INTRODUCTION

Allergy is a state of sensitization of the immune system, a disease mechanism, rather than a specific disease entity ***(Krishnan and Dolen, 2012)***.

Programming of disease in fetal and early postnatal life has been hypothesized to be an important mechanism for atopic diseases ***(Jones et al., 2000)***.

Peripheral blood mononuclear cell responses to allergen can be detected from as early as 22 weeks gestation ***(Jones et al., 1996)***. While there is universal priming to allergens, enhanced responsiveness is associated with a higher risk of allergy. Furthermore, a number of studies have shown differences in cytokine profiles from allergen and mitogen stimulated peripheral blood mononuclear cells in those neonates destined to be allergic ***(Tang et al., 1994)***. This suggests that fetal life is a critical period for development of atopic diseases and may be an important "window of opportunity" for prevention of disease ***(Tang et al., 1994)***.

Many immune cells, including neutrophil, monocytes, macrophages and lymphocytes, express Fas (CD95) on their surface ***(Stuart et al., 1997),*** Fas Ligand (FasL or CD95L) is a type II transmembrane protein that belongs to the tumor necrosis factor (TNF) family. Its binding to its receptor produces apoptosis (programmed cell death). Fas ligand

/receptor interactions play an important role in the regulation of the immune system ***(Trautmann et al., 2000).***

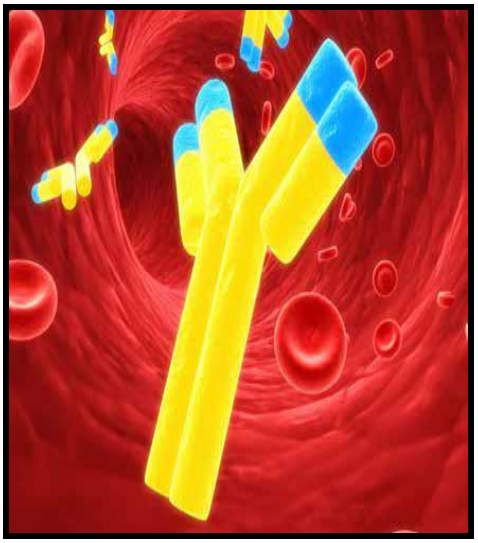
S.Fas Ligand has been correlated to allergic diseases in several studies as atopic dermatitis ***(Trautmann et al., 2000)***, asthmatic and allergic rhinitis in children ***(Mezei et al., 2006; Zakrzewska et al., 2003)***, however, whether s.FasL can serve as a predictor for allergy still under trials.

AIM OF THE WORK

This prospective cohort study aims to assess soluble Fas Ligand as a marker of fetal programming and early predictor of feto-maternal aeroallergen sensitization in relation to in-vivo IgE assessment and peripheral blood mononuclear cells.

Chapter One

ALLERGY



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The term allergy represents the clinical expression of IgE mediated allergic diseases that have a familial predisposition and that manifest as hyper-responsiveness in target organs such as the lung, skin, gastrointestinal tract, and nose ***(Akdis and Sicherer, 2016)***.

Prevalence and Epidemiology

There has been a significant increase in the prevalence of allergic diseases during the last few decades. This increase is attributed to changes in environmental factors (exposure to tobacco smoke, air pollution, indoor and outdoor allergens, respiratory viruses, obesity and perhaps a decline in certain infectious diseases [hygiene hypothesis]) ***(Akdis and Sicherer, 2016)***.

The growing worldwide burden of allergic rhinitis, asthma and atopic eczema has been properly defined as the "allergy epidemic" ***(Matricardi et al., 2002).***

Respiratory allergies (allergic rhinitis and asthma) appeared first among the high social class, then spread within the middle class and finally affected also the disadvantaged. Following a similar pattern, respiratory allergies and atopic eczema are nowadays on the rise in developing countries, especially in the urban areas ***(Matricardi et al., 2002)***.

Many researches are conducted in Egypt to study the prevalence and severity of allergic diseases among Egyptian

children and found the prevalence of childhood bronchial asthma in Menoufiya governorate was 6.5% ***(El Mashad et al., 2016)***, and overall prevalence in in Nile Delta was 7.7 % ***(Zedan et al., 2009)***, 6.2 % among prepatory school children in Assuit ***(Abdallah et al., 2012)***, and 26.5 % among children aged 11-15 years ***(George et al., 2005).***

Age and sex:

In the "Atopic March" infantile eczema and food allergy precede the onset of allergic airway disease (rhinitis and asthma). However, there are individuals with isolated allergic airway disease (for example hay fever) starting later in life at school age without any signs of other atopic disease during infancy and preschool age. Equally, there are children with infantile eczema without any signs of food or inhalant allergy. Furthermore, remission and relapse of disease entities are possible at any time ***(Lau, 2014)***.

