Introduction

Asthma, one of the common diseases of childhood, is a chronic inflammatory disease of the respiratory tract characterized by hyperresponsivness of tracheobronchial smooth muscles to variety of stimuli resulting in widespread narrowing of the airway accompanied with increase airway secretion, mucosal oedema and mucus plugging resulting in triad of asthma (dyspnea, wheezes and cough). Genetic and environmental factors are suspected in the pathogenesis of asthma ***(GINA, 2009).***

The increase in the prevalence of asthma over recent decades is associated with environmental factors one of which is nutrition ***(Yilmaz et al., 2011).*** The decreased amount of antioxidants in the diet in recent years has been reported to contribute to the increased incidence of asthma ***(Romieu et al., 2002).*** As inflammatory cells generate and release reactive oxygen species, asthmatic airway are liable to oxidative stress ***(Shanmugasundaram et al., 2001).*** Moreover, inflammatory cells from asthmatic patient generate more reactive oxygen species than those from controls. The extent of oxidative stress will depend on the antioxidant defense available within respiratory tract lining fluid ***(Kelly et al., 1999).***

Antioxidants are physiologic substances that are derived from both endogenous and exogenous sources and that act against oxidant stress. Oxidant-antioxidants balance is essential for normal lung function. Both an increased oxidant and or

decreased antioxidant may reverse the physiologic oxidant-antioxidant balance leading to lung injury. A number of diseases involved the lung such as COPD, Bronchiectasis and bronchial asthma have been associated with a disturbance of these balance ***(Buhi and Meyer, 1996).***

Glutathione peroxidase and superoxide dismutase, the important antioxidant enzymes of the body, contain zinc in their structure ***(Yilmaz et al., 2011).*** Copper (Cu) and zinc (Zn) are present in the structure of superoxide dismutase (SOD), this enzyme converts O2 to H2O.most of CuZn SOD enzyme is found inside cell cytosol while being also found in lysosomes between outer and inner membranes and in the nucleus ***(Aydin et al., 2001).***

Therefore, zinc is an important antioxidant element. It is found in the respiratory tract epithelium, plays a role in the regulation of cellular and humoral immune response and possesses anti-apoptotic and anti-inflammatory features indicating a possible role in asthma pathogenesis and treatment ***(Zalewski et al., 2005).*** It is clear that pround variations in copper and zinc status occur during the course of acute and chronic inflammation, and both zinc and copper are 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 cofactor in several important enzyme systems ***(Fraker et al., 1986).***

Zinc and copper are involved in cell and tissue growth. Changes in patterns of dietary consumption may have contributed to the rise in asthma over the past few decades ***(Seaton et al., 1994).*** World Health Organization suggests using zinc supplementation in children with pneumonia or diarrhea in developing countries ***(World Health Organization, 2004).*** Zinc content of hair shafts reflects the quantity of zinc available to hair follicles during certain time while serum levels are affected by different factors and may not reflect marginal deficiencies ***(Solomons, 1984).***

Aim of the Work

The aim of this work is to study the inter-relationships between asthma clinical grading and degree of control and both hair and serum zinc, copper together with growth status in children with bronchial asthma.

Pediatric Bronchial Asthma

Definition:

Bronchial asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. In susceptible individuals, the inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment ***(Myers and Tomasio, 2011).***

It is an inflammatory disorder of the conducting airways which undergo distinct structural and functional changes, leading to non-specific bronchial hyperresponsivness to variety of stimuli and airflow obstruction that fluctuates over time ***(Holgate et al., 2010).***

Epidemiology

Incidence and prevalence

Worldwide, the prevalence of asthma among children has increased steadily during the last 2 decades. Considerable evidence indicates that there is a significant regional variation in the prevalence of asthma and in the relative weight of risk factors ***(Zedan et al., 2009).*** It is among the commonest chronic conditions in western countries affecting 1 in 7 children and 1 in 12 adults ***(Holgate et al., 2010).***

Rates vary between countries with prevalence between 1 and 18%. It is more common in developed than developing countries ***(GINA, 2011).*** One thus sees lower rates in Asia, Eastern Europe and Africa. Within developed countries it is more common in those who are economically disadvantaged while in contrast in developing countries it is more common in the affluent. The reason for these differences is not well known. Low and middle income countries make up more than 80% of the mortality ***(WHO, 2007).***

