Study Of Glutathione S - Transferase M1 Genotype, Maternal Smoking During Pregnancy & Environmental Tobacco Smoke On Pediatric Asthma

Thesis
Submitted for partial fulfillment of the master degree in pediatrics

BY

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2005

Acknowledgment

I would like to express my deepest gratitude and cordial thanks to prof. Dr. Maged Ashraf Abdel Fattah prof. of pediatrics-university of Ain-Shams, For his knowledge, experience, advice and continuous supervision throughout the whole work. It certainly takes me a life time to say thank you.

I also wish to express my deep appreciation to Dr. Malak Aly Shaheen, Lecteurer of pediatrics-university of Ain-Shams, for everything she did for me, her generous helping and kind guidance given throughout the course of this work.

I wish to express my sincere appreciation to Prof. Dr. Naser Elhawary, fellow doctor of pediatric genetics-university of Ain-Shams, for his invaluable help, unfailing advise and continuous guidance during the study of the genetic aspects of this work.

To Dr. Hosam Abdel Hamid, I would like to express For this gentleman my sincere gratitude.

Last but not least, to my family who have been supportive and understanding in every way they could.

Finally, to my daughter……..

List of Abbreviations

ACEIs : Angiotensin converting enzyme inhibitors.

AHR : Airway hyper-responsiveness.

AP-1 : Activator protein1.

BAL : Broncho-alveolar lavage.

CAMP : Childhood Asthma Management Program.

DPI : Dry Powder Inhaler.

EPO : Eosinophil Peroxidase.

FeNO : Fractional exhaled concentration of nitric oxide.

FEV1 : Forced Expiratory Volume.

GM-CSF: Granulocyte Monocyte Colony Stimulating Factor.

GINA : Global Initiative of Asthma.

LABAs : Long Acting B agonists.

MPO : Myelo-peroxidase enzyme.

MIP-1a : Macrophage Inflammatory Protein-1a.

NF-B : Nuclear Factor B.

NAEPP : National Asthma Education and Prevention Program.

PMDI : Pressurized Metered Dose Inhaler.

PDE : Phosphodiesterase enzyme.

PC : Provocative concentration.

PD : Provocative dose.

PLA2 : Phospholipase A2.

RNS : Reactive Nitrogen Species.

RATES : Regulated on Activation normal T cell expressed and secreted.

TGF-B : Transforming Growth Factor-B

TBARs : Thio-Barbituric Acid Reactive products.

YAC : Yeast Associated Clone.

Introduction

Over the last 25 years, asthma has emerged as an increasingly important public health problem ***(Anderson et al, 2000).*** The rapid rise in childhood asthma prevalence suggests that the environmental factors and the genetic factors are both necessary for the eiteology ***(Cookson and Moffatt, 2000).***

Full spectrum of exposures and susceptibility genes involved in the pathogenesis of asthma and wheezing have yet to be established ***(Samet, 2000).*** Fetal exposure to maternal smoking may contribute to the occurrence of asthma and wheeze ***(Gilliand et al, 2002).*** Airway responsiveness was increased in infants with a family history of asthma, parental smoking, or both as compared with infants with no family history of asthma or smoking ***(Syoung et al, 1999).***

Glutathione S-transferase M1 enzyme is involved in detoxification of reactive tobacco metabolic intermediates ***(Hayes and Strange, 2000).*** It also may play a role in asthma and wheezing occurrence because antioxidant pathways are involved in asthma pathogenesis ***(Ivashchenko et al, 2001).*** Passive smoking was the risk factor for the un-favourable course of bronchial asthma for patients carrying GSTM1(-) genotype ***(Vavilin, 2001),*** while among children with GSTM1(+) genotype passive smoking either in utero or after birth is not associated with asthma or wheezing ***(Frank D. Gilliand, 2000).***

Aim of the work:

The aim of this work was to estimate the role of Glutathione S-transferase M1 (GSTM1) genotype with passive smoking either intra-uterine or after birth in the prevalence of asthma and wheezing during childhood.

We used life time tobacco smoke exposure histories, parental reports of wheezing and physician diagnosed asthma and blood DNA preparation for GSTM1 presence and expression to assess the relationships of GSTM1 genotypes, maternal smoking during pregnancy and childhood exposure to environmental tobacco smoke with asthma or wheezing.

Bronchial Asthma in Childhood

Definition of asthma:

Asthma can be defined as chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells ***(Nadel and Busse, 1999)***.

In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing particularly at night or in the early morning. These episodes are usually associated with wide spread but variable airflow obstruction. Broncho-constriction is reversible either completely or partially spontaneously or with treatment (***Fishman et al, 2000).***

Classification of asthma:

For many years asthma has been classified as extrinsic or intrinsic depending on the suspected role of allergens as etiologic factor.

