

**Study The Role of Natural Killer T Cells
in Asthmatic Children.**

Thesis

Submitted for Partial Fulfillment
of
Master Degree in Pediatrics
By

Shawky Abd Elaziz Zaki

M.B., B.CH, 2000, Ain Shams University

Under Supervision of

Professor Dr. Karima Ahmed Abd Elkhalek

Professor of Pediatrics
Faculty of Medicine – Ain Shams University

Dr. Malak Ali Shaheen

Assistant Professor of Pediatrics
Faculty of Medicine – Ain Shams University

Dr. Abeer Atia Sad Eldeen

Lecturer of Clinical Pathology
Faculty of Medicine – Ain Shams University

Faculty of Medicine – Ain Shams University

CAIRO 2009

Acknowledgment

*First of all, I would like to thank **Allah** the merciful and compassionate for making all this work possible and granting me with the best teacher, family, friends and colleagues that many people would wish and dream of having.*

*I am honored to have **prof. Dr. Karima Ahmed Abd El khalek** professor of pediatrics, Faculty of Medicine, Ain Shams University, as a supervisor of this work. I am greatly indebted to her for her valuable supervision, generous help, kind advice, constructive opinions and kind guidance.*

*Words can not express my deep gratitude and sincere appreciation to **Ass. Prof. Malak Ali Shaheen** Assistant professor of pediatrics Faculty of Medicine, Ain Shams University, for her great help and effort to make this work possible. I was very honored to work with her.*

*I do feel greatly indebted to **Dr. Abeer Atia Sad Eldeen** Lecturer in clinical pathology, Faculty of Medicine, Ain Shams University, for her great effort in doing the work of this study and assisted me in most of the practical work.*

Shawky Abd elaziz zaki

Study The Role of Natural Killer T Cells in Asthmatic Children.

Thesis

Submitted for Partial Fulfillment

of

Master Degree in Pediatrics

By

Shawky Abd Elaziz Zaki

M.B., B.CH, 2000, Ain Shams University

Under Supervision of

Professor Dr. Karima Ahmed Abd Elkhalek

Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Dr. Malak Ali Shaheen

Assistant Professor of Pediatrics

Faculty of Medicine – Ain Shams University

Dr. Abeer Atia Sad Eldeen

Lecturer of Clinical Pathology

Faculty of Medicine – Ain Shams University

Faculty of Medicine

Ain Shams University

CAIRO 2009

دراسة دور الخلايا تي الليمفاوية القاتلة الطبيعية في مرضى الربو الشعبي من الأطفال

رسالة

مقدمة من الطبيب /شوقي عبد العزيز ذكى

بكالوريوس الطب والجراحة 2000

طب عين شمس

توطئة للحصول على درجة الماجستير في طب الأطفال

تحت إشراف

ا.د / كريمة أحمد عبد الخالق

أستاذ طب الأطفال

كلية الطب – جامعة عين شمس

د / ملك على شاهين

أستاذ مساعد طب الأطفال

كلية الطب – جامعة عين شمس

د / عبير عطية سعد الدين

مدرس الباثولوجيا الاكلينيكية

كلية الطب – جامعة عين شمس

القاهرة 2009

List of contents

<i>Title</i>	<i>Page No</i>
Introduction	1
Aim of work	2
Review of literature.....	3
Part 1: Pediatric bronchial asthma	3
- Definition & Risk factors.....	3
- Etiology of asthma	19
- Diagnosis of asthma	28
- Management of asthma	35
- Asthma score system.....	40
Part 2: Natural killer T cells	43
Part 3: Role of NKT cells in asthma	53
Subjects and methods.....	63
Results	71
Discussion	93
Summary and conclusion	101
Recommendations	106
Appendix	107
References	108
Arabic summary	--

List of Figures

<i>Figure</i>	<i>Subject</i>	<i>Page</i>
(1)	Prevalence and mortality from asthma.	4
(2)	Pathogenesis of asthma; Interaction between host factors and environmental exposures	19
(3)	Asthma inflammatory cascade summary of proposed mechanisms.	21
(4)	The NKT system as a functional bridge between innate acquired immunity.	44
(5)	Inflammatory cells as (NKT) cells moves into lung produce asthma even Th ₂ cells are absent.	44
(6)	Action of NKT cells and activation by α -Gal-Cer.	52
(7)	Interaction between polarized Th ₂ responses, NKT cells and regulatory T cells.	53
(8)	MIR Spirobank used by this study to measures pulmonary function tests.	67
(9)	Flowcytometry system.	68
(10)	Sex distribution of studied children.	71
(11)	Age distribution of studied children.	72
(12)	Severity distribution of asthmatic children.	73
(13)	Statistical comparison between cases& controls as regards absolute eosinophil count in blood.	76
(14)	Statistical comparison between cases &controls as regards absolute N K T cells count in blood.	77
(15)	Statistical comparison between cases &controls as regards N K T % of WBCs in blood.	78

List of Figures (Cont.)