Asthma is the leading serious chronic illness of children in the U.S. in 2006, an estimated 7 million children under 18 years, of which 4.1 million suffered from an asthma attack or episode in 2008 ***(O'Reilly et al., 2013).*** While asthma is twice as common in boys as girls, severe asthma occurs at equal rates ***(Bush and Menzies, 2009).*** Global rates of asthma have increased significantly between the 1960s and 2008**,** with it being recognized as a major public health problem since the 1970s**.** Rates of asthma have plateaued in the developed world since the mid-1990s with recent increases primarily in the developing world ***(Robert et al., 2010).***

The prevalence of asthma in males and females in age group 5-10 years was 13% and 12.5% respectively while in age group 11-15 years the prevalence was 8.5-12.5 respectively. However, the prevalence of asthma in males and females among the whole study group was 10.3%- 11.9 % respectively. Asthma prevalence was higher in 5-10 years old students (13%) compared to 11-15 years old students (10%) ***(Ali et al., 2010).***

Prevalence in Egypt

In order to study the recent prevalence of asthma in Egypt, a stratified random sample was chosen from schools of 3 urban cities and 2 large villages located in the Nile Delta region of Egypt through relevant questionnaire. Out of 2720 children, 209 children fitted the diagnosis of asthma including 106 (8.6%) males and 103 (6.9%) females. Thus the overall prevalence of asthma was 7.7% (8% in urban and 7% in rural areas. The asthma prevalence is more evident in urban areas as compared to rural areas. Exposure to environmental tobacco smoke, air pollution and bad housing conditions are important determinant of asthma and may explain the trend of increase asthma in Egyptian school children ***(Zedan et al., 2009)***.

Morbidity and mortality:

Asthma is the main cause of morbidity worldwide. Morbidity and mortality associated with asthma have increased over the last 2 decades. This increase is attributed to increasing urbanization. According to WHO data, asthma death are more than 250000 persons per year allover the world ***(GINA, 2010).***

Despite significant advances in therapy over recent years, there are still more than 71,000 hospital admissions each year and 1400 deaths each year in the United Kingdom directly attributable to asthma. It is estimated that approximately 90% of asthma deaths could have been prevented if the patient, caregiver or health care professional had acted differently ***(GINA, 2010).***

Risk factors for asthma

Factors that influence the risk of asthma can be divided into those causes the development of asthma and those trigger asthma symptoms, some do both. The former include host factors which are primarily genetic and the latter are usually environmental factors ***(Busse and Lemanske, 2001).*** The mechanisms whereby they influence the development and expression of asthma are complex and interactive; for example, gene likely interacts both with other genes and with environmental factors to determine asthma susceptibility ***(Ober, 2005).***

1-Family history:

The strongest risk factor for developing asthma is a family history of atopic disease this increases one's risk of hay fever by up to 5 folds and the risk of asthma by 3-4 folds. Having one parent with asthma increases a child's risk of developing asthma three-fold, while both parents having asthma increases a child's risk by six times ***(Bracken et al., 2002).*** In children between the ages of 3-14 years, a positive skin test for allergies and an increase in immunoglobulin E increase the chance of having asthma ***(Anandan et al., 2010).***

2-Genetic factors:

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, CTLA4, SPINK5, LTC4S, IL4R and ADAM33 among others ***(Elward et al., 2010).*** 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 ***(Ober and Hoffjan., 2006).***

Some genetic variants may only cause asthma when they are combined with specific environmental exposures. An example is a specific **s**ingle nucleotide polymorphism in the CD14 region and exposure to endotoxin, 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 ***(Halabi and Bjoornsdottir, 2009).***

The severity of asthma and response to treatment have also been suggested to be dependent on genetic modulators, such as polymorphism of the B2 receptor found on chromosome 5, which is involved in the bronchodilator response to B-agonists ***(Ligget, 2000).*** TIM1 **(**T cell immunoglobulin mucin 1) has been identified as a susceptibility gene for asthma. New research in mice now

suggest that targeting TIM1 protein might have therapeutic benefit in treating the humanized mouse model of experimental asthma, ameliorating inflammation and airway and hyperresponsivness**.** A gene that encodes a protein responsible for determining whether certain immune cell live or die shows subtle difference in some people with asthma ***(Sonar et al., 2010)***.

