the brain's dark horse" because it also can be a neurotoxin, suggesting zinc homeostasis plays a critical role in normal functioning of the brain and central nervous system. (Berdanier.et al, 2007)

1.2.3.1.1 Enzymes

Zinc is an efficient Lewis acid, making it a useful catalytic agent in hydroxylation and other enzymatic reactions. The metal also has a flexible, which allows proteins using it to rapidly shift conformations to perform biological reactions. Two examples of zinc-containing enzymes are carbonic anhydrase and carboxypeptidase, which are vital to:

- The processes of carbon dioxide.
- Regulation and digestion of proteins, respectively.

- In vertebrate blood, carbonic anhydrase converts CO into bicarbonate and the same enzyme transforms the bicarbonate back into CO for exhalation through the lungs. Without this enzyme, this conversion would occur about one million times slower. (Bitanihirwe BK; Cunningham MG, 2009) at the normal blood pH of 7 or would require a pH of 10 or more. The non-related β-carbonic anhydrase is required in plants for leaf formation, the synthesis of indole acetic acid (auxin) and alcoholic fermentation. (Stipanuk and Martha, 2006)

Carboxypeptidase cleaves peptide linkages during digestion of proteins. A coordinate covalent bond is formed between the terminal peptide and a C=O group attached to zinc, which gives the carbon a positive charge. This helps to create a hydrophobic pocket on the enzyme near the zinc, which attracts the non-polar part of the protein being digested. (Hershfinkel. et al, 2007)

1.2.3.1.2 other proteins

Zinc serves a purely structural role in zinc fingers, twists and clusters. Zinc fingers form parts of some transcription factors, which are proteins that recognize DNA base sequences during the replication and transcription of DNA. Each of the nine or ten Zn2+ ions in a zinc finger helps maintain the finger's structure by coordinately binding to four amino acids in the transcription factor. (Blake and Steve, 2007) The transcription factor wraps around the DNA helix and uses its fingers to accurately bind to the DNA sequence.

In blood plasma, zinc is bound to and transported by albumin (60%, low-affinity) and transferrin (10%). Because transferrin also transports iron, excessive iron reduces zinc absorption, and vice versa. A similar antagonism exists with copper. The concentration of zinc in blood plasma stays relatively constant regardless of

zinc intake. Cells in the salivary gland, prostate, immune system and intestine use zinc signaling as one way to communicate with other cells. (Colin Tidy, 2010)

Zinc may be held in metallothionein reserves within microorganisms or in the intestines or liver of animals. Metallothionein in intestinal cells is capable of adjusting absorption of zinc by 15–40%. However, inadequate or excessive zinc intake can be harmful; excess zinc particularly impairs copper absorption because metallothionein absorbs both metals. (Ensminger. et al, 1993)

1.2.3.2 Dietary intakes

In the U.S., the Recommended Dietary Allowance (RDA) is 8 mg/day for women and 11 mg/day for men. Median intake in the U.S. around 2000 was 9 mg/day for women and 14 mg/day in men. Oysters, lobster and red meats, especially beef, lamb and liver have some of the highest concentrations of zinc in food. (Rosado J, 2003)

Zinc supplements should only be ingested when there is zinc deficiency or increased zinc necessity (e.g. after surgeries, traumata or burns). Persistent intake of high doses of zinc can cause copper deficiency.

The concentration of zinc in plants varies based on levels of the element in soil. When there is adequate zinc in the soil, the food plants that contain the most zinc are wheat (germ and bran) and various seeds (sesame, poppy, alfalfa, celery, mustard). Zinc is also found in beans, nuts, almonds, whole grains, pumpkin seeds, sunflower seeds and blackcurrant. (DiSilvestro.et al, 2008)

Other sources include fortified food and dietary supplements, which come in various forms. A 1998 review concluded that zinc oxide, one of the most common supplements in the United States, and zinc carbonate are nearly insoluble and poorly absorbed in the body. This review cited studies which found low plasma zinc concentrations after zinc oxide and zinc carbonate were consumed compared

with those seen after consumption of zinc acetate and sulfate salts. However, harmful excessive supplementation is a problem among the relatively affluent, and should probably not exceed 20 mg/day in healthy people, although the U.S. National Research Council set a Tolerable Upper Intake of 40 mg/day. (Freeland-Graves J. H. et al, 1980)

For fortification, however, a 2003 review recommended zinc oxide in cereals as cheap, stable, and as easily absorbed as more expensive forms. A 2005 study found that various compounds of zinc, including oxide and sulfate, did not show statistically significant differences in absorption when added as fortificants to maize tortillas. A 1987 study found that zinc picolinate was better absorbed than zinc gluconate or zinc citrate. However, a study published in 2008 determined that zinc glycinate is the best absorbed of the four dietary supplement types available. (Hambidge M, 2003)

1.2.3.3 Deficiency

Zinc deficiency is usually due to insufficient dietary intake, but can be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses. Groups at risk for zinc deficiency include the elderly, children in developing countries, and those with renal insufficiency.