The incidence of atopic eczema and food allergy to cow`s milk, hen`s egg, wheat and soy is highest during the first 2 years of life in childhood, however there is a second peak of new onset of atopic eczema in puberty for females ***(Lau, 2014)***.

Male Sex is a risk factor for asthma in pre-pubertal children in the US. 15 % of boys compared to 13 % of girls have asthma. Boys with asthma are more likely to grow out of their asthma during adolescence than girls. Female sex is a risk factor for persistent asthma ***(Sing, 2014; Liu et al., 2016).***

Risk factors for allergy

Genetic and environmental factors likely interact in a complex manner to produce both disease susceptibility and disease expression.

1- Genetic basis of atopy

The development of allergic disease is complex and not fully understood, with both environmental and genetic components.

The hygiene hypothesis, which attempts to explain the increasing prevalence of allergy, is based on the immuno-modulation potentially induced by bacterial and viral infections early in infancy, which can later modify the chances of developing an allergic response. However, environmental factors do not fully explain the increase of allergic disease ***(Liu and Leung, 2006).***

The first study to identify the heritability of allergy found that 48.4% of a group of 621 sensitized individuals had a family history of sensitization to common environmental allergens, compared with only 14.5% of the control group of 76 non-sensitized individuals ***(Cooke and Vander, 1916).*** A few years later, the term atopy was first coined, to mean inherited hypersensitivity ***(Coca and Cooke, 1923).*** Heritability estimates for allergic disease vary, but have been described as high as 95%

for asthma, 91% for AR and 84% for AD (reviewed ***(Ober and Yao, 2011).***

More recent studies in twins provide further evidence for allergy heritability, due to the higher levels of concordance for allergic phenotypes in monozygotic, compared with dizygotic twins, where atopy heritability was estimated between 50 and 84 % ***(Bazaral et al., 1974; Clarke et al., 2000; Andiappan et al., 2001).***

Asthma and atopy are example of complex disorders that show a clear hereditary component, however the mode of inheritance does not follow any simple Mendelian pattern. Furthermore, unlike single-gene disorders, they tend to have an extremely high prevalence ***(Collins et al., 2016).***

GWAS is the current method of choice for gene identification in complex disorders, which involves examining association with typically 500 000+ common (> 5% frequency) polymorphisms spanning the entire genome in cases and controls with very stringent statistical thresholds, e.g. $P < 5 X10^{-8}$.

Table (1): Showing susceptibility genes for asthma, allergic rhinitis and atopic dermatitis recently identified by Genome Wide Association Studies (GWAS) ***(Portelli et al., 2014)***

Gene(s)	Chrs	Association	Potential Function	GWAS
IL6R	1q21	Asthma	Regulatory T-cell function, T-cell differentiation	1
DENND1B	1q31		Memory T-cell functions	2†
IL1RL1	2q11		IL-33 receptor-recruitment of inflammatory cells	3–5‡, 6†, 11
PDE4D	5q12		Cell signalling, inflammation, ASM function	7
TSLP	5q22*		Activates dendritic cells, Th2 immune responses	3
SLC22A4/RAD50/IL13	5q31		Organic cationic transporter/DNA repair/Th2 cytokine	6†, 8‡
HLA-DRA/DRQ	6p21*		T-cell responses/many additional genes in region	3, 9, 11
CDHR3	7q22		Epithelial polarity, cell–cell contact and differentiation	6†
IL33	9p24		Recruitment/activation of inflammatory cells	3, 4, 6†
C11orf30/LRRC32	11q13		Regulates gene expression, epithelial barrier/regulatory T-cell function	1
SMAD3	15q22		TGF-b signalling intermediate, fibrosis	3
ORMDL3/GSDMB	17q21		Sphingolipid synthesis/cell apoptosis	3, 5‡, 6†, 10§
IL2RB	22q12		Binds IL-2/IL-15, lymphoid cell differentiation	2, 3
C11orf30/LRRC32	11q13	Allergic Rhinitis	Regulates gene expression, epithelial barrier/regulatory T-cell function.	12
FLG, LCE3A	1q21*	Atopic Dermatitis	Epidermal differentiation and structure	13, 14, 15, 16 ¶
IL1RL1, SLC9A4	2q12*		IL-33 receptor/sodium-hydrogen exchanger	15¶

