Ministry of Higher Education and Scientific Research
University of Al-Qadisiyah
College of Medicine

Role of cysteine leukotriene receptors type I and II gene polymorphism in asthmatic patients in Kerbala province

A Dissertation

Submitted to the Council of the

College of Medicine / University of Al-Qadisiyah

in Partial Fulfillment of the Requirements for

the Degree of Philosophy of Doctorate in Medical Microbiology

By

Ahmed Abbas Hasan

B.Sc. Medical Laboratory Science Technology / 2006

M.Sc. Medical Laboratory Science Technology / 2010

Supervised by

Asst. Prof.

Dr. Ibtisam H.AL .Azawi

2018 A.D. **1439 A.H.**

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

نٓۚ وَٱلۡقَلَمِ وَمَا يَسۡطُرُونَ (١) مَآ أَنتَ بِنِعۡمَةِ رَبِّكَ بِمَجۡنُونٖ(٢) وَإِنَّ لَكَ لَأَجۡرًا غَيۡرَ مَمۡنُونٖ(٣) وَإِنَّكَ لَعَلَىٰ خُلُقٍ عَظِيمٖ (٤) فَسَتُبۡصِرُ وَيُبۡصِرُونَ (٥) بِأَييِّكُمُ ٱلۡمَفۡتُونُ(٦)إِنَّ رَبَّكَ هُوَ أَعۡلَمُ بِمَن ضَلَّ عَن سَبِيلِهِۦ وَهُوَ أَعۡلَمُ بِٱلۡمُهۡتَدِينَ (٧)

صدق الله العلي العظيم
سورة القلم(١-٧)

Supervisor Certification

We certify that this thesis **(Role of Cysteine leukotriene receptors types I and II gene polymorphism in asthmatic patients in Kerbala province)** was prepared under our supervision at the Department of Microbiology, College of Medicine/ University of Al-Qadisiyah, as partial fulfillment of the requirements for the degree of Philosophy Doctorate in Medical Microbiology.

Signature:

Asst. Prof.

Dr. Ibtisam H.AL.Azawi

Department of Microbiology

College of Medicine

University of Al-Qadisiyah

Date: / /2018

Recommendation of the Head of the Department of Microbiology

According to the recommendation presented by the supervisors of this dissertation, I forward this thesis for debate by the examining committee.

Professor

Dr. Adnan H.Al-Hamadani

Head of microbiology Dept./ Collage of Medicine

Al-Qadisiyah University

Date: / /2018

Certification of Examining Committee

We are the members of the examining committee; we certify after reading this thesis **(Role of Cysteine leukotriene Type I and II gene polymorphism in asthmatic patients in Kerbala province**) and examining the student **(Ahmed abbas hasan Rokan)** in its contents. We found adequate for the degree of Doctorate of Philosophy in Medical Microbiology with excellent degree.

Professor

Dr. Angham Jasim Mohamed ali

University of AL-Forat AL-Awsate

Chairman

Professor

Dr. Hawraa Abdulameer Ali Al_dahhan

University of AL-Kufa

Member

Professor Dr.

Mazin Zamel Muhammad Al-Shibani

M.B.Ch.B-F.I.B.M.S-med.-M.Sc.-Interventional Cardiology

Assist. Prof.

Dr. Thikra Adnan Jawad

University of AL-Kasim AL-Kadra

Member

Assist. Prof.

Dr. Ghada Basil Al Omashi

Collage of Medicine /University of AL-Qadisiyah

Assist. Prof.

Dr. Ibtisam H.AL.Azawi

Collage of Medicine /University of AL-Qadisiyah

Member & Supervisor

Assist. Prof .

Dr. Aqeel R-AL-Barqaui

Dean of College of Medicine /University of AL-Qadisiyah

/ / 2018

DEDICATION

TO….. MY DEAREST LATE FATHER & MY DEAREST MOTHER

TO ….. MY GORGEOUS BROTHERS

TO ….. MY DARLING WIFE

TO ….. PURE HEARTS

MY CHILDREN HASAN , MOKHALAD & ASENAT

I OFFER THE SIMPLEST WORK

AHMED

Acknowledgments

I would like to express my sincere thanks, gratefulness and appreciation to the memory of my dearest supervisor, Prof. Dr. Hammadi Al-Helali for his supervision and suggestion the topics of this work, Special thanks for my supervisor Asst .Prof.Dr Ibtisam H.AL-

Azawi for her supervsion, invaluable advice and comments during the course of investigation and writing of this thesis, endless patience, generousness and kindness, efforts and care are highly appreciated and will never be forgotten .

It is only my duty to thank and appreciate to the Deanery of College of Medicine, University of Al-Qadissiya and Department of Microbiology for giving me the opportunity to complete Doctorate degree . My deep thanks for all staff in Kerbala Allergy Specialized Center for helps during collection of blood samples from asthmatic patients espcially Dr. Mozahim for their helping. My sincere gratittude is due to all postgraduate students with no exception of the departement of Microbiology – Medicine College / University of Al-Qadissiya for their support.

Ahmed

Summary

A total 100 patients with asthma (52 females and 48 males)with age range from (20-50 years) during the period between January/ 2017 to June/ 2017. They were out-patients clinic of the allergy department in Al-Hussein Teaching hospital and specialized center of allergy in Kerbala Province. 60 apparently healthy people were included in this study .

Blood specimens were collected from both groups, eosinophil directly detected , serum samples separated for testing of IgE, IL-5 and genomic DNA

was extracted from peripheral blood for molecular study to reveal any association between CyLTR1 &CyLTR2 polymorphism in asthmatic patients. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used for this purpose and digestion of the amplified DNA products by restriction endonuclease (*NlaIII* and *Hpy188I* enzymes) gave fragments with different molecular sizes , which express certain genotypes.

The study specimen showed that the asthmatic patients were included in this study their ages ranging between 20-50 years old, (26%) of them their ages ranging between (20-30) years old, (24%) of them their ages ranging between (30-40) years old and(50%) in age rang(40-50).

There are statistically significant increasing in serum levels of IgE , IL-5and eosinophil in asthmatic patients compared to healthy control group with P.value < 0.001 . There is non-significant difference in IgE levels and highly significance in(IL-5 and Eosinophil levels P.value < 0.05) according to severity groups and there are no significant according to the gender and age groups .

The genotyping of CyLTR1 972 T/C revealed three genotypes; the wild homozygous TT type, the heterozygous TC & the mutant homozygous CC type, the frequency of these genotypes(42%, 46%, 12% in patients and 40% 33, 3% 26, 7% in controls group) respectively .The CC genotype gives statistically significant difference according to asthma severity ,also all these genotypes (TT,TC and CC) and the T and *C* alleles give statically significant difference according to each asthmatic severity (mild, moderate and severe)groups.

There are increase in levels of IgE, IL-5 and eosinophil but has statistically non-significant difference according to difference in three genotypes of CyLTR1 972 T/C, also there are a positive correlation between these three parameters .

The genotyping of CyLTR2 M01 V revealed three genotypes; the AA type, AG and GG type, the frequency of these genotypes (24%,54%,22% in patients and 20% 60% 20% in controls group) respectively .All these genotype give statistically significant difference according to asthma severity ,also all these genotypes (AA,AG and GG) and the *A* and *G* alleles give statically significant difference according to each asthmatic severity (mild ,moderate and severe)groups.

The mean levels of IgE, IL-5 and eosinophil show increases but not reach to give statistically significant difference according to CyLTR2 M01 V difference in three genotypes, also there are a positive correlation between these three parameters

List of Contents

Titles	Page
Summary	I-III
List of contents	9-VIII
List of tables	IX-X

List of figures	XI-XIII
List of Abbreviations	XIV-XVII
Chapter one: Introduction and Literatures Review	
1.1 Introduction	1-4
1.2. Literatures review	5
1.2.1. Asthma	5
1.2.2. Prevalence of asthma	5
1.2.3. Sign and symptoms of asthma	6
1.2.4. Etiology and risk factors	7
1.2.4.1.Genetic factors	7
1.2.4.1. 2.Genes associated -asthma	8
1.2.4.2. Allergic trigger	9
1.2.4.3. Hygiene hypothesis	11
1.2.4.4.Medical condition	12
1.2.5. Classification of asthma	13
1.2.6. Pathophysiology of asthma	13
1.2.7. Eosinophils in Asthma	16
1.2.8. Evaluation and diagnosis	17
1.2.8.1. History and Examination	18

1.2.8.3.Physical examination	19
1.2.9.Biomarkers in asthma	19
1.2.9.1.B-cell-associated biomarkers	19
1.2.9.2.T-cell-associated biomarkers	20
1.2.10.IgE (immunoglobulin E)-associated asthma	20
1.2.11.Interleukins and its importance	21
1.2.11.1.Interleukin -5 (IL-5)	21
1.2.11.2.Role of Interleukin- 5 and Eosinophils	22
1.2.12.Cysteine leukotriene	23
1.2.12.1.Receptors of cysteine leukotriene:	24
1.2.12.1.1Cysteinyl leukotriene receptor 1	24
1.2.12.1.2.Function of cysteine leukotriene receptor 1	25
1.2.12.1.3.Gene polymorphism of CysLTR1	26
1.2.12.1.4.Clinical significant of CysLTR1	26
1.2.12.2.Cysteinyl leukotriene receptor 2	27
1.2.12.2.1.Polymorphism of CysLTR2	28
1.2.12.2.2.Clinical significant of CysLTR2	29
Chapter Two: Materials and Methods	30
2.1 Materials	30
2.1.1. Instruments and Equipments:	30

2.1.2 Commercial kits	31
2.1.3 Marker and primers	33
2.1.3.1.Primers	33
2.1.4. Chemicals	34
2.1.4.1 Restriction enzyme	35
2.2.Methods	35
2.2.1 Collection of specimens	35
2.2.1.1. Blood Samples	36
2.2.2. ELISA method	36
2.2.2.1. IL-5 Assay	36
2.2.2.1.1Test principle	36
2.2.2.1.2.Preparation of Items and Solutions for ELISA Technique	37
2.2.2.1.3.Assay procedure	37
2.2.3.Total IgE kit.	38
2.2.3.1. Test principle :	38
2.2.3.2 Manual test performance	38
2.3. Haematological study	39
2.3.1 Eosinophil counts .	39
2.4. Molecular Methods	39
2.4.1 Genomic DNA extraction	39

2.4.2.Genomic DNA Profiling	40
2.4.3. RFLP-PCR Technique	41
2.4.3.1.PCR master mix preparation	41
2.4.3.2.PCR Program	42
2.4.3.2.PCR product analysis	43
2.4.3.3 .RFLP-PCR mix preparation	43
2.5. Statistics analysis	44
Chapter Three: Results & discussion	
3.1. Demographic distribution of subject .	45
3.2. Levels of selected outcome measurements in asthmatic patients	47
3.3.The association between asthma severity and selected outcome measurements	49
3.5. The association between selected outcome measurements and age group.	50
3.5.The distribution of selected outcome measurements in gender groups.	51
3.6. Genetic study:	53
3.6.1. Cystine leukotriene receptor I (CysLTR1T/C).	53
3.6.2. Polymorphism in cysteine leukotriene receptor gene (CyLTR1 927 T/C)	55

3.6.2.1 Alleles frequency in Cysteine leukotriene receptor 1 (927 T/C) .	57
3.6.2.2.Association of Cys LTR1(927 T/C) gene polymorphism and asthma severity.	58
3.6.2.3.The levels of selected outcome measurements according to Cysteine leukotriene receptor 1(CysLR1 927 T/C) gene polymorphism.	61
3.6.2.4 The levels of parameters among asthmatic patients according to difference in each two cysteine leukotriene receptor 1	65
3.6.2.5.The correlation among three parameters depending on CysLR1 927 T/C polymorphism.	66
3.6.3. polymorphism in cysteine leukotriene receptor gene 2 (CyLTR2 M201V).	68
3.6.3.1Association of Cys LTR2(M201V) gene polymorphism and asthma severity.	69
3.6.3.2.The levels of three Parameters in difference in cysteine leukotriene receptor 2 (CysLR2 M01 V) genotypes.	72
3.6.3.4. Levels of three parameters between each two cysteine leukotriene receptor 2 (CysLR2 M01 V) genotypes groups .	74
3.6.3.5.Correlation of three parameters in CysLR2 M01 V gene polymorphism	76
Conclusions and Recommendations	

Conclusions	78
Recommendations	79
References	80-108
Arabic summary	أ-ب

List of Tables

Table No.	Title	Page
2-1	Instruments and Equipments.	30

2-2	The commercial kits.	31
2-3	IL-5 ELISA kits with its components	32
2-4	Contents of IgE kit:	32
2-5	Marker and primers.	33
2-6	The Multiplex PCR primers with their sequence and amplicon size:	34
2-7	Chemical substances.	34
2-8	Restriction enzyme	35
2-9	Mixture of PCR.	41
2-10	Amplification conditions of 927 C/T gene.	42
2-11	Amplification conditions of M201V gene.	42
2-12	Mixture for genotype CysLTR1 927 T/C:	44
2-13	Restriction enzymes and DNA Fragments size	44
3-1	The distribution of patients and control group according to different parameters .	46
3-2	Distribution of Alleles frequency for of Cys LTR1(927 T/C) in asthmatic cases and healthy groups.	58
3-3	Distribution of patients with Cys LTR1(927 T/C) gene polymorphism according to asthma	57

	severity.	
3-4	Distribution of three parameters among asthmatic patients & control group according to difference in cysteine leukotriene receptor 1 (CysLR1 972 T/C) genotypes.	59
3-5	Distribution of three parameters among asthmatic patients according to difference in three cysteine leukotriene receptor 1 (CysLR1 972 T/C) genotypes.	61
3-6	Distribution of three parameters among asthmatic patients according to each two CysLR1 972 T/C genotypes groups .	63
3.7	Correlation of three parameters among asthmatic patients according to difference in cysteine leukotriene receptor 1(CysLR1 927 T/C)genotypes	65
3.8	Distribution of patients with CysLTR2(M201V)gene polymorphism according to asthma severity	68
3.9	Distribution of three parameters among asthmatic patients & control group in Each gene of cysteine leukotriene receptor 2 (CysLR2 M01 V)	69

	genotypes.	
3-10	Distribution of three parameters among asthmatic patients according to difference in cysteine leukotriene receptor 2(CysLR2 M 201 V)genotypes .	71
3-11	Distribution of three parameters among asthmatic patients according to difference in each two cysteine leukotriene receptor 2 (CysLR2 M01 V) genotypes groups	73
3-12	Correlation of three parameters among asthmatic patients according to difference in cysteine leukotriene receptor 2(CysLR2 M 201 V)genotypes	75