Symptoms of mild zinc deficiency are diverse. Clinical outcomes include depressed growth, diarrhea, impotence and delayed sexual maturation, alopecia, eye and skin lesions, impaired appetite, altered cognition, impaired host defense properties, defects in carbohydrate utilization, and reproductive teratogenesis. Mild zinc deficiency depresses immunity, although excessive zinc does also. Animals with a diet deficient in zinc require twice as much food in order to attain the same weight gain as animals given sufficient zinc. (Buhl and Meyer, 1996)

Despite some concerns, western vegetarians and vegans have not been found to suffer from overt zinc deficiencies any more than meat-eaters. Major plant sources of zinc include cooked dried beans, sea vegetables, fortified cereals, soyfoods, nuts, peas, and seeds.(Urushidate S, et al 2010) However, phytates in many whole-grains and fiber in many foods may interfere with zinc absorption and marginal zinc intake has poorly understood effects. The zinc chelator phytate, found in seeds and cereal bran, can contribute to zinc malabsorption. There is some evidence to suggest that more than the US RDA (15 mg) of zinc daily may be needed in those whose diet is high in phytates, such as some vegetarians. These considerations must be balanced against the fact that there is a paucity of adequate zinc biomarkers, and the most widely used indicator, plasma zinc, has poor sensitivity and specificity. Diagnosing zinc deficiency is a persistent challenge. (Ghaffari J.et al, 2013)

Nearly two billion people in the developing world are deficient in zinc. In children it causes an increase in infection and diarrhea, contributing to the death of about 800,000 children worldwide per year. (Sagdica A.et al, 2011) The World Health Organization advocates zinc supplementation for severe malnutrition and diarrhea. Zinc supplements help prevent disease and reduce mortality, especially among children with low birth weight or stunted growth. However, zinc supplements should not be administered alone, because many in the developing world have several deficiencies, and zinc interacts with other micronutrients. (Behmanesh F. et al, 2011)

Rationale:

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. The aim of the present study was to investigate serum levels of Zinc, Copper in asthmatic patients

Objectives of the study:

General objective:

Assessment of serum levels of the trace elements Zn and Cu in asthmatic patients

Specific objectives:

1- To measure plasma copper and zinc levels in blood samples of patients with bronchial asthma.
2- To compare plasma zinc, and copper level in patients with asthma with control
3- To correlate plasma zinc and copper level with age, body mass index, and duration of disease

2.1. Materials

2.1.1 Study design:

This is case-control study to determine copper, and zinc levels in bronchial asthmatic patients.

2.1.2 Study area:

Blood sample were collected from alshaab teaching hospital, asthma room, in Khartoum state and Sudan University for sciences and technology faculty of laboratory science.

2.1.3 Sample size:

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

2.1.4 Study duration:

The study took time from 18 may to 18 June 2015

2.1.5 Sample type:

Serum blood samples were used in this study.

2.1.6 Sample collection:

5 ml of venous blood was drawn from each volunteer in this study using a disposable plastic syringe. The blood is poured in a plane container and then centrifuged after clotted. The Serum is kept at -20°C in sterile condition till used.

2.1.7 Study population:

In this study, bronchial asthmatic patients in acute phase was tested for trace elements (copper and zinc)

2.1.8 Exclusion criteria:

We excluded patients have diabetes mellitus and also hypertensive patients

2.1.9 Inclusion criteria:

We included patients with bronchial asthma

2.1.10 Ethical consideration:

The objectives of the study were explained to all individuals Participating in this study. All participants who agreed were gave Verbal approval.

2.2 Biochemical measurement of plasma zinc and copper

2.2.1 Instrument

The estimation of plasma zinc and copper were performed in environment and natural resources and desertification research institute in Khartoum by using buck scientific atomic absorption spectrophotometry (AAS) model 210 VGP apparatus, D2 corrector nitrous oxide burner, conditions N2O/Acetylene flame (temperature range : 2650-2800ć

2.2.2 Principle of AAS

The principle based on estimation of zinc and copper by measuring the amount of absorbed light of unknown concentration of these elements. The diluted sample aspirated, aerosolized and mixed with acetylene/ nitrous oxide gas. The mixture is ignited in the flame. During the combustion, atoms of the elements of interest in the sample are reduced in free, un excited ground state atoms which absorb light at a characteristic wavelengths , the characteristic wavelengths are element specific (Cu: 324nm,Zn: 214nm). To provide elements specific wavelengths, a light beam from a lamp (hollow cathode lamp) whose cathode is made of the element being

determined in passed through the flame. A device such as photomultiplier detector can detect the amount of reduction of the light intensity due to absorption by the analyte, and this can be directly related to the amount of the element in the sample. The electronic convert the amount of light absorbed to the actual sample concentration.