Epigenetics is the study of mitotically heritable changes in gene expression that occur without directly altering the DNA sequence. It includes methylation of DNA by the covalent addition of a methyl group to a cytosine residue in a CpG site, as well as the post-translational modification of the amino acid tails of histones by acetylation, phosphorylation, methylation, sumoylation or ubiquitylation ***(Salam et al., 2012).***

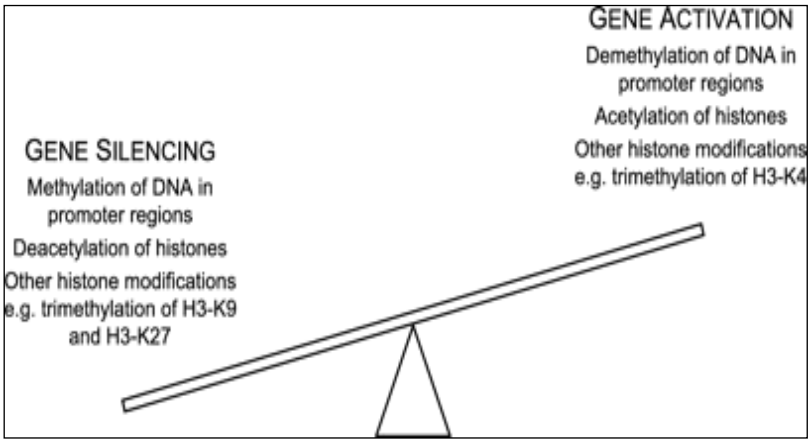

Figure (1): Epigenetic regulation of gene ***(Rakesh et al., 2009).***

Epigenetic regulation of gene expression involves shifting the balance between changes that cause gene silencing or activation. Epigenetic changes are observed across the IL-4 locus in cells exhibiting Th2 differentiation. Stem cells, or cells

that retain phenotypic plasticity, may exhibit both types of marks at key loci. Additional regulation is provided by control mechanisms such as microRNA ***(Rakesh et al., 2009).***

Epigenetic regulation provides an attractive mechanistic explanation for some of the molecular events linking early-life environmental exposures with the subsequent development of disease ***(Miller and Ho, 2008)***. Most epigenetic alterations are believed to occur prenatally and shortly after birth, thus coinciding with the specific time periods when individuals are apparently most susceptible to the effects of environmental exposures and other triggers that induce asthma ***(Baccarelli et al., 2009).***

More studies have examined the influence of environmental exposures on DNA methylation than other epigenetic alterations. Emerging evidence indicates that some of the exposures that have been associated with asthma risk (e.g., maternal smoking, ambient air pollution, diet and microbial exposure during pregnancy) modulate DNA methylation at several CpG sites. In addition, low birth weight and maternal allergy have been found to modulate DNA methylation ***(Salam et al., 2012).***

Recently, the effect of smoking on gene-specific methylation has been reported. In a genome-wide methylation study where Infinium 27K methylation array (Illumina, Inc.) was used, tobacco smoke exposures resulted in differential CpG methylation and placental expression of more genes in the

oxidative stress pathways compared with the expression and DNA methylation in placenta from nonsmokers ***(Suter et al., 2011).***

Air pollution exposure also influences DNA methylation in genes in the immune pathways. In a study among children with asthma, a 3-year average exposure to polycyclic aromatic hydrocarbon was associated with higher methylation of *FOXP3*, reduced Treg function and increased asthma severity ***(Nadeau et al., 2010).***

Many miRNAs are implicated in key patho-physiological aspects of asthma such as immune development and differentiation, and airway inflammation. DNA sequence variations in miRNA genes (including the pri- and pre-miRNAs) have been found to influence miRNA function ***(Duan et al., 2007).*** In an experimental study, stretch stimulation of human airway smooth muscle cells induced transcription of miR-26a and resulted in hypertrophic responses in human airway smooth muscle cells ***(Mohamed et al., 2010).***

3-Age:

Asthma may have its onset at any age. 30% of patients are symptomatic by age of one year, whereas 80-90 % of asthmatic children have their first symptoms before 4-5 years of age. However of all young children who experience recurrent wheezing. Only a minority will go on to have persistent asthma in later childhood ***(Liu et al., 2004).***

In most children asthma develops before age 5 years, and, in more than half, asthma develops before age 3 years ***(Girish et al., 2012).***

4- Sex:

Males have severe airway hyper responsiveness' this may be one factor contributing to the higher prevalence of asthma in boys. Before puberty, the prevalence of asthma is 3 times higher in boys than in girls. During adolescence, the prevalence is equal among males and females ***(Girish et al., 2012).***

Asthma predominantly occurs in boys during childhood, with a male to female ratio 2:1 until puberty, when male to female ratio become 1:1. boys are more likely to decrease symptoms by adolescence ***(Townsend et al., 2012).***

5- Residence and race:

Asthma is more common in urban compared to rural communities and is more affluent than in poorer communities. All children living in an urban setting, regardless of race or income, are at increased risk of asthma ***(Aligene et al., 2000).*** Evidence has rapidly accumulated to suggest that growing up on a farm may reduce the risk of developing atopic disease ***(Von Hertzen and Haahtela, 2004).***

The pollution in urban environments can decrease lung growth and function by narrowing airways, the pathogenesis of the effect of pollution on the airways may be caused by enzymes from dust mites that harm the lining of the airway and cause

narrowing. Urban children may have underlying air disease secondary to the pollution even though they may not have any clinical manifestation of disease ***(Malik et al., 2012).***

6- Obesity:

Obesity and asthma are both common complex traits responsible for substantial morbidity in the developed world. The consistency of the relationship between obesity and asthma, and its temporal and dose-response association, suggest that the obesity-asthma link is causal. All of the existing epidemiological studies show a consistent, positive association of obesity with both incidence and prevalence of asthma in children and adults. In obese humans, even in the absence of any overt inflammatory insult, there is chronic low grade systemic inflammation. The origin of this inflammation appears to be, at least in part of the adipose tissue itself because expression of a variety inflammatory genes is up regulated in adipose tissue derived from obese human ***(Lang, 2012).***

Worldwide there are now more than 40 million overweight or obese children below the age of 5**.** This fact makes the appreciation for the relationships between obesity, nutrition, and lung disease important for any clinician who cares for children in the fields of allergy, immunology, or pulmonology ***(WHO, 2011).***

One hypothesis for the obesity–asthma link is that obesity-related systemic inflammation primes the lung for exaggerated responses to environmental triggers, leading to asthma-like

symptoms. Adipose tissue releases pro-inflammatory 'adipokines' (e.g., adiponectin, IL-6, TNFα, leptin) that influence multiple organ systems, including the lung's responses to external stimuli ***(Lang, 2012).*** Therefore, obesity-related inflammation may play a role in the development and severity of asthma. Obesity-related comorbidities such as gastroesophageal reflux and sleep apnea have yet to be conclusively linked to increased asthma risk in children ***(Michelson et al., 2009).***

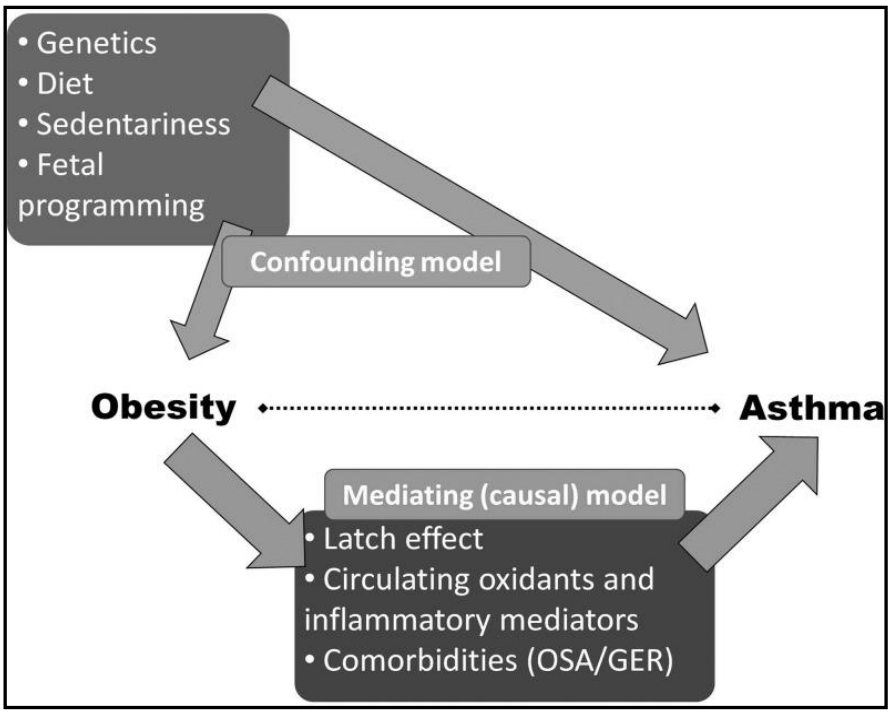

Figure (2): Potential models of association between obesity and asthma. OSA: obstructive sleep apnea; GER: gastroesophageal reflux ***(Lang, 2012)***.

7- Diet:

Infants fed formulas of intact cow's milk or soy protein have a higher incidence of wheezing illnesses in early childhood compared with those fed breast milk ***(Friedman and Zeiger, 2005).***