List of Figures

Figure No.	Titles	Page
1.1	Scheme for two different pathways lead eosinophilic airway inflammation in asthma	15
3.1	Distribution of patients according to age groups.	45
3.2	The distribution of mean levels of IgE ,IL-5 and Eosinophil according to severity groups.	48
3.3	Differentiation in means of selected outcome measurements between the three age groups of asthma cases.	50
3.4	Differentiation of mean levels of selected outcome measurements between male and female gender of Asthma cases .	51
3.5	Agarose gel electrophoresis image that show the PCR product analysis of CysLTR1gene	52
3.6	Agarose gel electrophoresis image that show the RFLP-PCR product analysis of CysLTR1 927 T/C by using Hpy188I restriction enzyme.	52
3.7	Agarose gel electrophoresis image that show the PCR product analysis of CysLTR2 gene	53

3.8	Agarose gel electrophoresis image that show the RFLP-PCR product analysis of CysLTR2 (A/G) M201V by using NlaIII restriction enzyme.	54
3.9	Distribution of percent of Cysteine leukotriene receptors gene type 1 (CyLTR1 927 T/C)polymorphism in asthmatic patients and controls groups.	55
3-10	Distribution of percent of cysteine leukotriene receptors type 2 gene (CyLTR2 M01 V)polymorphism in patients and controls groups.	67

List of Abbreviation

Symbol	Abbreviation
A	Adenine
AR	Allergic rhinitis
Ab	Antibody
Ag	Antigen
AHR	Airway hyperresponsiveness
AM	Alveolar macrophages
AR	Allergic rhinitis
ARI	Acute respiratory illness
ASM	Airway smooth muscle
BAL	Broncho-alveolar lavage
BA	Bronchial asthma
BHR	Broncho hyper responsiveness
B- lymphocytes	Bone marrow- (or bursa)-derived lymphocytes
BMI	Body mass index
bp	Base pair
B_{reg}	Regulatory B-lymphocytes
BSA	Bovine serum albumin
CAM -1	Cell adhesion molecule
C-C	Chemokine
CCR	Chemokine receptor
CD	Cluster differentiation
CDC	Center for disease control and prevention
CSF	Cerebrospinal fluid

CTL	Cytotoxic T lymphocyte
CysLT	Cysteine leukotriene
CysLTR1	Cysteine leukotriene receptor 1
CysLTR2	Cysteine leukotriene receptor 2
CysLTA	Cysteine leukotriene antagonists
DNA	Deoxyribonucleic acid
EAR	Early allergic response
ECP	Eosinophils-chemotactic protein
EDTA	Ethylene-diamin-tetra-acetic acid
EGPA	Eosinophilic granulmatosis with polyangitis
FDA	Food and Drug Administration
ELISA	Enzyme linked immunosorbant assay
FeNO	Forced exhaled nitric oxide
FEV_1	Forced expiratory volium in first second
G-CSF	Granulocytes colony-stimulating factor
GM-CSF	Granulocytes -macrophages colony-stimulating factor
HLA	Human leukocyte antigen
HDMs	House dust mite
H/O	History of
HRP	Horse radish peroxidase
Hpy1881	*H. pylori 1881*
ICAM	Intercellular adhesion molecule
ICS	Inhaled cortecosteroids
IFN	Interferon
Ig	Immunoglobulin
IgE	Immunoglobulin E
IgG1	Immunoglobulin subtype G 1

IgG2	Immunoglobulin G subtype 2
IgG3	Immunoglobulin G subtype 3
IL	Interleukin
IL-1	Interleukin-1
IL-3	Interleukin-3
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-5R	Interleukin-5 receptor
IL-9	Interleukin-9
ILC2s	Group 2 innate lymphoid cells
IL-13	Interleukin-13
LABAs	Long-acting beta-agonists
LT	Leukotriene
LTC4	Leukotriene C4
LTD4	LeukotrieneD4
LTE4	LeukotrieneE4
LTRAs	Leukotriene receptor antagonists
MCP-3	Monocyte chemotactic protein-3
MHC	Major histocompatibility complex
NKC	Natural killer cells
mRNA	Messenger ribonucleic acid
NHDS	National hospital discharge survey
NOD	Nucleotide-binding oligomerization domain
NPA	Nasopharyngeal aspirate
NlaIII	*Nassieraia lactamica III*
NSAIDs	Non-steroidal anti-inflammatory drugs
OD	Optical density

OR	Odds ratio
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PFTs	Pulmonary function test
PG	Prostaglandin
RANTES	regulated upon activation normal T-cell expressed and secreted
RNA	Ribonucleic acid
RFLP	Restriction-fragment length polymorphism
RSV	Respiratory syncytial virus
RT-PCR	Real time- polymerase chain reaction
rt-PCR	Reverse transcriptase- polymerase chain reaction
SNP	Single nucleotide polymorphism
SPT	Skin prick test
STAT	Signal transducer and activator of transport
TAE buffer	Triss-Acetic acid-EDTA buffer
TE buffer	Triss-EDTA buffer
T_C lymphocytes	Thymus-derived cytotoxic lymphocytes
TGF- β	Transforming growth factor- β
Th2	Thymus helper 2
TLR	Toll-like receptor
T- lymphocytes	Thymus-derived lymphocytes
TNF	Tumour necrosis factor
T_{reg}- lymphocytes	Thymus-derived regulatory lymphocytes
TMB	Tetra methyl benzadine
W1	Washing buffer 1
WBCs	White blood cells

1.Introduction and literature review

1.1.Introduction

The word asthma (initially from Greek) firstly described by the Corpus Hippocraticum as a medical term that showed breath shortness and trouble breathing globally. The prevalence of allergy and asthma is considered big problem with continual increase especially in the last few decades. It is considered as a public health problem for developed countries and also for developing countries (Gupta *et al* .,2018) .

Asthma is characterized as a heterogeneous disease, and usually characterized by chronic inflammation of airway. The heterogeneity of asthma may be attributed to great differences in characteristic of clinical and physical features, the pathway of disease development not same between the patients or there are great variation in therapy, which considered suitable for each patients, also it is considered as a complex disease and refers to a shared clinical expression of several or partially overlapping of biological conditions (Sterk,2016 ; Vogelmeier *et al.,* 2017and GIA ,2017) .

Asthma is a non-communicable disease and considered as a common chronic disorder that may be effect on about 334 million people in different parts of the world. It is usually cause worse of life quality, because of its psychological and social effects (Asher *et al* .,2006 and Asher *et al* .,2014).

Asthma is chronic respiratory disorder mainly affecting about 1-18% of population in the world with different types of symptoms like wheezing, chest

tightness and or chough ,shortness of breath, and limitation of respiratory airflow ,these symptom vary during the time and in intensity. There are different factors that trigger these symptoms and lead to that change in severity such as exercise ,weather changing, exposure for different types of irritants or allergens ,or infection with respiratory viruses (GIA .,2017). The environmental factors are very important because these factors cause increase of prevalence of disease specially in the last 50 years, that occur due to a rapid and long change in the exposure to environmental factors. Through studying many complex diseases such as asthma or allergy, one of consideration includes some individuals could be more susceptible to certain exposures to environmental factors due to their genetic make-up (Asher *et al* .,2014).

In the world the prevalence of asthma is markedly increasing due to the changing in the world behaviors also the prevalence of asthma has increased during the last three decades. According to the World Health Organization (WHO) estimate in 2005, around 300 million people were affected by asthma. According to the existing evidence, the prevalence of asthma and allergy in children is increasing around the world . It is estimated that this number will reach 400 million by 2025. It is not clear that this increase is due to a real increase in the prevalence of asthma and allergy, or it is due to a higher level of awareness and diagnosis. Overall, respiratory diseases cause 6.3 % of the total death around the world (WAO, 2011;Droste *et al.*,2014 and Mirzaei *et al*.,2017)

In Iraq , the study of Radwan *et al* .,(2017) which recorded the increase in the prevalence of asthma due to the effect of war and also the important of different chemical agents on different kinds of lung diseases in relationship to diverse war sectors, also (Al-Habboo *et al*.,2017) recorded that the asthma plays a

role on psychological status of patients especially in the females . Other study was done by Shakir *etal.,* (2012), founded that patients family history of allergic rhinitis, asthma, passive smoking associated with asthma in respectively. The prevalence of asthma was higher in females than males.

There are two different types of cysteine leukotriene receptors: CysLTR1 (Present in airway smooth muscles , leucocytes, spleen), and also the CysLTR2 (located in brain, heart, placenta, central nervous system, leukocytes, spleen) This difference and diversity in occurrence of CyLT receptors may lead to indicate a role of leukotrienes in numerous pathological and physiological conditions. CysLT1 receptors have ability to bind with high affinity to leukotriene D4 and low affinity to leukotriene E4 and leukotriene C4. These receptors play a major role in mucus secretion, bronchoconstriction, and edema in the airways. The another type of CysL is a CysLT2 receptors related to vascular permeability, inflammation as well as tissue fibrosis (James *etal* .,2001).

Aim of the study

The present study aimed to detect the correlation between asthma and presence of polymorphism in cysteine leukotriene receptors I,II

The following objectives were proposed :-

1-Determination of CysLTR I& CysLTR II gene polymorphism by using RFLP PCR.

2- Determine the predictive value of blood eosinophil concentrations in asthmatic patients .And Investigate the levels of IgE and IL-5 in serum of patients with asthma by using ELISA assay .

3- Identify the correlation between IgE and IL-5 levels with eosinophil concentration in asthmatic patients .

4- Correlate the linkage association between CysLs (T1 &T2) genes polymorphism, levels of IgE levels ,Eosinophilia and Interleukin- 5 .

1.2. Review of literatures

1.2.1 Asthma

Asthma is currently understood to be a fairly common but complicated chronic respiratory disease with multiple etiologies (Buttaro *et al* .,2012 and Zhang *et al* .,2013). The diagnosis of asthma is commonly made by the clinical setting through a history and physical exam, as well as with the use of pulmonary function tests (PFTs). More than simple bronchospasm, asthma is characterized by such clinical elements as short- and long-term inflammation of the respiratory airway, edema, narrowing of the airway, and increased secretions mediated by different allergic, lifestyle, environmental and inflammatory triggers (VanGarsse . *et al*.,2015). Those triggers range from pollen to humidity, cold air to exercise, smoke exposure to acute infection; the risk factors for asthma are also complicated but known to include low-socioeconomic status, obesity and primary and secondary smoke exposure (Vernon *et al* .,2012 and Zhang *et al*., 2013).

Asthma is one of the most common respiratory disorders in clinical practices, affecting up to 13% of people worldwide (Kamble *et al*.,2009). Most cases of asthma are atopic in nature, with trigger for acute asthma attacks and chronic worsening of inflammation being allergens inducing an immune response through immunoglobulins of IgE class (Cecchi *et al.*,2010).

1.2.2 Prevalence of asthma

Asthma is one of the most common chronic diseases among children (GIA,2017). Globally, the prevalence of childhood asthma has varied over the past few decades, with some countries reaching a high percent in prevalence after

decades of increase (Eder *et al.*,2006 ; Pearce *et al.*, 2007 and Anandan *et al.*, 2010). For example, in high-income countries, such as the U.K., the U.S.A., Australia, and New Zealand, the high prevelance have been observed. Among Swedish children, asthma prevalence is now stabilized at around 10% (Bjerg *et al.*,2010). In low- and middle-income countries in Africa and Latin America, asthma prevalence is lower, but a recent increasing trend has been reported. The genetic variations (i.e. heritability) do not seem to simply account for the differences in asthma prevalence over time by countries. The role of the environment, possibly associated with economic growth, seems to be increasingly meaningful to the development of asthma (Pearce *et al.*, 2007).

1.2.3. Sign and symptoms of asthma

Symptoms and airflow limitation vary between individuals and over time, either spontaneously, in response to triggers, or as a result of some treatment. Although treatment with effective inhaled, oral and parental therapies, there is no cure. Asthma therefore imposes a major burden on health systems, on societies through costs of treatment and lost productivity, and on personal and family life. As it is such a common condition, the bulk of diagnosis and management occurs in primary care in most economically developed countries, with specialist care generally reserved for those with severe disease, poor control, or diagnostic uncertainty (Holgate *et al.*,2017).

Asthma is characterized by frequent episodes of whistling or wheezing sound, shortness of breath, chest pain, and persistent coughing. The cough may be dry or productive with mucous containing; sputum may be expelled from the lungs by coughing but is usually hard to bring up (Jindal *et al.*,2017). These symptoms

might worsen at night or after exercise, and may also develop in response to different triggers, such as exposure to cold air or exposure to allergens (Janssens &Thomas ,2013).

1.2.4. Etiology and risk factors

Asthma is a disease with multi-factorial etiology. The genetic factors explain more than 50% of individual variations in the liability to asthma in the general population (Thomsen *et al.*, 2010 ; Javelle *et al.*,2015 and Pretolani *et al.* 2017). Consortium-based genome-wide association study identified several loci on chromosomes 2, 3, 6, 9, 15 and 22 that were associated with asthma at all ages (Moffatt *et al.*,2010). However, these could not contribute to all incident cases over the past few decades and explain all the variation by geographic locations mentioned previously. A growing body of evidence suggests both perinatal and early life environmental factors may play a significant role for the prevalence of asthma (Beasley *et al* .,2015). For example, perinatal factors including parental age, maternal body mass index (BMI), stress, smoking and diet been found to be associated with asthma (Almqvist *et al.*,2015). Early life infections, exposure to tobacco smoke, air pollution, and other allergens have all been linked to increased risk of asthma in childhood and adolescence, whereas some study recorded the exposure to dog and farm exposure were found to be negatively associated with asthma (Weinmayr *et al.*, 2013; Ortqvist *et al.*,2014and Milanzi *et al.*, 2017)

1.2.4.1.Genetic factors

In the genetically heterogeneous asthma the few common alleles are associated with disease risk at all ages of asthmatic patients . Some of these genes suggest a role for communication of epithelial damage to the adaptive immune system and

also activation of airway inflammation (Moffatt *et al.*, 2010 and Song *et al.*, 2017).

Asthma susceptibility is influenced by environment and genes; the important of these genes may indicate the pathways for therapeutic intervention. The detection of the risk factors of asthma may be useful in subtypes identification of asthma and determining whether intermediate phenotypes, such as the total serum IgE level elevation, are that may linked to disease, also the variation in genes coding for factors associated with the inflammatory response and increased risk for asthma or for respiratory physiological changes associated with asthma (Akhabir &Andrew 2011and Sordillo *et al.,* 2015).