2.2.3 Procedure

For the measurement of zinc the plasma was diluted 5 times by adding 0.2ml of plasma to 0.8ml of DW, and for the copper the plasma was diluted 2 times by adding 0.5ml of plasma to 0.5ml of DW. The diluted sample was well mixed and directly aspirated as fine mist of droplet in reproducible constant rate in to the flame of the device and the values were expressed as microgram per deciliter or milligram per liter. The analysis is performed against standards prepared in glycerol to approximate the viscosity characteristic of the diluted samples, and the DW used as blank.

The reference range for zinc is 50 – 120 µg/dl or 0.5 – 1.2mg/l while for copper is 70 – 140 µg/dl or 0.7 – 1.4 mg/l (appendix2).

2.2.4 Instrument calibration

Cu standards of serial concentration at level of ppm (mg/l) were prepared by diluted the Cu stock standard with 10% (v/v) glycerol also zinc standards of serial concentration were prepared by diluted the zinc stock standard with 5% (v/v) glycerol (appendix 2). Their absorbances were obtained against blank. The absorbance versus the concentration of the standards was used for blotting a linear calibration curve.

2.2.5 Quality control

The precision and accuracy of method used in this study were checked each batch, which performed by including commercially prepared control sera (appendix 3)

2.2.6 Statistical analysis

All data were analyzed by SPSS software version 11.5.

3. Results

The results of the biological determinants are given in tables and figures Mean and standard deviation of all parameters in serum were calculated. Students J t-test was used for comparison of means and $P<0.05$ was considered significant.

Statistical analysis of obtained results revealed that; mean of plasma levels of zinc was significantly lower among asthmatic patients compared to control group (p value 0.002), while the mean of copper levels was significantly higher (p value 0.003) as show in table (3.1). In asthmatic patients plasma levels of zinc and copper not influenced by age (p value copper 0.436, zinc 0.114) as show in table (3.3) and sex (p value copper 0.584, zinc 0.377) as show in table (3.2) and body mass index (p value copper 0.638, zinc 0.868) as show in table (3.5) and duration of disease (p value copper 0.356, zinc 0.652) as show in table (3.4)

Table (3.1) levels of trace elements in serum of asthmatic patients and control

Elements	Mean±SD Case	Mean±SD Control	P value
Copper Mg/l	0.900±0.278	0.729±0.293	0.003*
Zinc Mg/l	0.537±0.370	0.734±0.243	0.002*

- Table (3.1) Showed mean of copper and zinc in patients and control group, the results expressed as (M ± STD).
- * indicate P-value < 0.05

Table (3.2) levels of trace elements with gender

Elements	Mean±SD Male	Mean±SD Female	P value
Copper Mg/l	0.883±0.255	0.928±0.314	0.584
Zinc Mg/l	0.576±0.456	0.480±0.171	0.377

- Table (3.2) Showed mean of copper and zinc in male and female group, the results expressed as (M ± STD).
- * indicate P-value < 0.05

Table (3.3) correlation between age and trace elements levels

Elements		Age
Copper	Pearson Correlation Sig. (2-tailed) N	-0.113 0.436 50
Zinc	Pearson Correlation Sig. (2-tailed) N	-0.226 0.114 50

r = correlation coefficient

+ = positive correlation

- = negative correlation

N = number of patients

- Table (3.3) showed the correlation between copper and zinc levels and age, result expressed as (Pearson's r, P value).
- P value (copper 0.436, zinc 0.114) more than 0.05 (in significant)
- No correlation

Table (3.4) correlation between duration and trace elements levels

Elements		Duration
Copper	Pearson Correlation Sig. (2-tailed) N	-0.133 0.356 50
Zinc	Pearson Correlation Sig. (2-tailed) N	0.065 0.652 50