Gene(s)	Chrs	Association	Potential Function	GWAS
IL2-IL21	4q27		T-cell survival/B cell proliferation and IgE production	15¶
SLC22A4/RAD50/IL13/KIF3A	5q31*		Organic cationic transporter/DNA repair/Th2 cytokine/cilia protein	14, 15, 16 ¶
HLA-B (BAT1/TNXB/CREBL1)	6p21		T-cell responses/many additional genes in region	16
PRR5L	11p13		Cellular apoptosis	15¶
OVOL1	11q13*		Development and differentiation of epidermal/epithelial Tissues	14
C11orf30/LRRC32	11q13*		Regulates gene expression, epithelial barrier/regulatory T-cell function	13, 15¶, 16
CLEC16A	16p13		Inflammatory cell function (ITAM receptor)	15¶
ZNF652	17q21		Transcriptional repressor in epithelial cancers	15¶
ADAMTS10/ACTL9	19p13*		Extracellular matrix cleavage/epithelial morphology	14
TNFRSF6B	20q13*		Decoy receptor, immunomodulation of T cells	14, 15¶

*Association also observed in the Asian population GWAS, ¶Using Immunochip array, †Childhood severe asthma with exacerbation; ‡Severe Asthma and §Childhood onset asthma.

IL, interleukin; IL6R, IL-6 receptor; DENND1B, Denn/madd domain-containing 1b; IL1RL1, IL-1 receptor like 1; PDE4D, phosphodiesterase 4d, cAMP-specific; ASM, airway smooth muscle; TSLP, thymic stromal lymphopoietin; Th2, T helper 2; SLC22A4, solute carrier family 22 (organic cation/ zwitterion transporter), member 4; RAD50, S. cerevisiae, homolog of (DNA repair); IL13. IL-13; HLA-, Human Leukocyte Antigen, class II; CDHR3,cadherin-related family member 3; IL33, IL-33; C11orf30, chromosome 11 open reading frame 30; LRRC32, leucine rich repeat containing 32; SMAD3, mothers against decapentaplegic drosophila homolog 3; TGF-b transforming growth factor beta; ORMDL3, orm1-like protein 3; GSDMB, gasdermin b; IL2RB, IL-2 receptor beta; Ig, immunoglobulin; FLG, filaggrin; LCE3A, late cornified envelope 3A; SLC9A4, solute carrier family 9, subfamily A (NHE4, cation proton antiporter 4), member 4; IL2-IL21, interleukin 2/21; KIF3A, kinesin family member 3A; PRR5L, proline rich 5-like; OVOL1, ovo-like 1(Drosophila); CLEC16A, C-type lectin domain family 16, member A; ZNF652, zinc finger protein 652; ADAMTS10, ADAM metallopeptidase with thrombospondin type 1 motif, 10; ACTL9, actin-like 9; TNFRSF6B, tumour necrosis factor receptor superfamily, member 6b, decoy.

Genetic regulation of response to therapy: pharmacogenetics

Genetic variability may not only play a role in influencing susceptibility to allergy but may also modify its severity or influence the effectiveness of therapy ***(Bonnelykke et al., 2014)***. In asthma, patient response to drugs such as bronchodilators, corticosteroids and anti-leukotrienes is heterogeneous ***(Szefler et al., 2005)***.

One of the most investigated pharmacogenetic effects has been the effect of polymorphisms at the gene encoding the β2-adrenergic receptor, *ADRB2*, on the bronchodilator response to inhaled short and long-acting β agonists ***(Reihsaus et al., 1993).***

2- Epigenetics of allergy

Epigenetics is the study of heritable changes in gene activity that are not caused by changes in the DNA sequence but includes modifications to the structure supporting the DNA called histones.

Histone modification (adding or removing acetyl groups) determines DNA packaging and the cell's ability to access and read the associated sequence ***(Chinthrajah et al., 2014).***

DNA can also be modified directly by adding a methyl group to cytosine bases, which may restrict access to the DNA for transcription into mRNA; finally, gene expression can be

regulated at the post-transcriptional level by microRNAs, which can further modify mRNA transcripts and histones to alter the expression of genes ***(Chinthrajah et al., 2014)***.

Many environmental factors are thought to regulate gene expression through these mechanisms and studies are ongoing to identify specific exposures and pathways of effect.