The disease complexity associated with many genes and environmental factors, but the effect of each of these factors is mild, also there has been increased interest in gene-gene and gene-environment interactions, which may affect asthma pathophysiology. Although the importance of gene-gene , gene-environment interactions on asthma is still under documentation, in addition to a systematic analysis on the interaction between various genetic and environmental factors is still lacking (Wenzel *et al.,* 2007; Moore *et al.,* 2007 ; Stefanowicz *et al.,* 2012 ; Durack *et al.*,2017and Barnish *etal.*, 2017

1.2.4.1.2 Genes associated -asthma

Many linkage studies have recorded that the cluster of genes related to asthma susceptibility or atopy susceptibility map to a region on chromosome 5q31-q33 in certain populations, a region previously linked to atopy and enhanced IgE responsiveness. Many other study reported to other two regions, (2p21–p14 and

6p21 Smit *et al.*, 2010),and other similar study implicated the chromosome region (17q21.1 Moffatt *et al;* 2007).

In general, asthma susceptibility genes classify to four main groups: (1) Genes associated with innate immunity and immunoregulation (2) Genes associated with T helper 2 (T_H2)-cell differentiation and effector functions (3) Genes associated with epithelial biology and mucosal immunity and (4) Genes associated with lung function, airway remodelling and disease severity (Vercelli 2010; Tsai *et al.*,2018 and Stadhouders *et al* .,2018).

1.2.4.2 .Allergic trigger

There are many allergic trigger such as house dust mites (HDMs) are ubiquitous pyroglyphidae that live in human dwellings. The mite's gut contains potent digestive enzymes, notably proteases that persist in the feces, and these are thought to induce allergic sensitization and asthma (Tovey *et al.*, 2016). First suggest that in-bed exposure accounts for only 9% of the total exposure. Second, dust collected from the homes of patients includes many other air contaminants besides HDMs and HDM allergens, such as bacteria and endotoxins, molds, glucans originating from mold membranes, mycotoxins, and microbial volatile organic compounds (Nevalainen *et al.*, 2015) .

Microscopic fungi, or molds, represent one of the main groups of microorganisms present in all buildings. Although there are thousands of mold species, (only ~80% indoor molds) are thought to be responsible for adverse health effects in humans. There are few published interventional studies regarding dampness and mold in homes (Cox-Ganser ,2015). According to research statistics published by animal food suppliers, ~50% of families in developed countries have a pet (AVMA ,2016

and FEDIAF ,2016). There are as many dogs more than cats as pets,the cats are more allergenic than dogs. (Pyrhönen *etal* ., 2015). Cockroach allergens are mainly found in the kitchens of low income housing. The combination of cockroach allergen exposure and allergic sensitization contributes to asthma morbidity (Rosenstreich *et al.,* 1997). Mouse allergens originate from mouse urine, shed skin cells, and hair follicles, and they are mostly found in inner-city dwellings. High levels of allergens may be found in schools and homes, but their relationship with asthma morbidity is still controversial (Sheehan *et al.*,2009)

Allergenic pollens are thos small enough to be transported by the wind. With most patients, allergy to pollen induces rhino-conjunctivitis. Nonspecific bronchial hyper-reactivity increases with grass-pollen allergy as well as with parietaria pollen allergy during pollination (Kurt *et al.,* 2010). The outdoor mold such as alternaria and cladosporium species are considered to be major outdoor allergens responsible for sensitization development of rhinitis and asthma (Fukutomi *et al*.,2015).

Viral infections have been implicated in most asthma (> 80%) specially in seasonal period (Busse *et al.,* 2010) such as rhinoviruses are the most commonly encountered, although other viruses such as respiratory syncytial virus, enterovirus, coronavirus and human metapneumovirus, can also be involved. Viruses interact with allergens to induce asthma exacerbation and hospitalization (Murray *et al.,* 2006). According to the Centers for Disease Control and Prevention in the USA, The are 21% of americans with asthma are active smokers (CDC ,2016)(Fernandes *et al* ., 2016). Air pollution has an important role in developing of asthma and any changes in gaseous and particulate outdoor air

pollutants are associated with daily asthmatic symptoms, a decrease in lung function (Li *etal*.,2012)

In children with moderate persistent asthma, the occurrence of symptoms and the decrease in lung function are more pronounced in those patients who do not follow a maintenance therapy (Zheng *et al.*,2015) . An increase in daily temperatures correlated with an increase in emergency room department visits for asthma, especially in patients older than 65 years (Kim *et al.*,2014). However, cold exposure also trigger asthma attacks. In general, the effect of cold weather appears to last for several weeks, whereas the effect of hot weather was more short term (Zhang *et al.,* 2014). Work-exacerbated asthma can occur when current asthma symptoms are made worse due to work; it does not refer to work as the cause of asthma (Tarlo, 2016) . Avoidance is mainly based on occupational hygiene and the use of personal protective equipment. It also relies on an adequate knowledge of the potential triggers of work-related asthma by asthmatic patients (Lipszyc *et al.*,2016).

1.2.4.3 .Hygiene hypothesis

Allergic diseases are now affecting 20–40% of the population of highly industrialized and economically advanced regions of the world,also incidence of these disorders appears to have dramatically increased in the United States of America and Europe, but also in developed Asian countries (Versini *et al*., 2015). Changes of lifestyle are most evident among young children, and therefore, children are the most vulnerable to develop allergic diseases (Versini *et al*.,2015). A detailed account of the historical recordings of the allergy epidemics, from the

first reported hay fever case in 1870 until 2010, was published by (Platts-Mills, 2015)

The hygiene hypothesis proposes that cleaner environments have led to less immunological stress and reducing the development of an asthma-protective Th1 cytokines phenotype (Scirica *et al.*,2007).

1.2.4.4. Medical conditions

There are highly association between history of eczema allergic rhinitis and development of asthma, also the asthma occurring at a much greater rate in those who have either eczema or hay fever (Rapini *et al* ., 2007) Asthma has been associated with eosinophilic granulomatosis with polyangiitis (formerly known as Churg–Strauss syndrome), an autoimmune disease and vasculitis ,also the individuals with certain types of urticaria may also experience symptoms of asthma. (Jennette *et al* .,2013) .

There is a correlation between obesity and the risk of asthma occurrence (both having increased in recent years) ,several factors may be play role to affect on respiratory function due to a buildup of fat and the fact that adipose tissue leads to a pro-inflammatory state (Holguin &Fitzpatrik 2009; Beuther ,2010 and Dixon *et al.,* 2010)

Beta blocker medications such as propranolol can trigger asthma in those who are susceptible ,Other medications that can cause problems in asthmatics including aspirin and NSAIDs and use of acid suppressing medication during pregnancy is associated with an increased risk of asthma in the child (Lai *et al.*,2018)

1.2.5 . Classification of asthma

In general, bronchial asthma can be classified into an allergic asthma (Th2-weighted) and a non-allergic asthma (non-Th2-weighted,the Th2- weighted form gives rise to an infiltration of eosinophil cells into the bronchial wall, in addition to the differentiation between allergic and non-allergic bronchial obstruction, an evaluation of symptoms associated with occupational asthma in workplaces (work related asthma) must take place. Furthermore, history of intolerance to aspirin (aspirin exacerbated respiratory disease) or exercise induced symptoms (exercise-induced asthma) should be informed, In addition to that classification of asthma need careful interpretation of clinical symptoms and allergy test findings (Schneider *et al.*, 2014).

1.2.6. Pathophysiology of asthma

Airway inflammation is the principal mechanism of asthma, and the main treatment target. Typical features of airway inflammation are increased eosinophils, mast cells, and lymphocytes the predominance (Th2 cells), which produce mediators such as IL-3, IL-4, IL-5, IL-13, (GM-CSF). However, some patients exhibit different patterns of inflammation, including neutrophilic bronchitis or, less frequently, few inflammatory cells (pauci-granulocytic phenotype) ,(Ricciardolo *et al* .,2018).

Asthma is the result of chronic inflammation of the airways which subsequently results in increased of the surrounding smooth muscle contraction in response to exposure to a different of stimuli including microbial allergens or irritants. Allergens induced an acute bronchoconstriction and many signs and symptoms result from IgE dependent release of preformed mediators from mast cell that

involve histamine, tryptase, leukotrienes neutral proteases and prostaglandins that directly contract airways smooth muscles besides other factors leads to episodes of narrowing of the airway and the classic symptoms of asthma (Busse *et al.,* 2010).

Asthma is characterized by structural changes in the airway that may precede the development of asthma including epithelial damage, sub-epithelial fibrosis, increased airway vasculature and increased smooth muscle mass. (Bergeron *et al* .,2006). Mucous hyper-secretion is associated with an increase in the number of secretory glands and goblet cells. Airway inflammation is the dominant abnormality, occurring even in the earliest stages. Airway mucosa inflammatory cells include lymphocytes, plasma cells, mast cells, and macrophages, all typically associated with eosinophils (Shaver *etal.,* 1997). Neutrophils are found in some patients particularly smokers (Bessa *etal.*,2008). In allergic asthma most cells exhibit (Th2) profile of cytokine secretion, characterized by production of interleukin-4 (IL-4), IL-5, IL-9, GM-CSF, and IL-13(Hamid *et al.,* 1991) .

However, some patients do not show eosinophilic inflammation and T h2 cytokine responses. These respond less well to inhaled corticosteroids (Woodruff *etal.*,2009) and other interventions targeting Th2 cytokines. They have a predominantly mononuclear inflammatory cell airway response, with T lymphocytes and activated macrophages.

It has been proposed that asthma can be classified according to two major endotypes. Endotype is a disease subtype defined by a distinct functional or pathological mechanism (Anderson, 2008 and Green *et al.,* 2002). "Th2-high"

asthma is characterized by increased levels of type 2 inflammation, mainly mediated by eosinophils, mast cells and Th2 cells, Group 2 innate lymphoid cells (ILC2s), and IgE-producing B lymphocytes (Fahy, 2015),the patients with Th2-high asthma have eosinophilia and other signs of type 2 inflammation,by contrast to "Th2-low" asthma is less well characterized and probably represents a mix of multiple endotypes involving subgroups of patients (Wenzel, 2012and Choy *et al.*, 2015).

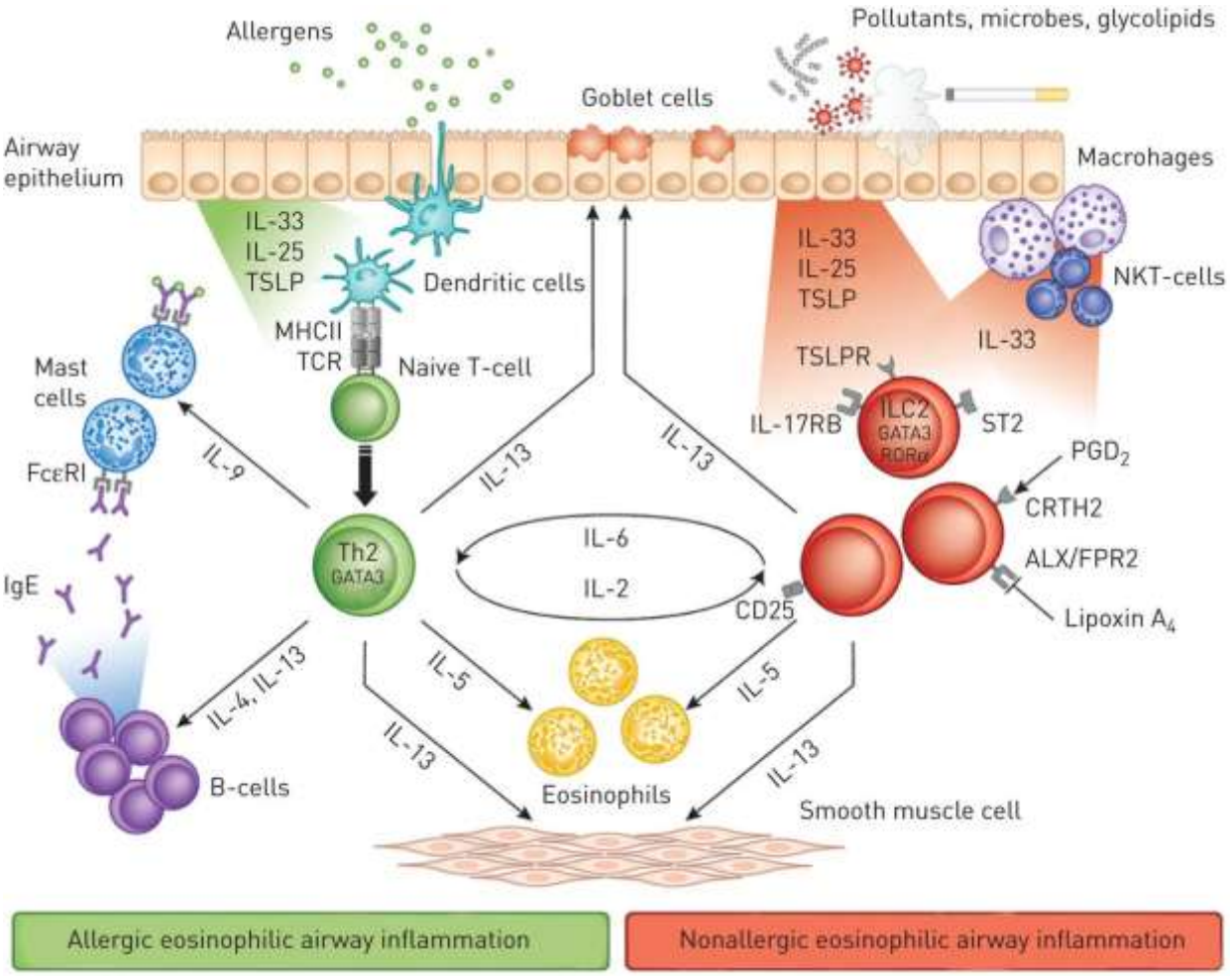


Figure (1-1) Scheme for two different pathways lead eosinophilic airway inflammation in asthma. (Brusselle *et al.*,2013)

1.2.7.Eosinophils in Asthma

Eosinophils are bone marrow–derived cells of the granulocyte lineage. They have an approximate half-life of 8 to 18 hours in the bloodstream, and mostly reside in tissues where they can persist for at least several weeks. Their functional roles are multifaceted and include antigen presentation; the release of lipid-derived peptide, and cytokine mediators for acute and chronic inflammation (Kovalszki &Weller, 2016).

Any patients start with nasal allergies and asthma produce a more robust eosinophilic response that can be in the mild to moderate range , but then develop abnormal arachidonic acid metabolizing cascades and hence have a more dramatic presentation both of both disease disease entity and of the eosinophilia (Stevens *etal.*,2015 and Laidlaw *et al* .,2016).