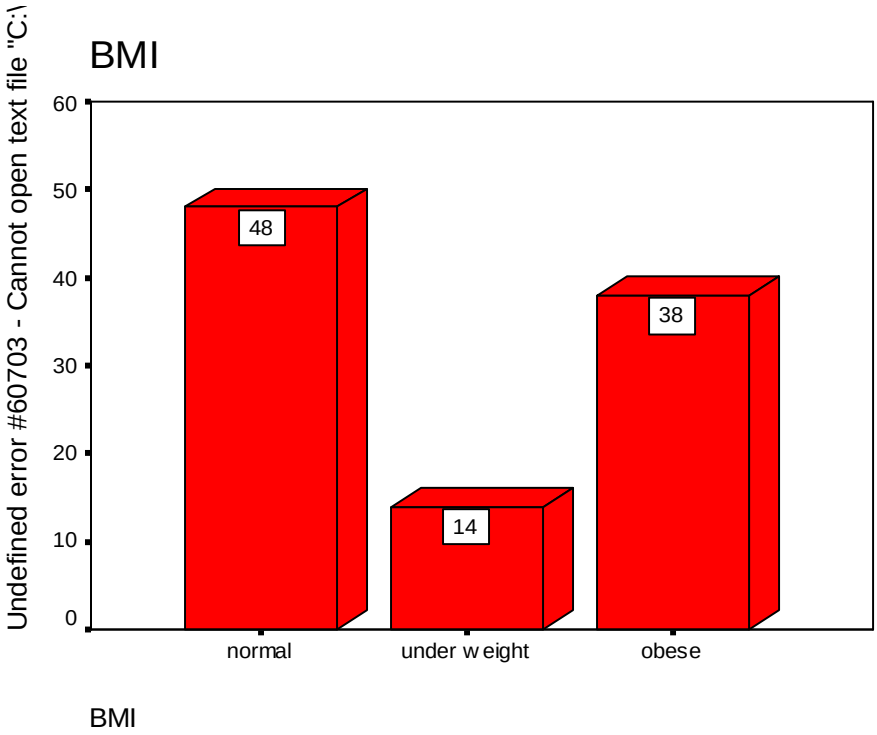
- Table (3:4) showed the correlation between copper and zinc levels and duration of disease, result expressed as (Pearson's r, P value).
- P value (copper 0.356, zinc 0.652) more than 0.05 (in significant)
- No correlation

Table (3.5) correlation between BMI and trace elements levels

Elements		BMI
Copper	Pearson Correlation Sig. (2-tailed) N	-0.068 0.638 50
Zinc	Pearson Correlation Sig. (2-tailed) N	0.024 0.868 50

- Table (3.5) showed the correlation between copper and zinc levels and body mass index, result expressed as (Pearson's r, P value).
- P value (copper 0.638, zinc 0.868) more than 0.05 (in significant)
- No correlation

Figure (3.1) percentage of obesity among asthmatic patients



4.1 Discussion

Bronchial asthma is an atopic disease characterized by chronic airway inflammation and hyper-responsiveness. Severe acute asthma is a medical emergency and sometimes difficult to treat. (Urushidate S. et al, 2010)

Oxidants-antioxidants balance is essential for the normal lung function. Both, an Increased oxidant and/or decreased antioxidant may reverse the physiologic oxidant-antioxidant balance in favor of oxidants, leading to lung injury. A number of diseases involving the lung, such as bronchial asthma, COPD, emphysema, bronchiectasis and have been associated with a disturbance of these balances. Following the understanding of the role of free oxygen radicals in pathogenesis of Asthma, most studies have recently paid attention to the role of antioxidant defense systems. The most important antioxidant systems are the superoxide dismutase (SOD) which contains zinc and copper in their structures .Zinc is important elements in the preservation of immune resistance and is required for numerous biochemical functions and for optimal activity of the immune system. Zinc plays an important role in DNA and protein synthesis and is intimately involved with copper as cofactors in several important enzyme systems. (Truong-Tran AQ, et al, 2001)There is increasing evidence from observations studies that there are a strong relationship between diet and respiratory disease. (El-Kholy MS.et al, 1990)

The results of the current study show a significant decrease in serum zinc level in patients with BA in comparison with the healthy controls(P value 0.002) showed in table (3.1) which in agreement with the findings observed by other investigators) and other researchers . (Wellinghausen and Rink, 1998)

On the other hand, elsewhere hyperzincemia were found in severe cases of asthma by others which inconsistent with the findings of the present study. (TekinD.et al, 2000) In other studies no significant difference in the zinc levels was found

between patients and controls which also disagree with results of this work. (Smith LJ, et al, 1997) In Japanese adult asthmatic case, no significant decrease of zinc was detected in the asthmatic patients, which was disagreement with the present study. (De Raeve HR.et al, 1997) There is more than one explanation for the mechanism of hypozincemia in enhancing respiratory diseases in adults. It was proposed that several intrinsic factors may contribute to a low Zn status in asthmatics. (Multi A.et al, 2006) First, like other inflammatory diseases, redistribution in plasma Zn to the liver can occur during excessive stress. This has been attributed to the release of leucocyte endogenous mediator from activated phagocytes, which then stimulates movement of Zn from plasma to hepatocytes in allergic reactions. (Evans and Halim, 1974) Second, the immune system is extremely dependent on the availability of Zn for maintaining its homeostasis. Inflammatory diseases can cause an increase in the demand for Zn as: (i) Zn is essential for producing the thymic hormone thymulin necessary for regulating T-cell development and activation; and (ii) Zn is crucial for the activation of natural killer cells, phagocytic cells and for granulocytes, such as mast cells and eosinophil. As a result, greater demand for Zn by the immune system could be a contributing factor to the Zn deficiency noted in inflammatory diseases. Zinc deficiency itself is detrimental for inflammation as it results in dramatic increases in the number, size and activation state of mast cells. This further exacerbates damage via increasing chemotaxis of eosinophils and neutrophils, which create a continuous cycle of oxidative damage, all of which have been implicated in promoting the pathogenesis of allergic diseases such as asthma (Wellinghausen and Rink, 1998). Otherwise in table (3.1) showed a significant increase in copper level in asthmatic patient when compared with control group (P value 0.003) Hypozincemia in patients with BA may be due to hypercupremia. Zinc and copper