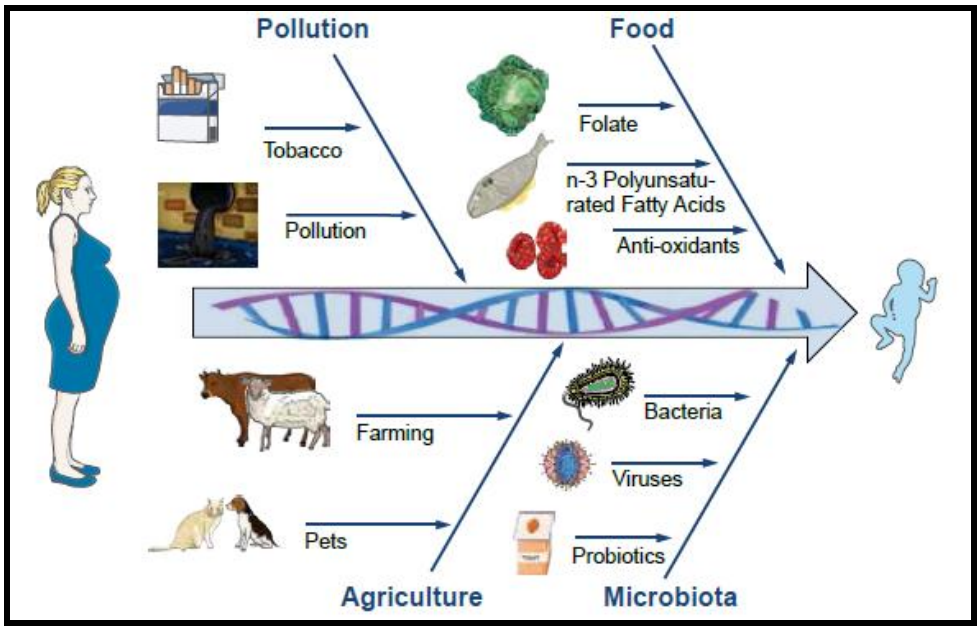

Fig. (1): Environmental exposure constituting prenatal epigenetic changes to immunity ***(Chinthrajah et al., 2014)***.

Environmental factors such as pollution, food, agriculture and microbiota have been shown to have significant implications on early immune programming and development. Prenatal exposure to these elements is postulated to cause epigenetic changes to genes and signaling pathways of fetal immunity that may have lasting effects during the child's life (Fig. 1).

One of the main characteristics of epigenetic changes is that it is passed on to daughter cells with each cell division so it may have a long lasting effect on cell function.

The importance of epigenetics in determining allergic phenotype was illustrated in identical twin studies, in which one twin suffered from asthma and the other did not. Asthmatic twins were found to exhibit DNA methylation patterns that differed from their healthy counterpart. Most notably, they had increased methylation and decreased expression of the FOXP3 gene, which is important for the anti-inflammatory function of T regulatory cells ***(Kohli et al., 2012).***

Furthermore, there is evidence that some epigenetic marks can be transmitted from parents to children trans-generationally, with cumulative effect over multiple generations. This is particularly relevant when considering the epidemiology of allergic disease, which seems to be amplified with subsequent generations ***(Chinthrajah et al., 2014)***.

Considering that the increase in the prevalence of allergic diseases cannot be ascribed solely to genetic factors, most studies on development of allergic diseases have focused on the influence of in environmental factors, e.g. early feeding (breastfeeding vs. cow's milk formula), diets/nutrients, exposure to allergens, tobacco smoking, pollution, farm vs. urban environment, and infectious load. Also age and dose and duration of exposure are important synergistic effects ***(Host, 2014).***

3. Environmental factors

a) Indoor allergen

The primary indoor allergens include allergens from house dust mite, pets such as dogs and cats, molds, and pests such as cockroach and rodents. Indoor allergens have diverse biologic functions and may be enzymes, lipid-binding proteins, ligand-binding proteins, structural proteins or regulatory proteins. Allergens promote T cells to differentiate along the Th2 pathway to produce IL-4 and IL-13 and to initiate isotype switching to IgE.

Biologic functions of allergens, such as proteolytic enzyme activity or other adjuvant-like effects, can enhance IgE responses, damage lung epithelium and cause allergic inflammation ***(Sheehan and Phipatanakul, 2016)***.

Childhood asthma is more closely linked to allergic sensitization and allergen exposure than adult asthma. Sensitization to indoor allergens likely occurs earliest in life as young children have been shown to have higher rates of sensitization to indoor allergens as compared to outdoor allergens ***(Sheehan et al., 2010).***

b) Outdoor risk

Outdoor aeroallergenic particles include intact pollen grains or spores, as well as cell fragments and submicronic particles.