Approximately 5–10% of asthmatic patients have severe asthma that is poorly controlled by drugs including high-dosage of ICS and/or systemic glucocorticoids. The mechanisms of glucocorticoid sub-sensitivity or insensitivity in severe asthma are largely unknown (Mukherjee *etal.*,2017).

Eosinophilic inflammation is present in a significant proportion of patients with severe asthma (Zhang &Wenzel, 2007) and is associated with exacerbations and decreased lung function (Price *et al.,* 2016). Moreover, progressive increase in sputum and blood eosinophils is accompanied with poor pharmacological asthma control (Belda *et al.,* 2006).

There is compelling evidence that eosinophils and their mediators are critical effectors to severe eosinophilic asthma and EGPA (Vaglio *et al.,* 2013and Varricchi *et al.,* 2016). EGPA is a systemic vasculitis frequently occurring in

patients with severe asthma and eosinophilia. EGPA patients often have severe respiratory involvement that requires treatment with oral glucocorticoids (Detoraki *et al;*2016).

Due to their rarity (approximately 1% of peripheral blood leukocytes), eosinophils have been erroneously neglected for decades (Kay ,2015) .

During the last years, researchers of immediate hypersensitivity appreciated that these cells represent repositories of a wide spectrum of pro-inflammatory mediators such as several cationic proteins (major basic protein; eosinophil cationic protein; eosinophil peroxidase, and eosinophil-derived neurotoxin), cytokines/chemokines. and lipid mediators (Varricchi *et al.*,.2016). Importantly, eosinophils have the capacity to adhere to activated endothelial cells, to leave the blood-stream and to concentrate at the site of allergic inflammation (Furuta *et al.*, 2014). These cells and their mediators are found in airway tissue and sputum of patients with asthma. In addition, human eosinophils play a major role in the modulation of the functions of a wide spectrum of cells of the innate and adaptive immune system, including subsets of lymphocytes, macrophages, mast cells, basophils, neutrophils, dendritic , plasma cells, and platelets (Sehmi *et al.*,2016).

1.2.8. Evaluation and diagnosis

The lack of a simple gold standard diagnostic test and limited availability of diagnostic tests may lead to difficulties in primary care and there is evidence of both over- and under-diagnosis (Aaron *et al.*,2008).

1.2.8.1. History and examination

Asthma is a symptomatic condition and symptom pattern and triggers should be documented. However symptoms do not accurately predict pulmonary function and are not reliable to establish a diagnosis in isolation. (Thomas &Wilkinson, 2014) Physical examination is often normal unless the disease is severe or the examination is performed during an exacerbation or exposure to triggers, when wheezes can be heard on auscultation with prolonged expiratory time. Assessing of patient at high risk of mortality from asthma, can be carried out by asking questions needed to determine the background persistent asthmatic severity and exacerbation (Turner *et al.,* 1998 , Palange *et al.,* 2018). Taking high doses of β-agonist regularly by the patient is also informative- and associated with a progressively greater probability of a hospital admission and risk of death (Aldington &Beasly, 2007).

For some individuals, asthma is a disease whose symptoms seem to remit with time. Numerous patients develop disease that is persistent throughout their lifetimes and is associated with more serious symptoms and lung function impairment. These patients usually have a positive family history of bronchial asthma and demonstrate airway hyper-reactivity and atopy in childhood (Guilbert *et al* .,2006 and Bisgaard &Bonnelykk, 2010).

1.2.8.3.Physical examination

The physical examination should be carried out to confirm the diagnosis of asthma quickly and to assess its severity. The general appearance of the patient, including difficulty in talking, an increased respiratory and heart rates form the basis of the clinical assessment of severity (Neville *et al.,* 1991). High pulse rate has a close correlation with worsening asthma severity (Aldington&Beasely, 2007). Sounds of wheezing during breathing or a prolonged phase of forced exhalation indicate of airflow obstruction correlated with asthma severity. In some cases there are increase in nasal discharge secretion, mucosal swelling, and/or nasal polyps ,also may developed atopic dermatitis, eczema or any other symptoms of an allergic skin condition (National Heart,lung and blood Institute 2007).

1.2.9.Biomarkers in asthma

1.2.9.1.Biomarkers of B cells

The assessment of the disease status and its classification into mono-, oligo- and polysensitized (atopic) is already providing important prognostic information for clinical outcomes. For instance, polysensitized endotypes are more frequently prone to hospital admissions (Lazic *et al.,* 2013). Profiling of immunoglobulin specificities is currently performed on the level of IgE (multicomponent protein array analysis). Other studies demonstrate that distinct patterns of IgE responses to different protein families are associated with different clinical symptoms .Furthermore, the balance of Ig subclasses underestimated are IgGs (like IgG1, IgG2, IgG3). (Simpson *et al.,* 2015)

1.2.9.2.T-cell-associated biomarkers

T cells are also infiltrating the affected tissue and are anticipated to play an important role in disease development and progression (Leckie ,2003). Their assessment in the peripheral blood is difficult due to the low frequency of allergen-specific Th2 cells, while local Th2 endotypes are most visible in sputum (Peters *et al.*,2014). However, sputum T lymphocytes are predominantly of activated intraepithelial phenotype (CD103+CD69+) that are known to belong to the long-lived memory pool, which rapidly responds to antigen challenge (Leckie ,2003).

IL-4, IL-5 and IL-13 are detectable in the serum of asthma patients (1–50 pg/ ml range) in acute episode (Lee *et al.*, 2001).

1.2.10.IgE (immunoglobulin E)-associated asthma

A IgE -related allergies are the major health threat in industrialized countries influencing over 25% of the population (Pawankar *et al* .,2012). Asthma represents one of the most serious signs of allergy because of its chronic course, strong impact on quality of life and severe life-threatening symptoms (Holgate , 2012). Sensitization to airborne allergens from indoor allergen sources indicated IgE formation such as house dust mite, pets, mold and cockroach is a causative factor for asthma (Konradsen *et al.*, 2015). Moreover, severe exacerbations are frequently observed after seasonal exposure to allergens from birch, grass, cypress and ragweed pollen (Schäppi *et al.*,1997 , Erbas *et al.*,2012).

IgE antibodies have important role in the pathophysiology of allergic diseases (Oettgen,2016).In hypersensitive asthma, symptoms are caused by allergen-induced crosslinking of IgE antibodies on the surface of mast cells and basophils and the subsequent release of inflammatory mediators. IgE may also play an

important role in T cell activation through IgE-facilitated allergen presentation T cell-derived cytokines such as IL-5 and IL-13 contribute to mucus secretion and eosinophilic infiltration, respectively, both of which are involved in airway remodeling (Holgate, 2012).

1.2.11.Interleukins and its importance

Interleukins are a group of cytokines (secreted proteins and signaling molecules) that were first known to be produced by white blood cells (Brocker *et al.,* 2010). The function of the immune system depends in a large part on interleukins and rare deficiencies of a number of them have been described, all featuring autoimmune diseases or immune deficiency. The majority of interleukins are synthesized by helper CD4 T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. They promote the development and differentiation of T and B lymphocytes and hematopoietic cells (Menachem-Zidon *et al.*,2011).

1.2.11.1. IL-5 .

IL-5 is a key mediator in eosinophil activation produced by Th-2 cells and mast cells, involved in allergic and infectious diseases, stimulates B cell growth and development also play a role in increase Igs secretion (Zaynagetdinov *et al.*,2015).

The IL-5 gene is situated on chromosome 11 in the mouse and chromosome 5 in human , in close proximity to the genes encoding IL-3, IL-4 (Lee *et al.,* 1989), which are regularly co-expressed in Th2 cells. IL-5 is also expressed by eosinophils and has been observed in the mast cells of asthmatic airways by immunohistochemistry (Kaminuma *et al.*, 2005).

1.2.11.2.Role of IL- 5 and eosinophil in asthma

IL-5 plays principle role in eosinophil differentiation in the bone marrow, recruitment and activation at sites of allergic inflammation (Broughton *et al.*,2015). Human eosinophils express around a three-fold more elevated amounts of IL-5Ra compared with basophils (Kolbeck *et al.*, 2010). Th2 cells, mast cells, CD34 progenitor cells, invariant natural killer T, group 2 innate lymphoid cells, and eosinophils themselves are major cellular source of interleukin-5 (Phillips *et al.*,2003, Fallon *et al.*,2006 and Nussbaum *et al.*,2013). Group 2 ILCs are an important source of IL-5 contributing to tissue and blood eosinophilia (DeKruyff *et al.*,2014).

Interestingly, blood eosinophils demonstrate circadian cycling and ILC-2 control eosinophil number through the production of IL-5 (Nussbaum *et al.*,2013). Interleukin-5 modulates the differentiation and maturation of eosinophil in the bone marrow, their migration from blood to tissue sites (Shahabuddin *et al.*, 2000), and the prevention of eosinophil apoptosis (Ochiai *et al* .,1997). IL-5 additionally seems to modulate the development and functions of human basophils and mast cells. IL-5 enhances the release of mediators from human basophils (Dahinden *et al.*,1997) by means of the engagement of IL-5 receptor (Nussbaum *et al.*, 2013).

There is expanding proof that eosinophilic inflammation of the lungs is a sign of eosinophilic asthma and has been correlated with raised levels of interleukin-5 in bronchial biopsies from asthmatic patients (Hamid *et al.*, 1991 and George &Brightping, 2016).

Additionally, IL-5 mRNA is up regulated in the bronchial mucosa upon allergen challenge and IL-5 concentrations correlate with clinical features of asthma (Robinson *et al.,* 1993 ; Humbert *et al.,* 1997and Roufosse 2018).

1.2.12.Cysteine leukotriene

Leukotrienes (LTs) are considered to assume a significant role in the pathomechanism of a lot of diseases, such as bronchial asthma, and allergic rhinitis (Poff & balazy, 2004 and Pergola & werz, 2010).

In the some investigation have demonstrated that an increasing generation of the CysLTs in asthma contributes significantly to exacerbations of asthma symptoms (Kanaoka & Boyca, 2014).

The cysteinyl leukotriene ligands including leukotriene C4 (LTC4), D4(LTD4) andE4 (LTE4) are intense broncho-constrictors and pro-inflammatory mediators of atopic asthmatic (Bisgaard, 2001). They are released by mast cells and macrophages during asthma attacks (Severien *et al.*,2000). These verity of an asthmatic attack can be influenced by the cellular concentrations of these CysLTs (Maekawa *et al.,* 2002). These ligands regulate human airway contractions upon binding to the CysLT receptors (Kormann *et al.*, 2005).

LT assume to play a central pathophysiological role in asthma especially in particular subgroups of patients with asthma. Cysteinyl-LTs induce pathophysiological responses similar to those associated with asthma and elevated CyslT concentrations have been detected in biological fluids, including BAL and EBC from patients with asthma (Busse *et al*.,2005; Dahlen 2006 ; Peters-Golden& William,2007; Montuschi, *et al.,* 2007 ; Hallstrand *et al.*,2010 and Aggarwal *et*

al.,2010). The CysLTs are probably to contribute to airway remodelling that characterises persistent asthma (Holgate *et al.,* 2003 and Mehrotra *et al.*,2009). Two G-protein coupled receptor subtypes for CyLTs (CysLT1 and CysLT2) have been identified (Lynch *et al.*,1999). The vast majority of the effects of CyLTs relevant to the pathophysiology of asthma are mediated by activation of the CysLT1 receptor (Peters-Golden *et al.*, 2007and Montuschi *et al.*,2007), which is expressed in various types of inflammatory and structural cells in the airways (Lynch *et al.*, 1999 and Figueroa *et al.*,2001).

1.2.12.1. Receptors of cysteine leukotriene:

Including CysLTR1 ,CysLTR2 and CysLTE, represented by GPR99/OXGR1 and may constitute a third CysLTR (Kanaoka *et al.*,2013).

1.2.12.1.1.Cysteinyl leukotriene receptor 1

CYSLTR1, is a receptor for cysteinyl leukotrienes (LT) CYSLTR1, by binding to LTC4, LTD4, and to a significantly lesser degree , LTE4) that mediating different allergic and hypersensitivity reactions in humans as well as in animals models in that reactions (Singh *et al.*,2013).

The human CysLTR1 gene maps to the X chromosome at position Xq13-Xq21, contains three exons with the entire open reading frame located in exon 3, and codes for a protein made of 337 amino acids. The CYSLTR1 gene promoter region is separated from 665 to 30 bp upstream of its transcription start site(Zhang *et al*., 2006 and Liu & Yokomizo, 2015).

1.2.12.1.2.Function of cysteine leukotriene receptor 1

CysLTR1 is a G protein–coupled receptor that connects when bound to its CysLT ligands and lead to activates the Gq alpha subunit and additionally Ga subunit of its coupled G protein, contingent on the cell type. Acting through these G proteins and their subunits, ligand-bound CysLTR1 initiate and activates a series of pathways that lead to promote cell work .The request of strength of the cysLTs in stimulating CysLTR1 is LTD4, LTC4, LTE4, with LTE4 most likely lacking sufficient potency to have much activity that operates through CysLTR1 in vivo (Singh *et al.*, 2013).

1.2.12.1.3.Gene polymorphism of CysLTR1

The 927T/C (nucleotide thymine replaced by cytosine at position 97 of the CysLTR1 quality) gene polymorphism in the coding locale of CysLTR1 has been appeared to be predicative of the seriousness and severity of atopy (i.e. a predisposition toward developing certain allergic hypersensitivity reactions), yet not related with asthma, in humans of 341 caucasians families from the United Kingdom. This atopy seriousness was most clear in female kind, yet the frequency of this polymorphism is to a great degree low and the usefulness of the 927T/C gene and its product protein are up 'til now unknown.(Allen ,1980 and Hao *et al* .,2006)

1.2.12.1.4.Clinical significant of CysLTR1

These receptor work in responsive to CysLTs, CysLTR1 has all appear of being basically critical in medication a large number of the pathological reactions to CysLTs in people. Montelukast, Zafirlukast, and Pranlukast are specific receptor antagonist for the CysLTR1 but not CysLTR2. These medications are being used as well as appeared to be compelling as prophalaxis and chronic medicines for allergy and non-allergic sicknesses, (eg. allergen-prompted asthma and rhinitis, no steroidal anti-inflammatory drug-incited asthma and rhinitis , exercise-and cold air initiated asthma ; and young sleep apnea due to adenotonsillar hypertrophy) .(Haeggström *et al.*,2011 ;Anwar *et al.*, 2014; Kar *et al.*,2016 and Oussalah *et al.*,2016) However, reactions to these lukast drugs shift enormously with the medications demonstrating fairly high rates of poor reactions and ~20% of patients revealing no change in manifestations after treatment with these agents.(Szefler *et al.*,2005; Thompson *et al.*, 2006 and Kanaoka *etal.*,2013). It appears to be

conceivable that the reactions of CysLTR2, GPR99 or different receptors to CysLT's may associated to these diseases.(Austen *et al.,* 2009 and Bhosle *et al.,*2017)

The number of inhabitants in the little remote far South Atlantic Ocean island of Tristan da Cunha (266 perpetual, hereditarily separated occupants) endures a high predominance of atopy and asthma. The CysLTR1 gene product variation, 300G/S (i.e. amino corrosive glycine replaces serine at the 300 position of the CysLTR1 protein), has been appeared to be altogether connected with atopy in this population. The CysLTR1 300S variation exhibited significant increased sensitivity to LTD4 and LTC4 suggesting that this hypersensitivity underlies its relationship with atopy (Thompson *et al* .,2007 and Yaddaden *et al.,*2016).