are required for antioxidant enzymes such as sodium oxide dismutase (SOD) and hence the optimal functions of immune system, as both zinc and copper form the prosthetic of SOD.any change in their concentration will affect the activity of the enzyme. The negative correlation between zinc and copper may explained by their competitive either for the same absorptive binding sites on the enterocytes or for similar functional protein systems. (Van Huisstede and Branushahi, 2010) Table (3.2) showed mean of copper and zinc in male (0.838 mg/l) and female (0.928 mg/l) there is no variation in mean of copper and zinc levels in male and female p value(copper 0.584, zinc 0.377) .also in the current study there is no correlation between copper and zinc level and age as showed in Table (3.3).the result also express there is no correlation with duration of disease as showed in table (3.4). Also the current study showed there is no correlation between copper and zinc level with body mass index of patients (3.5) this result opposite to the other researches Hypozincemia in asthma may be due to obesity that asthma was related to obesity .Obese people consume food with less national value, fewer vitamins and more fat. A high amount of intake is associated with asthma. (Weiss ST, 2005)The results of current revealed that 38% of patients with BA were obese in agreement with the investigation observed by other researchers.

4.2 Conclusion

These results show that, there is decrease in zinc level in patients with bronchial asthma, also this study revealed that there is elevated level of copper among asthmatic patients and according to this study there is no effect of age and body mass index on the serum trace elements levels

4.3 Recommendations

1- Measurement of zinc and copper levels could be recommended in asthmatic adults, especially in countries with a higher prevalence of zinc deficiency.

2- Zinc supplementation might be suggested in asthmatic patients with hypozincemia, while such a defect could aggregate to the severity of disease. However, further multicenter studies with greater sample sizes are needed to warrant the results of this study.

3- We speculate that, dietary deficiencies in zinc 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.

References:

Amrani Y and Panettieri Jr,(2002), *Modulation of calcium homeostasis as a mechanism for altering smooth muscle responsiveness in asthma*. Curr Opin Allergy Clinical Immunology:2:39-45.

Anderson, HR; Gupta R; Strachan DP and Limb ES ,(January 2007), *"50 years of asthma: UK trends from 1955 to 2004".* Thorax **62** (1): 85–90. doi:10.1136/thx.2006.066407. PMC 2111282. PMID 17189533.

Burney PGJ, Chinn S, Rhona RJ, (1990), *Has the prevalence of asthma increased in children?* Evidence from the national study of health and growth. B M J; 300: 1306-10

Bornehag, CG; Nanberg, E, (April 2010), *"Phthalate exposure and asthma in children".* International journal of andrology **33** (2): 333–45. doi:10.1111/j.1365-2605.2009.01023.x. PMID 20059582.

Been, Jasper, (March, 2014), *"Effect of smoke-free legislation on perinatal and child health: a systematic review and meta-analysis".* Lancet **383** (9928): 1549–60. doi:10.1016/S0140-6736(14)60082-9. PMID 24680633.

Bousquet, J; Bousquet, PJ; Godard, P; Daures, JP, (July 2005), *"The public health implications of asthma".* Bulletin of the World Health Organization **83** (7): 548–54. PMC 2626301. PMID 16175830.

Bonham, Maxine; O'Connor, Jacqueline M.; Hannigan, Bernadette M.; Strain, J, (2002), *"The immune system as a physiological indicator of marginal copper status?".* British Journal of Nutrition **87** (5): 393–403. doi:10.1079/BJN2002558. PMID 12010579.

Berdanier, Carolyn D.; Dwyer, Johanna T.; Feldman, Elaine B, (2007), *Handbook of Nutrition and Food*. Boca Raton, Florida: CRC Press. ISBN 0-8493-9218-7.

Bitanihirwe BK; Cunningham MG, (2009), *"Zinc: The brain's dark horse"*. Synapse **63** (11): 1029–49. doi:10.1002/syn.20683. PMID 19623531.