<i>Figure</i>	<i>Subject</i>	<i>Page</i>
(16)	Correlation between total leucocytes count and absolute count of eosinophil cells in blood in cases group.	89
(17)	Correlation between FEV1% and absolute count of NKT cells in blood in cases group.	90
(18)	Correlation between FEV1% and absolute count of eosinophil cells in blood in cases group.	91
(19)	Correlation between FEV1% and NKT % of WBCs in blood in cases group.	92

List of Tables

<i>Table</i>	<i>Subject</i>	<i>Page</i>
(1)	Triggers of asthma.	27
(2)	Differential diagnostic possibilities for asthma in Infants and Children.	33
(3)	Classification of Asthma Severity by Clinical Features before Treatment.	34
(4)	Showing stepwise approach for managing asthma in children <5 Yr of age.	38
(5)	Stepwise Approach for Managing Asthma in adults and children >5 Yr of age treatment.	39
(6)	Asthma score questions.	42
(7)	Classification of NKT cells.	48
(8)	Classification of asthma severity by clinical features before treatment.	64
(9)	Demographic data of the studied children.	71
(10)	Severity distribution of asthmatic children.	73
(11)	Grading of asthma score among asthmatic children.	74
(12)	Frequency of family history of asthma among asthmatic children.	74
(13)	History of bad housing among asthmatic children.	75
(14)	History of associated allergy among asthmatic children.	75
(15)	Statistical comparison between cases and controls as regards absolute eosinophilic count in blood.	76
(16)	Statistical comparison between cases and controls as regards absolute natural killer T cells count in blood.	77

List of Tables (Cont.)

<i>Table</i>	<i>Subject</i>	<i>Page</i>
(17)	Statistical comparison between cases and controls as regards natural killer T cells % of WBCs in blood.	78
(18)	Statistical comparison between cases subgroups and controls as regards absolute natural killer T cells count in blood.	79
(19)	Statistical comparison among cases subgroups and controls as regards absolute eosinophil cells count in blood.	80
(20)	Statistical comparison between cases subgroups and controls as regards natural killer T cells % of WBCs in blood.	81
(21)	Statistical comparison between cases and controls as regards pulmonary function tests (PFTs) parameters (expressed as % of predicted for age & sex).	82
(22)	Statistical comparison between cells as absolute eosinophil &NKT cells and NKT % of WBCs as regards family history of asthma.	83
(23)	Statistical comparison between cells as absolute eosinophil &NKT cells and NKT % of WBCs as regards bad housing.	84
(24)	Statistical comparison between cells as absolute eosinophil &NKT cells and NKT % of WBCs as regards associated allergy.	85
(25)	Intergroup comparison as regards FVC.	86
(26)	Intergroup comparison as regards FEV ₁	87
(27)	Correlation between cases.	88

INTRODUCTION

Pediatric bronchial asthma is considered a common chronic illness in childhood (**Sidwell and Thomson, 2000**).

Studies suggested that therapies targeted at depletion or limiting of natural killer T cells may be a possible strategy for the treatment of asthma (**Bendelac et al., 2003**).

Many natural killer T cells express a highly restricted repertoire of T cells receptors consisting of V alpha 24 in humans and are called invariant T cell receptor- positive natural killer T cells (invariant NKT cells) (**Taniguchi et al., 2003**).

On activation of invariant natural killer T cells, They rapidly produce large quantities of both type I helper (Th₁)-biased (interferon-gamma) and Th₂-biased cytokines (interleukin-4), Which enhance the function of dendritic cells, and B cells, as well as the function of conventional CD4+ and CD8+ T cells (**Kronenberg M and Gapin L, 2002**).

As the role of invariant natural killer T cells in asthma is not well evaluated, We study the frequency distribution of restricted invariant natural killer T cells (TCR V α 24 NKT cells) in peripheral blood of known cases of asthmatic children (**Akbari et al., 2006**).



The aim of study was:

To assess the role of TCR V alpha 24 invariant natural killer T cells in asthmatic children and detection of TCR V alpha 24 invariant NKT cells in peripheral blood in asthmatic children .

PEADIATRIC BRONCHIAL ASTHMA

Definition:

Asthma is a major health problem. It is the most common chronic childhood diseases (**Liu et al., 2004**). Acute exacerbation of asthma are the leading cause of emergency department visits in the pediatric patients (**Fernandez, 2005**). In addition asthma is responsible for a significant problem of school day loss (**Liu et al., 2004**).