1.2.12.2.Cysteinyl leukotriene receptor 2

CyLTR 2, additionally named CYSLTR2, is a receptor for cysteinyl leukotrienes (LT) .CyLTR2, by restricting these CyLTs (LTC4, LTD4, and to a substantially lesser degree, LTE4) adds to intervening different allergy and extreme hypersensitivity responses in people CysLTR1.appears to play the major role in mediating these reactions (Van Keer *et al.,*1976 and Takasaki *et al.,*2000).

The human CysLTR2 quality maps to the long arm of chromosome 13 at position 13q14, a chromosomal locale that has for quite some time been connected to asthma and other susceptible allergic diseases (Thompson *et al.,*2006) .The gene continent of four exons with all introns situated in the genes ' 5' UTR region and the whole coding area situated in the last exon. 'CysLTR2 encodes a protein made out of 347 amino acids and shows just unassuming closeness to the CysLTR1

quality in that its protein sharing just 31% amino acid character to the CysLTR1 protein (Fukai *et al.*,2004 and Singh *etal.*,2013) .

CyLTR 2 mRNA is co-communicated alongside CysLRR1 in human blood eosinophils and platelets, tissue mast cells, macrophages, airway route epithelial cells, and vascular endothelial cells. It is additionally communicated without CysLTR1 all through the heart, including Purkinje cells, adrenal organ and additionally some vascular endothelial, airway route epithelial, and smooth muscle cells (Zhang *et al.,* 2006, Singh *et al.*,2013, Kanaoka *et al.,* 2013, Bankova *etal.*,2014 and Cattaneo, 2015.)

1.2.12.2.1.Polymorphism of CysLTR2

Polymorphism in the CysLTR2 gene resulting in a single amino acid substitution, M201V (i.e. amino acid methionine changed for valine at the 201 position of CysLTR2 protein) has been negatively associated in transmission disequilibrium testing with the inheritance of asthma in separate populations of:

a) white and African-Americans from 359 families with a high prevalence of asthma in Denmark and Minnesota, USA.

b) 384 families with a high prevalence of asthma from the genetics of Asthma International Network.

The M201V CysLTR2 variant exhibits decreased responsiveness to LTD4 suggesting that this hypo-responsiveness underlies its asthma transmission-protecting effect (Brochu-Bourque *et al.*,2011) . Gene polymorphism variant in intron III the upstream region of CysLTR2 has been associated significantly with development of asthma in a Japanese population; the impact of this polymorphism

on the genes expression or product has not been determined (Fukai *et al.*, 2004) These results suggest that CYSLTR2 contributes to the etiology and development asthma and that drugs targeting CYSLTR2 may work in a manner that differs from those of CYSLTR1 antagonists (Fukai *et al.*, 2004)

1.2.12.2.2.Clinical significant of CysLTR2

The CysLT has ability to activate CysLTR2 that induces many of *in vitro* responses of cells and involved in allergic reactions as well as the in vivo allergic responses in different animal models as that occur in CysLT-induced CysLTR1 (Liu &Yokomoizo, 2015) .Otherwise, CysLT2 requires in about the 10-fold higher concentrations of LTD4, the mostly potent CysLT for CysLTR1, that lead to activate CysLTR2. However, the hypersensitivity and allergic responses in humans and animal models are significantly decreased by treatment with Zafirlukast, Montelukast, and Pranlukast drugs, which are considered as selective receptor antagonists of CysLTR1 while not CysLTR2 (Haeggström *et al.*,2011 ; Anwar *et al.*,2014 ;Kar *et al.*,2016 and Oussalah *et al.*,2016) .Allergic reactions models in CyslTR2-deficient mice also in a human mast cell line determine that mouse CyslTR2 and its human homolog CysLTR2 act to inhibit Cysltr1 and CysLTR1, respectively, therefore suggest that CysLTR2 may similarly inhibit CysLTR1 in human allergic diseases .The role of CysLTR2 in the allergic and hypersensitivity diseases of humans must await the development of selective CysLTR2 inhibitors (Jiang *et al.*,2007and Austen *et al.*,2009).

2.Materials & Methods

2.1 Materials

2.1.1 Instruments and Equipments

The instruments and equipments used in this study are illustrated in table(2.1).

Table (2.1): Instruments and Equipments.

NO	Instruments and Equipment	Company	Origin
1	Camera	Canon	Japan
2	Centrifuge	Kokusan	Japan
3	Deep freezer -80C°	Jermaks	Germany
4	Disposable syringe 5 ml	Sterile EO.	China
5	Disposable tips	Netheler-Hinz	Germany
6	Distillator	GFL	Germany
7	EDTA tube	AFCO	Jordan
8	Electrophoresis	Biometra	USA
9	ELISA Reader and Washer	BioTek	USA
10	Eppendorf Tubes 5.0 mL	Geneaid	Taiwan
11	Eppendrof Mini spin centrifuge	Hamburg	Germany
12	Gel Tube	AFCO	Jordan
13	Incubator	Memmert	Germany
14	Micropipettes 5-50, 0.5-10, 100-1000µl	Gillson Instruments	France
15	Nano drop	Biometra	USA
16	Sysmex XT-2000i autoanalyser	Sysmex	USA
17	Thermocycler PCR	Labnet	USA

18	UV–transilluminater	Optima	Italy
19	Vortex	IKA	USA
20	Water bath	Memmert	Germany

2.1.2 Commercial kits

The commercial kits used in the present study are illustrated in table (2.2).

Table (2.2): The commercial kits.

No.	Kit	Company	Country
1	**Genomic DNA Extraction Kit**	**Geneaid**	**USA**
	Collection tube 2ml		
	Elution buffer		
	GB buffer		
	GT buffer		
	GD column		
	W1 buffer		
	Wash buffer		
	Proteinase K 10mg/ml		
2	**AccuPower^{TM} PCR PreMix**	**Bioneer**	**Korea**
	dNTPs (dATP, dCTP, dGTP, dTTP)		

	KCl		
	Loading dye		
	$MgCl_2$		
	Tris.HCl pH 9.0		
	Taq DNA polymerase		
3	IgE- Kit	**Euroimmun**	**German**
4	Interleukin-5 Kit	**Peprotech**	**UK**
5	Restriction Enzym	**Biolab**	**Canada**

Table (2.3): IL-5 ELISA kits with its components

No	Items	Specifications
1	Biotinylated Detection Ab Diluent	1vial 10mL
2	Concentrated Biotinylated Detection Ab	1vial 120µL
3	Concentrated HRP Conjugate	1vial 120µL
4	Concentrated Wash Buffer (25×)	1vial 30mL
5	HRP Conjugate Diluent	1vial 10mL
6	Micro ELISA Plate	8 wells ×12 strips
7	Plate Sealer	5pieces
8	Reference Standard	1 vials
9	Reference Standard and Sample Diluent	1vial 20mL
10	Stop Solution	1vial 10mL
11	Substrate Reagent	1vial 10mL

Table (2-4) :Contents of IgE ELISA kit:

No	Items	Specifications
1	Antibody-coated microplate wells	12*8
2	Calibrator 1 500 IU/ml	1* 2 ml
3	Calibrator 2 100 IU/ml	1* 2 ml
4	Calibrator 3 10 IU/ml	1* 2 ml
5	Calibrator 4 0 IU/ml	1* 2 ml
6	Chromogen /substrate solution TMB/H2O2	1vial 12mL
7	Enzyme conjugate (peroxidase labeled)	1* 12 ml
8	Plate Sealer	5pieces
9	Positive control 1 (high IgE concentration)	1* 2 ml
10	Positive control 2(Low IgE Concentration)	1* 2 ml
11	Sample buffer	1* 100 ml
12	Stop solution (0.2 M sulfuric acid)	1vial 12mL
13	Wash buffer 10 * concentration	1* 100 ml

2.1.3 Marker and primers

Table (2.5) Illustrate the DNA ladder and primers used in present study.

Table (2.5): Marker

No.	**Marker**	Manufacturing company	Origin
1	50 bp DNA ladder	Intronbiotechnology	Korea
2	100 bp DNA ladder	Intronbiotechnology	Korea

2.1.3.1 Primers

CysLTR1 927 T/C and CysLTR2 (A/G) M201V gene polymorphism primers were designed (Thompson *et al.*, 2003; Kadry *et al.*, 2014) and these primers were provided from (Bioneer company, Korea) as following tables:

Table (2.6): The PCR primers with their sequence and amplicon size:

Primer	Sequence		Amplicon Size	
CysLTR1 927 T/C	F	CTCTCCTATATTTCTTTTCTGC	237bp	Kadry *et al.*, 2014
	R	CTATACTTTACATATTTCTTCTCC		
CysLTR2 M201V	F	GCTTAGAGCTGAATCTCTATA	150bp	Thompson *et al.*, 2003
	R	GTGTGTGATAGGGCAGGAAAC		

2.1.4 Chemicals

The chemicals used in the present study are mentioned in table (2.7).

Table (2.7): Chemical substances.

No.	Chemical	Company and Origin
1	Absolute Ethanol	Scharlau (Spain)
2	Agarose	BioBasic (Canada)
3	Ehidium Bromide	
4	Free nuclease water	Bioneer (Korea)
5	TBE buffer 10X	BioBasic (Canada)

2.1.4.1 Restriction enzymes

The restriction enzymes used in RFLP-PCR assay with their company and country of origin are mentioned in table (2.8)

Table (2.8) :Restriction enzymes

Restriction enzymes	SNP	Company/Country

Hpy188I	**T/C**	**New England Biolabs. UK**
NlaIII	**A/G**	

2.2 Methods

2.2.1 Patients

A total of 100 patients with asthma who have referred to Kerbala Allergy Specialized Center, they were suspected to have asthma according to their clinical manifestations, radiological changes, skin test and confirmed by allergen assay, and venous blood samples were collected .

The age of the patients are ranging from (20 - 50) years old including (48 males) and (52 females) .

Venous blood samples were taken from apparently healthy persons through the same period, these persons not suffering from any respiratory problems and have negative family history to asthma , include 60 persons (30 males and 30 females) with age range from (20-50) year approximately matched to that of patients all control group confirmed diagnosed by radiological X – ray and allergen assay who give negative results .

2.2.1.1 Blood Samples

After sterilized the area of aspiration of blood from cubital fossa vein by alcohol 70%, blood sample (5ml) was collected from each patients and control groups. The collected sample was transferred immediately into 2 tubes as follows:

A. 2 ml of blood in 5 ml tube (EDTA tube) used for eosinophil concentration and PCR technique for detection of CysLTR I 927 T/C and CysTLR II M201V Gene Polymorphism .

B. 3 ml of blood in plain tube (serum tube), then the blood samples were centrifuged (at 4700 RPM for 5 min) to obtain serum then frozen at -80 ºC for screening of IL-5 cytokine and IgE levels.

2.2.2 .ELISA method

2.2.2.1. IL-5 Assay

2.2.2.1. Test principle

This ELISA kit used by Sandwich-ELISA method. The micro ELISA plate provided in this kit should be coated with antibodies specific to human IL-5 as first step . Standards or samples added to appropriate micro ELISA plate wells will bind to the antibodies. Detection antibodies specific to human IL-5 and Avidin- (HRP) conjugate can then be added successively to each micro plate well. After incubation, free components are washed away. When the substrate solution is added to each well, only those wells that contain human IL-5, detection antibody and Avidin-HRP complex will appear blue in color. The enzyme-substrate reaction will be terminated and appears yellow color by adding sulphuric acid solution. The (OD) can be measured with spectrophotometry at a wavelength of 450 nm. The OD value is proportional to the

concentration of human IL-5. We calculate the concentration of human IL-5 in samples by comparing the OD of the samples with the standard curve.

2.2.2.1.2 .Preparation of items and solutions for ELISA Technique:-

1. Capture antibody. A volume (11 µg) of Goat anti –human IL-5 + (0.5 mg) D-mannitol. Vial contents was centrifuged and reconstituted in (110 µL) sterile water at concentration of 100mg/ml.

2. **Detection antibody**. A Volume (11 µg) of Biotilyated Goat anti-humaan IL-5+ (0.5 mg D-manitol. Vial contents was centrifuged and reconstituted in (110 µL) sterile water at concentration of 100mg/ml.

3. **Human IL-5 standard**.A volume(1µg) of recombinant human IL-5 +(2.2 mg) BSA+ (11 mg) D-mannitol. Vial contents was centrifuged and reconstituted in(1ml) sterile water at concentration of 1µg/ml.

4. **Avidin-HRP conjugate**. A volume (18 ml) vial was a liquated into two (9ml) and stored at frozen -20c.

5. **Phosphate buffer saline**.(10xPBS) was diluted to (1x PBS) in sterile water.

6. Wash Buffer. Tween-20 (0.05%) was diluted in PBS.

7-Block buffer:1%BSAin PBS

8. Diluent. Tween-20 (0.05%), BSA(0.1%) was diluted in PBS.

9. Plat preparation. Capture Ab was diluted with PBS to concentration of (0.50 µg /ml) and (100µL) was added to each well in plate. Plat was sealed and incubated overnight. Wells were aspirated to remove liquid and the plate was washed 4 time by using (300 µL) of wash buffer. After last wish, plate was inverted to remove residual buffer and blotted on paper towel .Block buffer (300 µL)was added to each well and then incubated for 1 hour at room temperature. plate was aspirated and washed 4 time

2.2.2.1.3 Assay procedure

1. A volume 100µL of standard or sample was added to each well and incubated for 2 hours at room temperature.
2. The plate was aspirated and wished 4 time by using microplate washer, Detection Ab (100µ) added and incubate for 2 hours at room temperature.
3. Aspirated and washed 4 times by using microplate washer.
4. A volume 100µL HRP Conjugate was added and incubate for 30 minutes at roomtemperature.
5. Aspirated and washed 4 times by using microplate washer.
6. Substrate Reagent(100µL) added and incubate for 15 minutes at at room temperature.
7. Stop Solution (50µL) was added and read at 405 nm immediately
8. The results was Calculated

2.2.3. Total IgE kit.

2.2.3.1 Test principle :

The test kit contains microtiter strips each with 8 break-off reagents wells coated with poly clonal antibodies against human IgE. In the first reaction steps, diluted patients samples are incubated in the wells .IgE included in the sample will bind to the antibodies .To detect the bound IgE , a second incubation is carried out using an enzyme-labeled antihuman IgE(enzyme conjugate) catalyzing a color reaction .the determination of the IgE concentration is measured by means of the a calibration curve using the calibration sera 1 to 4.