Blake, Steve, (2007), *Vitamins and Minerals Demystified*. McGraw-Hill Professional. p. 242. ISBN 0-07-148901-0.

Buhl R, Meyer A,(1996), Volgelmeier C. *Oxidant-Protease interaction in the lung prospec* **B**ehmanesh F, BanihashemAA,Hiradfar S and Ansari E.A, (2011), comparative *study of serum zinc level between asthmatic and control group*. Med j Mashhad Univ Med Sci; 53(4):240-244.

Covar, RA; Macomber, BA; Szefler, SJ,February (2005), "*Medications as asthma triggers"*. Immunology and allergy clinics of North America **25** (1): 169–90. doi:10.1016/j.iac.2004.09.009. PMID 15579370.

Cates, CJ; Cates, MJ, Cates, Christopher J, ed, (July 2008) *"Regular treatment with salmeterol for chronic asthma: serious adverse events"*. Cochrane Database of Systematic Reviews (3): CD006363. doi:10.1002/14651858.CD006363.pub2. PMID 18646149.

Colin Tidy, (March 2010), [*Zinc Supplements "Patient.co.uk"]*. Retrieved November 2, 2013.

Dominguez LJ, Barbagallo M, Di Lorenzo G,(1998), *Bronchial reactivity and intracellular magnesium*: a possible mechanism for the bronchodilating effects of magnesium in asthma. Clin Sci ;95:137- 142.

Dietert, RR, (September 2011), *"Maternal and childhood asthma: risk factors, interactions, and ramifications*". Reproductive toxicology (Elmsford, *N.Y.)* **32** (2): 198–204. doi:10.1016/j.reprotox.2011.04.007. PMID 21575714.

Ducharme, FM; Ni Chroinin, M; Greenstone, I; Lasserson, TJ, (April, 2010), Ducharme, Francine M, ed. *"Addition of long-acting beta2-agonists to inhaled steroids versus higher dose inhaled corticosteroids in adults and children with persistent asthma"*. Cochrane Database of Systematic Reviews (4): CD005533. doi:10.1002/14651858.CD005533.pub2. PMID 20393943.

Decker, H. & Terwilliger, N, (2000), *"COPs and Robbers: Putative evolution of copper oxygen-binding proteins"*. Journal of Experimental Biology **203** (Pt 12): 1777–1782. PMID 10821735.

DiSilvestro, Robert A.; Swan, Melinda, (2008), *"Comparison of Four Commercially Available Zinc Supplements for Performance in a Zinc Tolerance Test"*. The FASEB Journal **22**. 693.3.

De Raeve HR, Thunnissen FB, kaneko FT et al, (1997), *Decreased Cu,Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroid in vivo*. Am. J. Physiol; 272:L148-54.

Ensminger, Audrey H; Konlande and James E, (1993), *Foods & Nutrition Encyclopedia* (2nd ed.). Boca Raton, Florida: CRC Press. pp. 2368–2369. ISBN 0-8493-8980-1.

El-Kholy MS, Gas Allah MA, el-Shimi S, el-Bas F,el-Tayeb H and Abdel-Hamid MS, (1990), *Zinc and copper status in children with bronchial asthma and atopic dermatitis*. Egypt Public Health Assoc; 65:657-68.

Evans GW and HalimCJ, (1974), *Copper and zinc binding components in rat intestine*.AdvExpBiol;48:285-297.

Fraenkel DJ and Holgate ST (eds). *Etiology of asthma*: Pathology and ediators. In:C.W.. Biermann, D.S earlman, G.G.Shapiro, W.W. Busse (Allergy, Asthma and Immunology from Infancy to Adulthood. 3rd ed . WB Saunders Company, Philedelphia, pp 443-72

Fanta CH, (March 2009), *"Asthma"*. New England Journal of Medicine **360** (10): 1002–14. doi:10.1056/NEJMra0804579. PMID 19264689.

Freeland-Graves J. H.; Bodzy P. W.; Epright M. A, (1980), "*Zinc status of vegetarians"*. Journal of the American Dietetic Association **77** (6): 655–661. PMID 7440860.

Godden DJ, Devereux GS, (1999), Anderson WJ. *Environmental lung disease: the role of diet.* Monaldi Arch Chest Dis; 54: 479– 84

George, Ronald B,(2005), *Chest medicine : essentials of pulmonary and critical care medicine* (5th ed.). Philadelphia, PA: Lippincott Williams & Wilkins. p. 62. ISBN 978-0-7817-5273-2.

Grant EN, Wagner R, Weiss KB, (August 1999), *"Observations on emerging patterns of asthma in our society"*. J Allergy Clin Immunol **104** (2 Pt 2): S1–S9. doi:10.1016/S0091-6749(99)70268-X. PMID 10452783.