The development of atopy is associated with increased risk of persistence and severity of asthma.

By convention atopic subjects are considered to have extrinsic asthma while non atopic subjects have intrinsic asthma.The presence of skin test sensitivity to allergens does not itself indicate that allergens are important triggers of asthma, since a large percentage of skin sensitive persons report no allergic symptoms ***(Godfrey, 1998)*** Moreover, exercise and viral respiratory infections may play a more important role than allergens as triggers of symptoms in some atopic subjects.

Classifying the patient as having intrinsic asthma is a problem also since it implies that all possible allergens in the environment have been excluded as etiologic factors (***Fishman et al, 1999).***

Prevalence of pediatric asthma:

Asthma is the most common chronic disease in children, it may affect up to 35% of the population in developed countries ***(Soussan et al, 2003)***.

The prevalence of asthma varies worldwide possibly because of different exposure to respiratory infections, indoor and outdoor pollution ***(Von mutius, 2000).***

In Egypt 23.5% of wheezy infants continued to be asthmatics but the incidence of asthma among school children aged from 5 to 15 years old was found 8.2%. ***(Elhefny et al, 1999).***

Figure (A): Prevalence of childhood asthma throughout the world (Kercsmar, 1998)

Pathogenesis of asthma:

Asthma is a multi-factorial complex disease of which several factors are involved in its pathogenesis, these include:

1-Airway obstruction:

Airway obstruction is responsible for the clinical manifestations of asthma as wheezing, dyspnea, and cough ***(Sheffer, 1998).***

Obstruction that is determined by the diameter of airway lumen can be influenced by several factors including airway smooth muscle contraction, edema of the wall, cellular infiltration and mucous secretions ***(Kaliner, 1998).*** Airway narrowing may worsen gradually and persists despite therapy, but it may also develop abruptly and produce acute respiratory insufficiency ***(Sheffer, 1998).***

2-Bronchial hyper-responsiveness:

Bronchial hyper-responsiveness is a characteristic feature of asthma and correlates to the severity of the disease. It is considered as an exaggerated broncho-constriction response to a variety of stimuli that produce little or no response in healthy subjects ***(Barnes, 1996).***

The precise mechanisms are not clear although many theories have been postulated. ***Martin, (1996)*** stated that the primary problem might start with one of the following mechanisms:

1- Neuro-humoral control, mainly the autonomic nervous system.

2- Bronchial smooth muscles responding excessively to normal concentrations of mediators.

3- Cellular dysfunction with increased mediator release leading to inflammatory response and excessive constriction of normal muscles.

3-Inflammation:-

Clinical observations have provided evidence for considering inflammation in the basis of asthma such as, the association of asthma with atopy and the presence of eosinophils in sputum and blood of patients during an active asthma attack ***(Halogate, 1996).***

Post-mortum examinations have shown presence of mucus plugs, serum proteins, inflammatory cells, and depris in both large and small airways ***(National institute of health, 1997).***

The airways of asthmatic patiens are infilterated with a number of different inflammatory cells that in turn secrete a large number of mediators ***(Lessof, 1996).***

Inflammatory cells in the asthmatic airways:-

Many types of cells may be involved in asthma:

Mast cells:

Are triggered by allergens to release the contents of their granules including mediators such as histamine and to synthesize lipid mediators e.g leukotriens and prostaglandins. These mediators produce bronchial smooth muscle contraction, increased capillary permeability leading to edema, increased bronchial mucus secretion, chemotaxis for other inflammatory cells e.g eosinophils, neutrophils ***(Robinson et al, 1996).***

Approximately 80% of asthmatics are atopic, forming IgE antibody on exposure to common allergens. Specific IgE binds to high affinity receptors on mast cells in the respiratory tract and a specific allergen then cross links bound IgE, this process leads to an influx of calcium ions, release of performed mediators (e.g Histamine) and synthesis and release of lipid mediators (e.g Prostaglandins, Leukotreins).

Mast cells may induce the immediate broncho-constrictor response to allergens but they are not responsible for either late responses or airway hyper-responsiveness ***(Barnes, 1998).***

Figure (B): Stimulus (antigen) initiates mast cell activation with release of mediators which promote bronchoconstriction and air way inflammation (Busse and Parry, 1998)

Eosinophils:

Are key effector cells in asthma, they contain basic granule protiens (e.g major basic protein, eosinophil cation protein, eosinophil peroxidase, eosinophil derived neurotoxin) which are rich in Arginine.

These proteins damage respiratory epithelial cells at the concentrations found in sputum from asthmatics. Eosinophils also produce lipid mediators (e.g platelet activating factor, leukotreine C4) which may provoke broncho-constriction and airway edema.