2.2.3.2 . Manual test performance

1-**Sample incubation** : 100µl of the calibrations , positive controls and patients samples were transferred into individual microplate wells according to the pipetting protocol .the plate were incubated for 30 minutes at room temperature (18 – 25 ºC).

2- **Washing :** Reagents wells were washed 3 times using 450µl of working strength wash buffer (program setting) .

3-**Conjugation incubation:** 100µl of enzyme conjugate (peroxidase –labeled anti-human IgE) were pipetted into each wells ,incubated for 30 minutes at room temperature (18 – 25 ºC).

4-Washing: The wells emptied,and washed as described above.

5- substrate incubation : 100µl of chromogen /substrate solution were pipetted into each wells .incubated for 15minutes at room temperature (18 – 25 ºC) (protect from sun light)

6-Stooping : 100µl of stop solution were pipetted into each wells in the same order and at the speed chromogen /substrate introduced.

7- Measurement :photometric measurement of the color intensity were made at the wavelength of 450nm and reference wave length between 620-650nm within 30 minutes after add stop solution (Euro immune, 2018).

2.3.Haematological study

2.3.1 Eosinophil counts

A total white blood cell counts (WBC) and absolute numbers were performed for all of EDTA-anticoagulated blood samples of patients and controls individuals using automated blood cell counter (Sysmex XT-200i).

2.4 Molecular Methods

2.4.1 Genomic DNA extraction

Genomic DNA from blood samples were extracted by using Accupower®Genomic DNA extraction kit (Whole Blood) Geneaid USA, and done according to company instruction as following steps:

1. A 200µl of whole blood was transferred to sterile 1.5ml microcentrifuge tube, and then added 20µl of proteinase K was added and mixed by vortex, then all tubes were incubated at 60℃ for 5 minutes.
2. Two hundred µl of GB buffer was added to each tube and mixed by vortex to achieve maximum lysis efficiency, and then all tubes were incubated at 60℃ for 5 minutes.
3. Two hundred µl of absolute ethanol was added to mixture and mixed by vortex for 10 second, and then briefly spin down to get the drops clinging under the lid. The lysate was carefully transferred into GD Binding filter column that fitted in a 2 ml collection tube, and then closed the tubes and centrifuged at 14000 rpm for 1 minute.
4. Lysate was discarded, and then 400µl Washing buffer 1 (W1) was added to each GD column, and centrifuged at 14000 rpm for 30 second.
5. Washing buffer 1 was discarded, GD column was transferred to 2 ml collection tube, and then 600µl Washing buffer 2 (W2) was added to each GD column, and centrifuged at 14000 rpm for 30 second.
6. Washing buffer 2 was discarded, GD backed to 2 ml collection tube, and then the tubes were centrifuged once more at 14000 rpm for 3 minute to completely remove ethanol.

7. GD column that containing genomic DNA was transferred to sterile 1.5ml microcentrifuge tube, and then added 100µl of pre -heated Elution buffer and left stand the tubes for 3 minutes at room temperature until the buffer is completely absorbed into the glass filter of Binding column tube.
8. Finally, all tubes were centrifuged at 14000 rpm for 30 second to elute DNA, and storage at -20℃ freezer.

2.4.2 .Genomic DNA Profiling

The extracted genomic DNA from blood samples was checked by using Nanodrop spectrophotometer (THERMO. USA), that check and measure the purity of DNA through reading the absorbance in at (260 /280 nm). as following steps:

1. After opening up the Nanodrop software, the appropriate application was chosen (Nucleic acid, DNA).
2. A dry wipe was taken and the measurement pedestals was clean several times. Then carefully pipetted 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then the pedestals were cleaned off and 1µl of DNA was added to measurement.

2.4.3. RFLP-PCR Technique

RFLP-PCR technique was performed for detection CysLTR1 927 T/C and CysLTR2 (A/G) M201V polymorphism genes polymorphism in asthmatic patients and in healthy control blood samples. This method was carried out according to (Thompson *et al.*, 2003; Kadry *et al.*, 2014) as following steps:

2.4.3.1. PCR master mix preparation

PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions as following in table 3-10.

Table (2-9): Mixture of PCR.

Mixture solution	**Volume**	**Concentration**
Target DNA	5µL	20 ng/µL
Nuclease free water	40µL	-
Forward primer	2.5µL of each primer	10pm/ µl
Reverse primers	2.5µL of each primer	10pm/ µl
Master mix	PreMix	1X
Total volume	50µL	-

PCR master mix components that previously mentioned in table above were placed in standard AccuPower PCR PreMix Kit that contains all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and loading dye). Then, all PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene. Korea).

2.4.3.2 PCR Program

This program was listed in table (3.11) and (3.12).

Table (2.10):Amplification conditions of CyTLRI 927 C/T gene.

Steps	Temperature	Time	No. of cycles
Initial denaturation	94 °C	5min	1 cycle
Denaturation	94 °C	30 sec	35 cycle
Annealing	59°C	30 sec	
Elongation	72 °C	20 sec	
Final elongation	72 °C	5min	1 cycle
Hold	4 °C	For ever	—

Table (2.11):Amplification conditions of M201V gene.

Steps	Temperature	Time	No. of cycles
Initial denaturation	95 °C	5 min	1 cycle
Denaturation	95 °C	30 sec	35 cycle
Annealing	55°C	30 sec	
Elongation	72 °C	30 sec	
Final elongation	72 °C	5 min	1 cycle
Hold	4 °C	forever	—

2.4.3.2. PCR product analysis

The PCR products were analyzed by agarose gel electrophoresis following steps:

1- 1% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µL of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixing the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 10ul of (100bp DNA Ladder) in First well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- The PCR products were visualized by using UV transilluminator was photographed using digital camera .

2.4.3.3. RFLP-PCR mix preparation

RFLP-PCR mix was prepared by using *NlaIII* and *Hpy188I* restriction enzyme for CysLTR1 927 T/C and CysLTR2 (A/G) M201V genes polymorphism, respectively. These reaction mix were done independent according to company instructions as following table:

Table (2.12) Mixture for genotype CysLTR1 927 T/C and CysLTR II M201V.

Mixture solution	Volume
PCR product	10µL

Restriction enzyme buffer 10X	2μL
Restriction enzyme (10 unit)	1μL
Free nuclease water	7μL
Total volume	20μL

After that, this master mix placed in Exispin vortex centrifuge at 3000rpm for 2 minutes, then transferred into incubation at 37°C overnight. After that, REFLP-PCR product was analyzed by agarose gel electrophoresis (2.5%) and product analysis as following :-

Table (2.13) Restriction enzymes and DNA Fragments size

Restriction enzymes	Gene	Genotype	Fragment size
NlaIII	**927 T/C**	CC	150 ,97 bp
		T/C	150 ,97 ,53 bp
		TT	150 bp
Hpy188I	**M201V**	GG	200 , 37 bp
		AG	237 ,200 ,37 bp
		AA	237bp

2.5. Statistics analysis

Data was translated into a computerized database structure . And using SSPS version 23.

3.Results& Discussion

3.1.Levels of selected outcome measurements in asthmatic patients

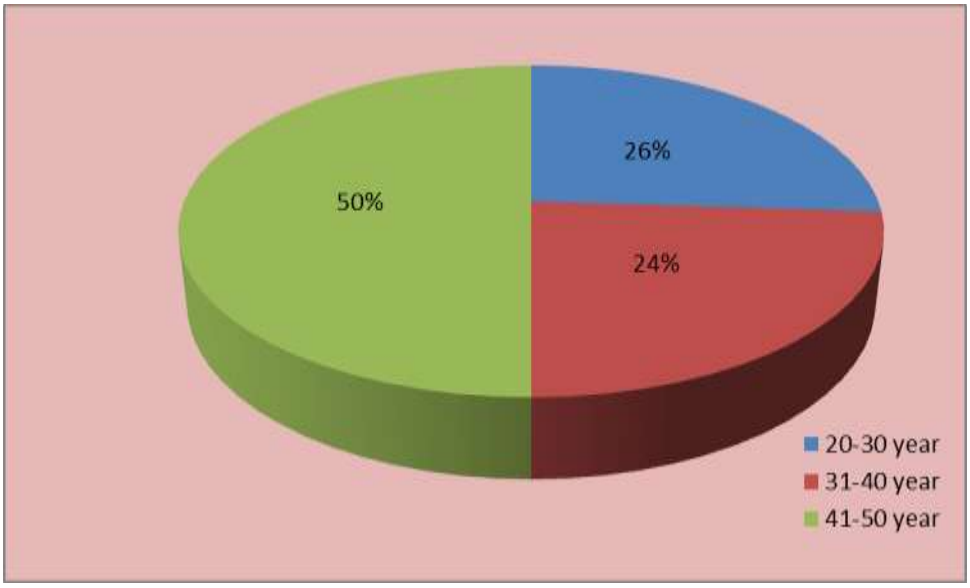


Figure (3-1) Distribution of patients according to age groups.

The results of this study recorded in figure (3-1) showed the highest percent of asthmatic patients in the age group more than 40 years old ,it is typically known as adult-onset asthma. Adult more than 40 may be more exposure to environmental allergen with decrease in immune activity also may be the result of common place irritants in the workplace (called occupational asthma) or home environments, and the asthma symptoms come on suddenly(Shaker *etal.*,2012).

These results agreed with (Shaker *etal.*, 2012) who found that most age with asthma patients in the range around 40

The results of table (3-1)show distribution of different parameters (IgE, IL-5 and Eosinophil)in asthmatic and health control group .These three parameters recorded highly significant difference between control and patients groups .

Figure (3-1) The distribution of patients and control group according to different parameters .

	groups	N	Mean	Std. Deviation	Std. Error Mean	P-Value
IgE	Patients	100	255.2047	208.90848	29.54412	.000
	controls	60	51.7754	27.33165	4.99005	
IL-5	patients	100	60.19314	23.588299	3.335889	.000
	controls	60	7.03540	3.670064	.670059	
Eosinophil	patients	100	.6132	.28590	.04043	.000
	controls	60	.2327	.07674	.01401	

There were increase mean levels of these three parameters may be due to the differentiation of naïve T cells within the Th2 subpopulation secreting many cytokine like IL-4 , IL-5 . IL-4 play a role for IgE synthesis that developed for immediate type of hypersensitivity reactions. IL-4 also important in isotype

switching from IgM to IgE which responsible for classic allergy and implicated in the pathophysiology of asthma . Expression of VCAM-1 on endothelial cells and for inducing the differentiation of Th2 cells .The production of IL-5 is very important for the eosinophils differentiation . mucus secretion and transmigration across endothelium (Miyazaki *etal.,* 2006). when Th2 cells produce the excessive IL-4 that associated with elevated IgE production and allergy (Cuvelier & patel, 2001).

The current study demonstrated additional support to the role of IgE in maintaining, mediating, and severity of the allergic response in asthmatic patients provided by the elevated levels of IgE when compared with healthy control , this is agreed with (Söderström *et al.,* 2011)and (Manoha &Selvakumaran, 2012), were founded an elevated IgE value is suggestive of the diagnosis, and explained test results T-IgE and there is markedly increase in levels of this antibody in patients with asthma compared with control .

In current study ,the mean levels of serum total IgE concentration were more than twice as high in asthmatic patients than that in controls, and the difference was highly significant ($P<0.0001$) in these groups of patients and controls this finding was agreed and compatible with (Agha *et al.,*1997 and Korn *et al.,* 2012).

In the presents study the mean level of blood eosinophil cells were statistically highly significant raised in patients with asthma when compared to healthy groups with P. Value ($P<0.0001$) .

Most asthmas are associated with Th2 cell-dependent production of IgE and the recruitment of eosinophils (Holgate ,2012).

The findings of present study were similar to study has recently been described that use any raise in blood eosinophil count as a useful biomarker to assess patients with eosinophilic asthma (Bleecker *et al* .,2016 and FitzGerald *et al.,* 2016) ,and other studies emphasize the importance of selecting patients based on raised in the number of eosinophils ($\geq$400 μL) in peripheral blood. Therefore , the blood eosinophils used as a baseline biomarker could help to select patients

who are likely to achieve more benefits in asthma control (Corren *et al.*, 2016 and Matera *et al.*, 2018).

According to the current study ,it shows an increase in eosinophil and IL-5 ,the interpretation of these results , that the immune response in patients with asthma characterized by the dominance of Th2 cell (Akdis *et al.*, 2004) , producing a different numbers of inflammatory cytokines specially including IL-5, which in turn affects other cells have an effective role in the pathogenesis of allergic diseases like eosinophil), by its activation , production ,and migration to the site of inflammation which is done by IL-5 binding with its specific receptors on the surfaces of these cells (Stafford *et al.*, 2002 ;Jacobsen *et al.*,2012 . At the end of this stimulation is a state called eosinophilia in addition to that there are production large amount of airway mucous and stimulate allergic hyper-responsiveness(AHR) (Cohn *et al.*,1998).

3.2.The association between asthma severity and selected outcome measurements.

Asthma severity classified into three groups to determine the difference in the three parameters according to GINA (2014) and Reddel & Levy2015) .The total peripheral IgE gives increase in mean levels but not high to give statistically significant difference among these three groups, but the IL5 and Eosinophil give significant difference between asthmatic patients and control group.