Gaskoin G ,(March 1872), *"On the treatment of asthma"*. British Medical Journal **1** (587): 339. doi:10.1136/bmj.1.587.339. PMC 2297349. PMID 20746575.

Gordon, Starkebaum; John, M. Harlan, (April 1986), *"Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine"*. *J.* Clin. Invest. **77** (4): 1370–6. doi:10.1172/JCI112442.

Ghaffari J, Rafatpanah H, Nazari Z, AbaskhaniaA,(2013), *Serum Level of trace Elements(Zinc,Lead, and Copper), Albumin and Immunoglobulins in Asthmatic* Children.Zahedan J Res Med Sci; 15(9):27-30

GuoCH, LiuPJ, HsiaS, ChuangCJ, ChenPC, (2011), *Role of certain trace minerals in oxidative stress; inflammation ,CD4/CDB lymphocyte ratios and lung function in asthmatic patients.*AnnClinBiochem;48(4):344-351.

Hershfinkel, Michal; Silverman, William F.; Sekler, Israel, (2007), *"The Zinc Sensing Receptor, a Link Between Zinc and Cell Signaling"*. Molecular Medicine **13** (7–8): 331–6. doi:10.1007/s10653-009-9255-4. PMC 1952663. PMID 17728842.

Hambidge, M, (2003), *"Biomarkers of trace mineral intake and status"*. Journal of Nutrition. 133 **3** (3): 948S–955S. PMID 12612181.

Jindal, editor-in-chief SK,(2011), *Textbook of pulmonary and critical care medicine*. New Delhi: Jaypee Brothers Medical Publishers. p. 242. ISBN 978-93-5025-073-0.

Johnson, MD PhD, Larry E., ed ,(2008), *"Copper"*. Merck Manual Home Health Handbook. Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. Retrieved 7 April 2013.

Kietizamann.A.(2000), immunotoxicology of Environmental and Occupational tals.Toxicon,38:735-741

Kindermann, W, (2007), *"Do inhaled beta(2)-agonists have an ergogenic potential in non-asthmatic competitive athletes?"*. Sports medicine (Auckland, N.Z.) **37** (2): 95–102. doi:10.2165/00007256-200737020-00001. PMID 17241101.

Lozano, R, (December 2012), *"Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010."*. Lancet **380** (9859): 2095–128. doi:10.1016/S0140-6736(12)61728-0. PMID 23245604.

Lippard J Berg M, (1994), *"Principles of bioinorganic chemistry"* University Science Books: Mill Valley, CA; 1994. ISBN 0-935702-73-3.

Martinez FD, (2007), *"Genes, environments, development and asthma: a reappraisal"*. European Respiratory Journal **29** (1): 179–84. doi:10.1183/09031936.00087906. PMID 17197483.

Moore WC, Pascual RM, (June 2010), *"Update in asthma 2009"*. American Journal of Respiratory and Critical Care Medicine **181** (11): 1181–7. doi:10.1164/rccm.201003-0321UP. PMC 3269238. PMID 20516492.

Masoli, Matthew, (2004), *Global Burden of Asthma* (PDF). p.9.

Manniche L, (1999), *Sacred luxuries: fragrance, aromatherapy, and cosmetics in ancient Egypt*. Cornell University Press. pp. 49. ISBN 978-0-8014-3720-5.

M C Linder; Wooten, L; Cerveza, P; Cotton, S; Shulze, R; Lomeli, N, (May 1998), *"Copper transport"*. The American Journal of Clinical Nutrition **67** (5): 965S–971S. PMID 9587137.

Maret, Wolfgang, (2013), "Chapter 14 Zinc and the Zinc Proteome". In Banci, Lucia. *Metallomics and the Cell*. Metal Ions in Life Sciences **12**. Springer. doi:10.1007/978-94-007-5561-10_14. ISBN 978-94-007-5561-1.

Multi A, CorradiM, GoldomiM, etal, (2006), *exhaleted metallic elemments and serum pneumo proteins in asymptomatic smokers and patients with COPD or asthma*.Chest;129(5):1288-1297.

Omran M, Russell G,(1996), *Continuing increase in respiratory symptoms and atopy in Aberdeen choolchildren*. BMJ; 312: 334-36

Pinnock H, Shah R, (2007), *"Asthma"*. BMJ **334** (7598): 847–50. doi:10.1136/bmj.39140.634896.BE. PMC 1853223. PMID 17446617.

Prasad A. S, (2008), *"Zinc in Human Health: Effect of Zinc on Immune Cells"*. Mol. Med. **14** (5–6): 353–7. doi:10.2119/2008-00033.Prasad. PMC 2277319. PMID 18385818.

Plum, Laura; Rink, Lothar; Haase, Hajo, (2010), *"The Essential Toxin: Impact of Zinc on Human Health"*. Int J Environ Res Public Health **7** (4): 1342–1365. doi:10.3390/ijerph7041342.