Eosinophils are activated by specific cytokines particularly IL-5 and have receptors for IgA and IgE on their cell surface ***(Robinson et al, 1996).***

Table (A): Eosinophil Granules.

Type of granule	***Protein constituents***	***Properties***
Primary	Charcot-Leyden crystal protein	Lysophospholipase
Secondary	- Major basic protein (MBP)	- Cytotoxic (Epithelium)
	- Eosinophil Cationic Protein (ECP)	- Promote histamine release from mast cell and basophils
	- Eosinophil-derived neurotoxin (EDN)	- Cytotoxic (Epitheleum)
		- Damages: myelinated neurons

(Busse and Parry, 1998).

Lymphocytes:-

There is evidence that a subpopulation of T-helper cells exists and that asthma may be a TH2-like disease ***(Nadel and Busse, 1998).***

Mucosal biopsies showed airway epithelial damage even when asthma was mild (***Beasly et al, 1996***).These findings suggested that the principle cellular infilterates in asthma included eosinophils and activated lymphocytes ***(Azzawl et al, 1996).***

Macrophage:-

Macrophages are professional antigen presenting cells that interact with and stimulate T-lymphocytes proliferation and cytokine production.

After antigen challenge, the percentage of macrophages in airways exceeds that of eosinophils, neutrophils and lymphocytes. Furthermore, in contrast to the high affinity of IgE receptors on mast cells and basophils, subpopulation of macrophages express low affinity IgE receptors.These receptors are increased in patients with atopic asthma and antigen IgE dependent mechanisms may lead to the release of inflammatory mediators by these cells ***(Busse and David, 1998***).

Basophils:-

Basophils are bone marrow derived cells that originate from myeloid precursors and contain meta-chromatic granules. They are activated by the interaction of membrane high affinity IgE receptors with antigen.

Complement fragments (C5a and C3a) and histamine releasing factors can also activate basophil through IgE dependant perturbation of the cell membrane, they release

pereformed mediators like histamine.They also generate (LTC4, LTD4, LTB4, PAF) and a number of cytokines including IL4, IL5. Basophil has been found in the sputum and blood of asthmatics during clinical exacerbations of the disease ***(Busse and David, 1999).***

Epithelial cells:-

The airway lumen is lined by epithelial cells which contain secretory epithelial cells (mucus goblet and serous cells) which may contribute to the excessive mucus production in asthma when stimulated by IL-4 or substance P.

Epithelial cells derived factors include lipid mediators PGE2, the potent broncho-constrictor (endothelin), Transforming growth factor B (TGF-B), Regulated on activation normal T cell expressed and secreted (RANTES), IL-11, macrophage inflammatory protein -1a (MIP-1a) and granulocyte monocyte-colony stimulating factor (GM-CSF) ***(Busse and David, 2000).***

Mediators In Asthma

Inflammatory Mediators In IgE Related Asthma.

1-Histamine:

A rise in plasma histamine concentrations is seen minutes after allergen inhalation (***Phillips, 1999***). Mast cells are the main source of this mediator as demonstrated by the increased levels of histamine seen in broncho-alveolar lavage from stable asthmatics, together with increased mast cell activation ***(Wardlaw, 1998).***

Histamine induces broncho-spasm in asthmatics through H1, H2 and H3 receptors and elicits activation of sensory reflexes and vasodiltation of Bronchial vessels through H1 receptors ***(Barnes, 2000).*** It mediates mucus secretion and vasodilatation through H2 receptors, whereas H3 receptors are responsible for the regulation of cholinergic and sensory nerve function.

2-Prostaglandins:

They are one of a group of local mediators believed to play a role in the asthmatic inflammatory process.

After immunologic or non-immunologic stimulation cytosolic phospholipase A2 (PLA2) is activated causing cleavage of arachidonic acid from the cell membrane phospholipids.

Arachidonic acid is then metabolized in either of two pathways.The cyclo-oxygenase pathway leading to the formation of prostaglandins, thromboxane and prostacyclin and the 5 lipo-oxygenase enzyme system pathway to from leukotreins ***(Dixen et al, 1995).***

3-Leukotrienes:

They are biologically activate fatty acids derived from the oxidative metabolism of arachidonic acid ***(Kaiser, 1995***) and can be produced by mast cells. Epithelial cells and macrophages have also been shown to produce leukotriens. LTC4, LTD4 and LTE4 bind to the same receptor (LT1) on the bronchial smooth muscles ***(Gardiner et al, 1999).***

Leukotriene C4 is detectable in significant amounts in broncho-alveolar lavage fluid of patients with chronic stable asthma ***(Wardlaw , 1998).***Both LTD4 and LTE4 have been reported to increase airway hyper- responsiveness ***(Caughy, 1998).***