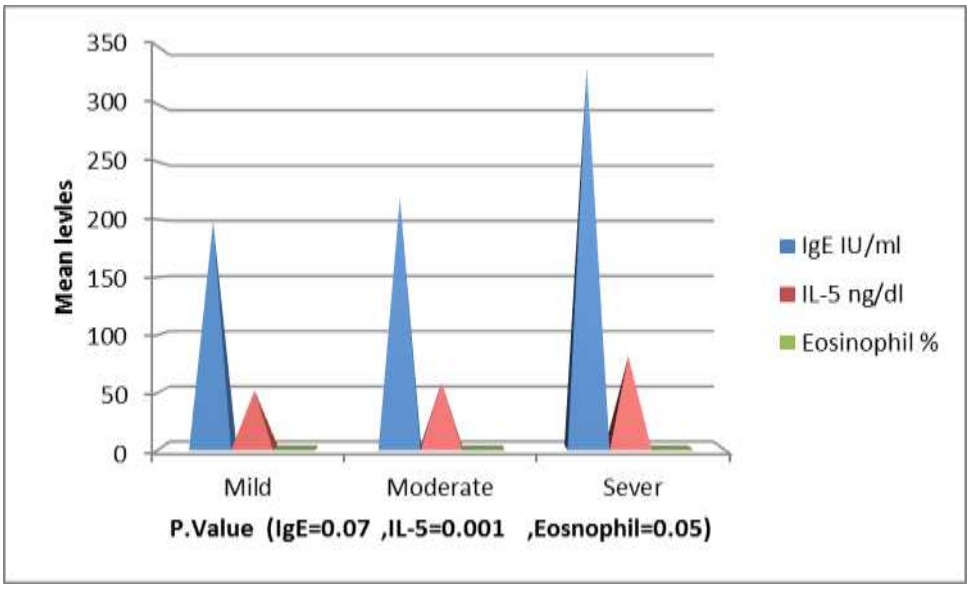


Figure (3-2) The distribution of mean levels of IgE ,IL-5 and Eosinophil according to severity groups.

Increase levels of total IgE may probably considered as nonspecific reaction secondary to airway inflammation in asthmatic cases.(Burrows *et al.*, 1995) The relative higher IgE levels may be proposed confined at the site of local inflammation and serum levels may not always necessarily reflect the level in lungs, also the asthma consider type I hypersensitivity and in this type the IgE binds to mast cells with high affinity. For that introduced previously can provide a possible explanation that why circulating IgE may not give an specific evidence for the severity of inflammation and that agreed with (Husain *et al.*,2007).

These agreed with other study done by (Kumar *et al* .,2017) that found no significant correlation between serum IgE and severity of asthma and similar study was observed by(Palomino *etal* .,2005 and Davila I *etal.*,2015) .In contrast the other studies achieved by(Borish L .,*et al* 2005 and de Marco *et al* .,2006) were reported a positive correlation between serum IgE and severity of asthma .

The blood eosinophil cells in this study recorded a significant correlation according to severity but consider not high significant .The explanation for reasons why blood eosinophil cells not give highly significant results according to severity , because its present in the intravascular space for short period only (Spector *et al*.,2012),also the important hypotheses explain the influx of peripheral blood

eosinophils at the site of local inflammation occur rapidly into the tissues that suggests the relationship between peripheral blood eosinophil count with inflammation of airway may be transitory (Hogan *etal.*,2008) ,and these results agreed with Kumar *et al* .,2017 was recorded there is a difference in blood eosinophil cells percent among different groups according to severity stages .

Varity of cytokines produced due to effect of IgE ,and these cytokine play an important role in developing of asthma pathogenesis and severity ,among of these cytokine is IL-5 (Linden, 2001).

This study reported that the serum concentration IgE, serum concentration IL -5 were slightly increased at the age range between 30-40 years but not enough to give statistically difference , The explanation of that age between 30-40 years represent by the immune system of patients at this age more active and these patients more contact with environmental allergens .

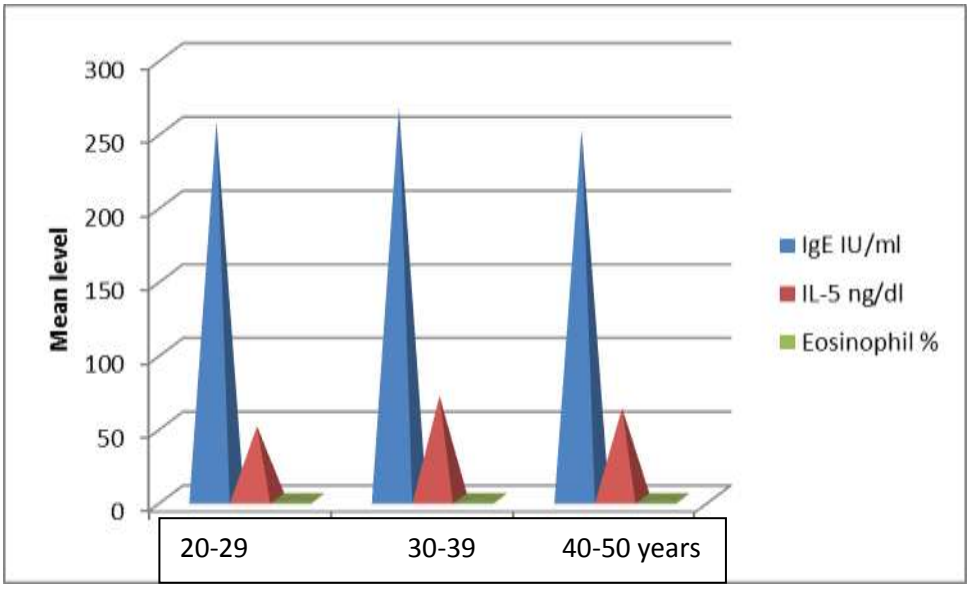


Figure (3-3) Differentiation in means of selected outcome measurements between the three age groups of asthma cases.

These agreed with other study that reported the total IgE levels in asthma cases were elevated and give significant differences in the levels of (Total -IgE) among different age groups in asthma, study achieved by Suaad&Jassim,2016) recorded that the age ranged between (30-39 years) give the highest level among other categories, also these findings are in agreement with the results of Brakhas *etal* .,(2015) achieved in Iraq, who reported that patients with certain levels of serum total IgE (more than 100 IU/ml) that gradually elevated with age, the high concentration being observed in the 31-40 year old group, and the level of T-IgE in allergic disease declined with age after 50 years old .the IL-5 and eosinophil recorded statically significant difference between age groups .

3.5.The distribution of selected outcome measurements in gender groups.

According to the results of this study , the mean of total serum concentration IgE recorded increase in females in comparison with males ,but these difference statistically non -significant with P.value (0.188) . Its known that the females as high risk for development of different types of disease especially immunological disease like autoimmune disorder . Because hormonal effects and differences between females and males ,some of these hormones have the ability to activate Th2 cells ,these cells have the ability to activate polyclonal B-cells and autoantibodies formation, with secretion of many pro-inflammatory mediators that play an important role in inflammatory process , disease pathogenicity and severity . that explain the majority of asthmatic patients are females rather than males. This results concurrence with study done by Wala'a *et al* .,(2006) .IL-5 and eosinophil also give non-significant difference between males and females.

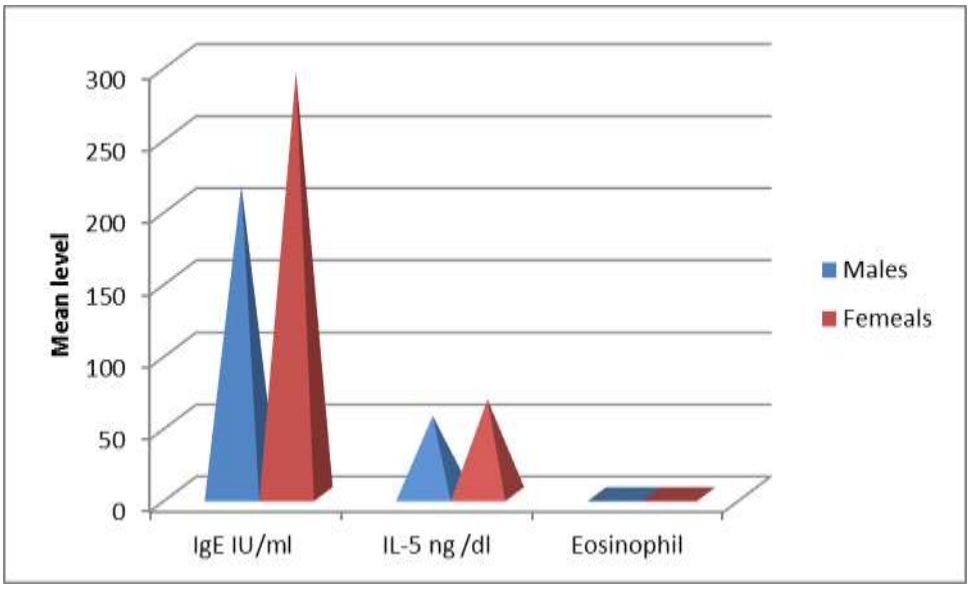


Figure (3-4) Differentiation of mean levels of selected outcome measurements between male and female gender of Asthma cases .

Although IL-4 regulating the production of IgE antibodies it possible give influence in the immunological mechanisms that lead to high rates of IgE expression in males compared with females Okano *etal.*,(2000). also the increase in mean of serum IL-5 and blood eosinophil cells in male than the females may be for the same resones make total serum IgE increase in males than in females.

3.6. Genetic study:

3.6.1.Cystine leukotriene receptor I .

In the study groups the DNA were extracted using (Genaid DNA extraction kit). Detection of results were done by electrophoresis on 1% agarose gel and exposed to U.V light in which the DNA appeared as compact bands figure (3-7).

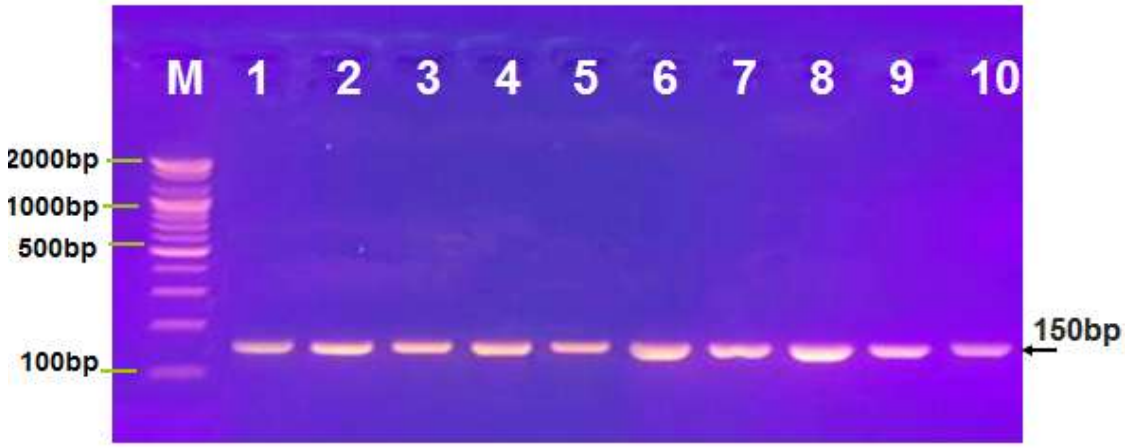


Figure (3-5): Agarose gel electrophoresis image that show the PCR product analysis of *CysLTR1*gene from blood patient samples(lane 1-5) and healthy control sample(lane 6-10). Where M: marker (2000-100bp), lane (1-10) positive gene amplification at 150bp PCR product size(100-2000).

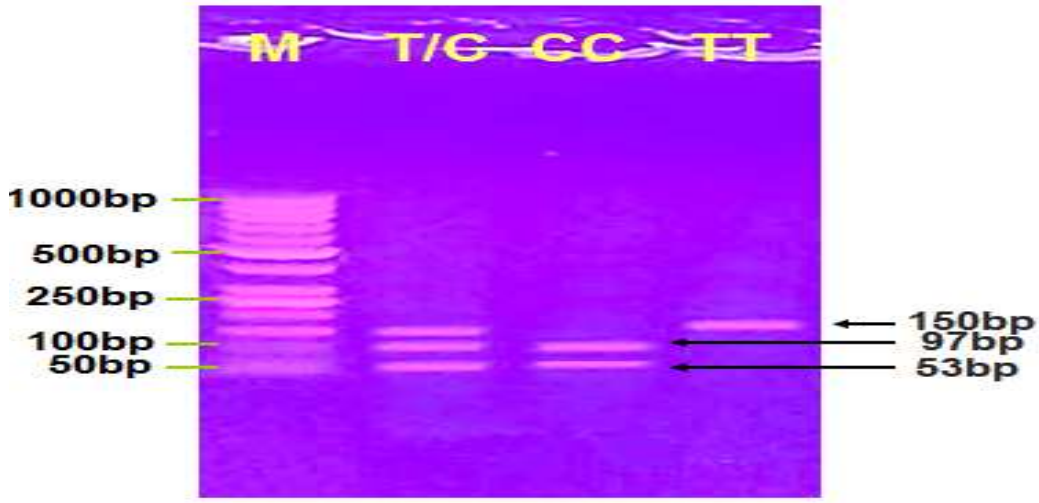
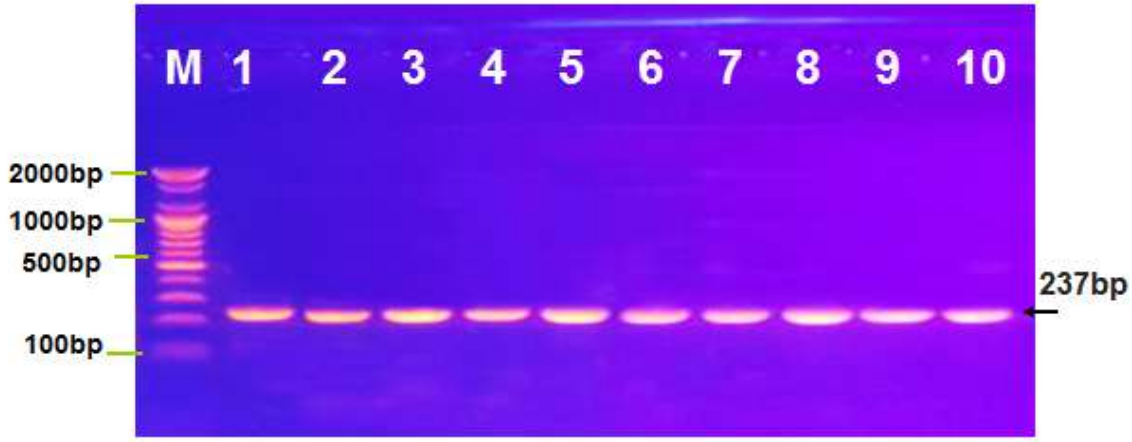


Figure (3-6): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of CysLTR1 927 T/C by using *Hpy188I* restriction enzyme. Where M: marker (50-1000 bp), lane (T/C) heterozygote, the product digested by restriction enzyme into 150bp, 97bp, and 53bp bands, lane (CC) homozygote, the product digested by restriction enzyme into 97bp, 53bp bands , and lane (TT) homozygote product undigested by restriction enzyme at 150bp bands.