Ripoll, Brian C. Leutholtz, Ignacio, (2011), *Exercise and disease management* (2nd ed.). Boca Raton: CRC Press. p. 100. ISBN 978-1-4398-2759-8.

Rosado, J. L, (2003), "*Zinc and copper: proposed fortification levels and recommended zinc compounds"*. Journal of Nutrition **133** (9): 2985S–9S. PMID 12949397.

Sommers, E., (1974), *the toxic potential of trace elements in foods* - a review, Environ. Res.Technology, 39 : 215 – 227

Seaton A, Godden DJ, Brown K,(1994), *Increase in asthma: a more toxic environment or a more susceptible population.* Thorax; 49: 171-74

Seaton A, Soutar A, Mullins J,(1996), *The increase in hay fever: pollen, particulate matter and SO2 in ambient air*. Q J Med; 89: 279-84

Scott JP, Peters-Golden M, (September 2013), "*Anti leukotriene agents for the treatment of lung disease"*. Am. J. Respir. Crit. Care Med. **188** (5): 538–544. doi:10.1164/rccm.201301-0023PP. PMID 23822826.

Schiffman, George, (18 December 2009), *"Chronic obstructive pulmonary disease"*. MedicineNet. Archived from the original on 28 August 2010. Retrieved 2 September 2010.

Shiber JR, Santana J, (May 2006), *"Dyspnea"*. Med. Clin. North Am. **90** (3): 453–79. doi:10.1016/j.mcna.2005.11.006. PMID 16473100.

Sugarman B, (1983), *Zinc's role in microorganisms is particularly reviewed in"Zinc and infection"*. Review of Infectious Diseases **5** (1): 137–47. doi:10.1093/clinids/5.1.137. PMID 6338570.

Stipanuk, Martha H, (2006), *Biochemical, Physiological & Molecular Aspects of Human Nutrition*. W. B. Saunders Company. pp. 1043–1067. ISBN 978-0-7216-4452-3.

Sagdica A, Senerb O and Bulucua F, (2011*), Oxidative stress status and plasma trace elements in patients with asthma or allergic rhinitis*. AllergolImmunopathol (Madr); 39(4):200-5.

Smith LJ, Shamsuddin M, Sporn PH, Denenberg M, Anderson j, (1997), *Reduced superoxide dismutase in lung cells of patients with asthma*. Free. Radic.Biol.Med; 22:1301-7.

Tripathi, R.M. ; Raghunath, R. and Krishnamoorthy, T.M.,(1997),.*Dietary intake of heavy metals in Bombay city*, India, Sci. of total Environ. 208, 49-159

Thorowgood JC, (November 1873), *"On bronchial asthma"*. British Medical Journal **2** (673): 600. doi:10.1136/bmj.2.673.600. PMC 2294647. PMID 20747287.

Truong-Tran AQ, Carter J, Ruffin R, Zalewski PD, (2001), *New insights into the role of zinc in the respiratory epithelium*. Immunology and Cell Biology; 79:170-177.

TekinD,Sin BA, Mungan D, Misirligil Z, Yavuzer S, (2000), *The anti-oxidative defense in asthma.* J Asthma; 37:59-63.

UrushidateS,MatsuzakaM,OkuboN,lwasakiH,HasebeT,TsuyaR,lwaneK,lnoue R,YamaiK,DanjoK,ashil,UmedaT,AndoS,ltaiK,Nakaj,(2010), *Association between concentration of trace elements in serum and bronchial asthma among Japanese general population Trace Elemement* Med Biol;24(4):236-42.

Urushidate S, Matsuzaka M, Okubo N, Iwasaki H, Hasebe T, Tsuya R, et al, (2010), *Association between concentration of trace elements in serum and bronchial asthma among Japanese general population*. J Trace Elem Med Biol; 24:236-42.

Vos, T et al, (December 2012), *"Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010".* Lancet **380** (9859): 2163–96. doi:10.1016/S0140-6736(12)61729-2. PMID 23245607.

Van Huisstede A, Branushahi GJ, (2010), *Obesity and asthma: co-morbidity or causal relationship?* .Monaidi Arch Chest; 73(3):116-123

WellinghausenN, RinkL, (1998), *the significance of zinc for leukocyte biology*. Leukoc. Biol; 64: 571-7.

Weiss ST, (2005), *Obesity: insight into origins of asthma*, Nature immunology; 6:537-9.

Yawn BP, (September 2008), *"Factors accounting for asthma variability: achieving optimal symptom control for individual patients".* Primary Care Respiratory Journal **17** (3): 138–147. doi:10.3132/pcrj.2008.00004. PMID 18264646. Archived from the original (PDF) on 2010-03-04