Asthma is defined as reversible obstruction of the airway, characterized by hyperresponsiveness to a variety of stimuli, caused by chronic inflammation. The airway obstruction is reversible, at least in part, and results in recurrent episodes of wheezing, cough, and shortness of breath that resolve either spontaneously or with treatment (**Keresmar, 2003**).

Asthma may have its onset at any age, 30% of patients are symptomatic by age of one year, which 80-90% of asthmatic children have their first symptoms before 4-5 years of age (**Sly, 2000**).

Although most cases begin before the age of 25 years asthma may develop at any time of life (**Drazen, 2000**).

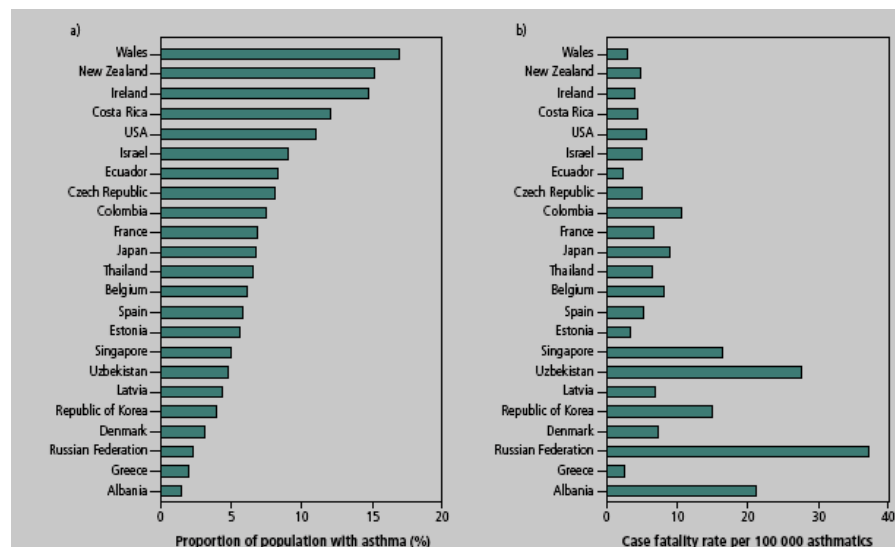
Epidemiology of pediatric asthma in Egypt:

In Egypt 23.2% of wheezy infants were proved to be real asthmatic (El Hefney et al., 1991).

The incidence of asthma among school children aged 5-15 years old was found to be 8.2% (El Hefney et al., 1994).

Abdel Latif (2000) studied the prevalence of asthma among 2321 secondary school (13-20 years old) in Four randomly selected districts (Misr El-Gedida, Helwan, Shoubra, Abbassia) and he reported a prevalence of pediatric asthma of 5.6%.

Figure (1): Prevalence and mortality from asthma :



(Bousquet et al., 2005)

Worldwide prevalence of asthma:

Asthma is one of the most common chronic diseases worldwide and the prevalence is increasing. Especially among children. The prevalence of asthma symptoms in children varies from 2 to 30 percent in different populations with the highest prevalence occurring in Australia, New Zealand and England (GINA, 2005).

Over the past 30 years, the prevalence of asthma has increased to epidemic proportion in developed countries, and asthma is the most common chronic disease in children (Arroda et al., 2006).

Risk Factors for Asthma:

(1) Genetic Factors:

The understanding of the genetic controls that lead to the development of asthma is essential to its proper diagnosis and management (Blumenthal, 2002).

Results from twin studies have consistently found evidence that genetic factors contribute importantly to asthma (Koeppen-Schomerus et al., 2002).

Asthma is a complex genetic disorder with variable phenotypes, largely attributed to the interactions of the environment and multiple genes (Arroda et al., 2006).

Asthma is essentially a polygenic disease in which many genetic variants determine small changes in immune responses

or in the manner in which the airway responds to the environment (**Holberg et al., 1996**).

The severity of asthma and response to treatment have also been suggested to be dependant on genetic modulators, such as the polymorphism of the $\beta 2$ -receptor (found on chromosome 5), which is involved in the bronchodilator response to β -agonists (**Ligget, 2000**).

(2) Gender and Asthma:

Gender differences in asthma prevalence and severity vary by age and may be attributed to differences in biologic susceptibility due to changes in hormonal milieu with aging, environmental exposures health care accessibility (**Caracta, 2003**).

Males have more severe airway hyperresponsiveness ;this may be one factor contributing to the higher prevalence of asthma in boys (**Jenssen and Cockroft, 2003 and Abd El Khalek et al., 2003**).

Epidemiological studies of both incidence and prevalence, have reported a male predominance of asthma and atopic conditions before puberty and a female predominance after puberty. There is evidence that airway development is different between the sexes. In females, there is proportionate growth of airways to lung volume and as a consequence