3.6.1.Cystine leukotriene receptor II (CysLTRII M201V).

The products of successful binding were detected by gel electrophoresis and product size was 237 bpfrom both patients and control groups .Figure (3-4).



Figure(3-7): Agarose gel electrophoresis image that show the PCR product analysis of *CysLTR2* gene from some blood patient samples(lane 1-5) and healthy control sample(6-10). Where M: marker (2000-100bp), lane (1-10) positive gene amplification at 237bp PCR product size(100-2000).

Figure (3-8): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of CysLTR2 (A/G) M201V by using *NlaIII* restriction enzyme. Where M: marker (50-1000bp), lane (AA) homozygote product undigested by restriction enzyme at 237bp bands, lane (G/G) homozygote, the product digested by restriction enzyme into 200bp, 37bp bands, and lane (A/G) heterozygote, the product digested by restriction enzyme into 237bp, 200bp, and 37bp bands.

3.6.2. polymorphism in gene coded for CyLTR1 972 T/C

The results of figure (3-9) included the polymorphism in cysteine leukotriene receptor gene (CyLTR1 972 T/C) in patients and controls groups ,recorded T/C genotypes frequency represented high present and more frequents (46.0%) in patients group and less frequency in genotypes CC(12.0%),but when comper these results with controls groups found that the genotypes TT(40.0%) represented high present ,while CC genotypes also showed less frequently in control groups .

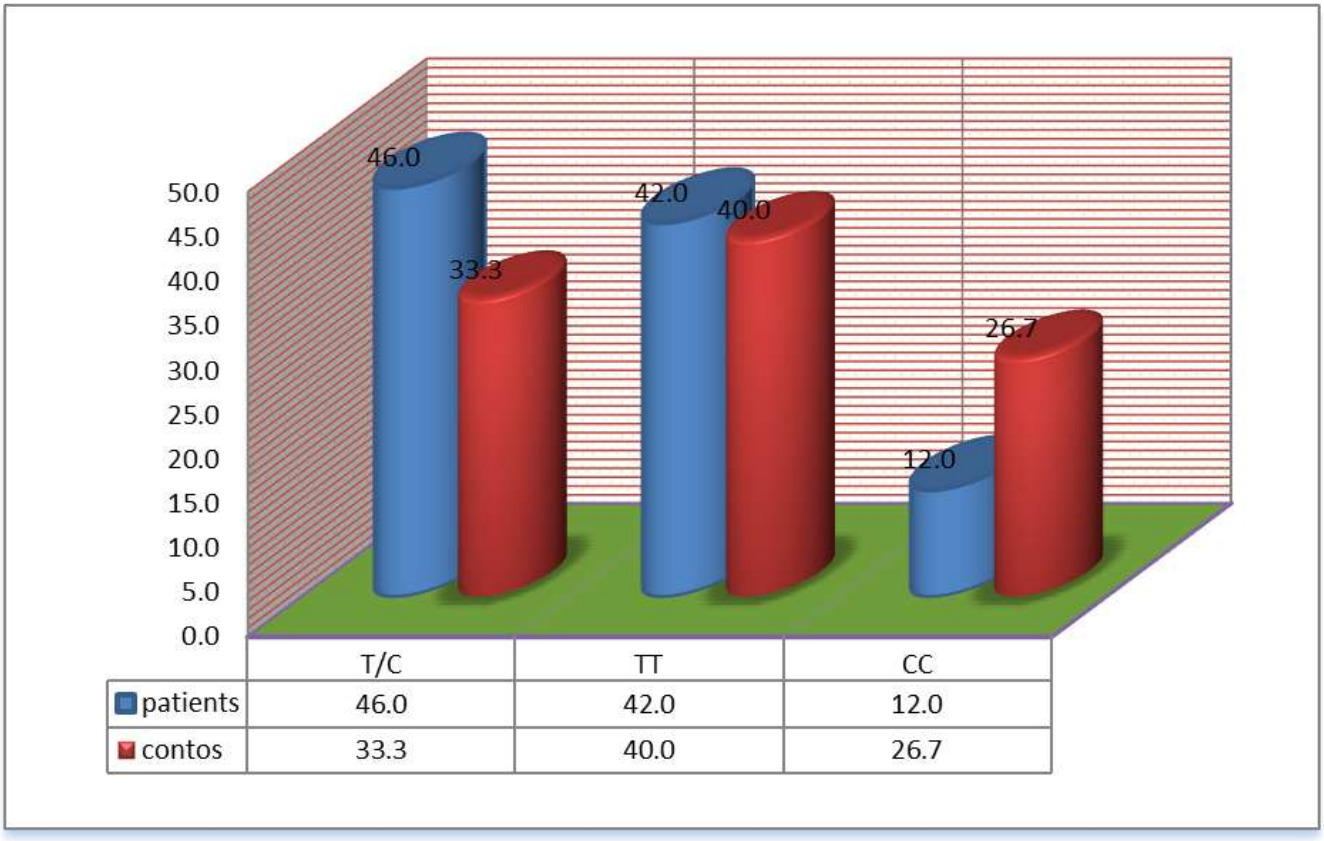


Figure (3-9) Distribution of percent of (CyLTR1 972 T/C)polymorphism in asthmatic patients and controls groups.

The presence of SNPs in both the CYSLTR1 and CYSLTR2 genes may increase the risk for asthma . In the present study, show different polymorphisms in two genes that are important in asthma development, CysLTR1 927 T/C and CysLR2 M201V. Although analysis the effects of the allelic and genomic combinations for both polymorphisms, suggested that most identified polymorphisms have influence on multifunctional diseases, the combinations of polymorphisms in genetic have higher functional effects than individual variants and that consider better explanation of susceptibility to asthma. Regarding to CysLTR1 927T/C gene polymorphism were done by RFLP PCR there is no statistical difference between control and asthmatic patients and these result was agreed with (Kadry *et al.*,2014). The CysLTR1 927T/C variant have been associated with asthmatic patients in population (Hong *et al.*,2009) .The polymorphism occur represented by this SNP was also found in Tristan da Cunha population (Thompson *et*

al.,2013),Usually not replicated in all studies about atopic asthma(Kadry *et al.*,2014), also there are many studies suggest that the variants of transcriptionally Active CYSLTR1 influence the onset and severity of asthma associated endophenotypes such as atopy (Sokolowska *et al.*, 2009).

3.6.2.1 Alleles frequency in Cysteine leukotriene receptor 1 (927 T/C) .

In table(3-2) depending on frequency of alleles for *CysLTR1*,the results show there was statistical difference in alleles frequency between the two groups of this studies in either females and males. However *T* allele was recorded more frequent in patient group (64.6 % for males & 65.4 % for females) differ in control group (53.6% for males & 59.4% for females). These differences were resulted from the presence only one copy of the X chromosome provided in males and two X chromosome in females These results agreed with the study of Arriba *et al.*, (2006) they recorded the statistically differences was a clear in the alleles distribution between genders group. And also other study done by Kadry *et al.*,(2014) they found T allele was reported increase in frequency in patient group (69.2% for males & 73.5% for females) than in healthy group (53.3% for males & 56.7% for females).

Table (3.2) Distribution of Alleles frequency for CyLTR1(927 T/C) in asthmatic cases and healthy groups.

	Patients		Controls		(OR) CI 95%
CysIR1 Positive Male	**No**	**%**	**No**	**%**	**1.58(0 .57-3.73)**
C	34	35.4	16	46.4	
T	62	64.6	30	53.6	

x^2=9.23					
P=0.002[HS]					

	Patients		Controls		(OR) CI 95%
CysIR1 Positive female	No	%	No	%	1.29(0.51-3.47)
C	36	34.6	26	40.6	
T	68	65.4	38	59.4	
x^2=13.5					
P=0.001[HS]					

3.6.2.2.Association of Cys LTR1(927 T/C) gene polymorphism and asthma severity.

The result of table (3.3) show there were no significant difference in TT and TC polymorphism, but significant difference in CC gene according to asthma severity. Both of C and T alleles show significant difference in severity groups that may be explain the CC gene polymorphism in patients with severe asthma have high expression of CYSLTR1 and that lead to causes: airways bronchoconstriction and hyper-responsiveness to bronchoconstriction agents such as histamine; increased vascular permeability, edema, influx of many cells like eosinophils and neutrophils, smooth muscle proliferation, deposition of collagen, and fibrosis in different tissue , also increase of mucin secretion by goblet cells, proliferation or metaplasia of goblet cells also other studies suggest that the variants of transcriptionally active influence the onset and severity of asthma associated endophenotypes such as atopy and that agreed with (kadry *et al.*,2014 and Sokolowska *et al.*, 2009).

Table (3.3) Distribution of patients with CyLTR1(927 T/C) gene polymorphism according to asthma severity.

Variable	mild	moderate	sever	P value

TT	16	20	*6*	0.917
TC	20	16	*10*	0.534
CC	2	2	*8*	0.044
Allele				
T	52	56	*22*	0.0358
C	24	20	26	0.0358

3.6.2.3.The levels of selected outcome measurements according toCysLR1 972 T/Cgene polymorphism.

The table (3.4) shows that the mean levels of serum IgE and serum IL-5 consider statistically highly significant difference among the CysLTR1gene morphology (T/C ,TT, C/C) when compered the mean levels of these parameters between asthmatic cases and health control group. The mean of blood eosinophil recorded the statistically significant difference in asthmatic patients and healthy control group those having T/C cysteine leukotriene receptor 1(CysLR1 972 T/C) .

Many studies demonstrated that the expression of CysLTRs in eosinophils was not unexpected (Bandeira –Melo *et al.,* 2002) that due to the contribution of cysteinyl LTs to their accumulation in airways of asthmatic patients ,and that has been well documented (Gauvreau *et al*., 2001; Ohshima *et al.,* 2002; Nagata and Saito, 2003 , Saito *et al.*, 2004).

Other study recorded the CysLT1R usually related with transduction signals of cytokine that lead to up-regulation of eosinophilopoiesis depending to the action of IL-13 in bone marrow of murine (Queto *et al.*, 2010). Other investigation recorded presence of inhibitory action of CysLT1R antagonists on activation and migration of eosinophil (Virchow *et al.,* 2001; Fregonese *et al.,* 2002; Suzuki *et al.,* 2003; Tanaka *et al.,* 2003; Saito *et al.,* 2004 and Nagata *et al.,* 2005), and also on adhesion (Fregonese *et al.,* 2002; Nagata *et al.,* 2002; Kushiya *et al.,* 2006; Meliton *et al.,* 2007 and Profita *et al.,* 2008).

There is other information usually available to determine the CysLTR antagonists ability to decrease eosinophili in airway and also eosinophil cationic protein (ECP) in humans (Laitinen *et al.*, 2005 and Kopriva *et al.*, 2006).And in animals (Ihaku *et al.*, 1999).

Table (3.4)Distribution of three parameters among asthmatic patients & control group according to difference in cysteine leukotriene receptor 1 (CysLR1 972 T/C) genotypes.

Group Statistics								
CysLR1 positive		**groups**	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**	**F-test**	**P-value**
TC	**IgE**	patients	46	243.09	205.92493	42.93832	16.34	**0.000 .HS**
		controls	20	56.237	30.47604	9.63737		
	IL-5	patients	46	56.206	25.464430	5.309701	17.50	**0.000 .HS**
		controls	10	8.0872	3.836939	1.213347		
	Eosinophil	patients	46	.5422	.31445	.06557	5.558	**0.025 .S**
		controls	20	.2450	.06852	.02167		
TT	**IgE**	patients	42	309.58	215.84235	47.10066	10.79	**0.003 .HS**
		controls	24	52.3945	32.32188	9.33052		
	IL-5	patients	42	66.815	23.115736	5.044267	14.18	**0.001 .HS**
		controls	24	6.18142	3.818082	1.102185		
	Eosinophil	patients	42	.6638	.26183	.05714	11.84	**0.002 .HS**
		controls	24	.2133	.07572	.02186		
CC	**IgE**	patients	12	111.29	129.67612	52.94005	42.59	**0.000 .HS**
		controls	16	45.2691	13.47139	4.76286		
	IL-5	patients	12	52.299	11.413595	4.659580	9.627	**0.009 .HS**
		controls	16	7.0016	3.336684	1.179696		
	Eosinophil	patients	12	.7083	.21665	.08845	10.84	**0.006 .HS**
		controls	16	.2463	.09102	.03218		

Expression of CysLT1Rs can demonstrate on B lymphocytes and CD34_ hematopoietic progenitor cells (Figueroa *et al.,* 2001). Many investigation recorded that LTD4 have ability to chemotaxis and transendothelial migration of CD34_ hematopoietic progenitor cells (Bautz *et al.,* 2001 and Mohle *et al.,* 2003) ,also related to their proliferation (Braccioni *et al.,* 2002; Parameswaran *et al.,* 2004 and Boehmler *et al.,* 2009) , also the different CysLT1 receptor antagonists have ability to reduce these activity , also these investigation recorded the a physiological role of cysteinyl LTs ,which considered as regulatory autocrine for hematopoiesis associated with expression of LTC4 in immature myeloid cells (Tornhamre *et al.,* 2003).

By association with cysteinyl LTs role in asthma, CysLT1Rs are expressed by different mucosal inflammatory cells of airway and these numbers of inflammatory CysLT1R-expressing cells (eosinophils, neutrophils, MCs, macrophages, and B lymphocytes but not T lymphocytes) and these consider as significantly elevated in patients with stable asthma and patients hospitalized for asthma exacerbation when these compared with control groups (Zhu *et al.,* 2005).

According to the results of table (3.5) found there is no significant difference in IgE and IL-5 and Blood eosinophil between these three genotype of CysLR1 972 T/C ,that mean the all patients recorded increase in these three parameters even with the difference in CysLR1 972 T/C genotypes and that lead to give non-significant difference between these three groups .

Some investigators demonstrated the activation of CysLT1R lead to propagation of the some inflammatory reaction that happened by the release of various cytokines and inflammatory mediators. On the other hand these cytokines have ability to regulate expression of CysLTR, that provided by the LTD4 triggered the release of metabolites of arachidonic acid into the culture medium, that effect that can suppressed by the antagonist (Mattern *et al.,* 1990). Many other investigator recorded that the CysLT1R have ability to stimulate release of cytokines from Th2 , Like IL-4 from cord blood-derived eosinophils (Bandeira-

Melo *et al.*, 2002), IL-5 from MNCs (Nabe *et al.*, 2002; Frieri *et al.*, 2003; Faith *et al.*, 2008).

Figure (3-5) Distribution of three parameters among asthmatic patients according to difference in three cysteine leukotriene receptor 1 (CysLR1 972 T/C) genotypes.

	CysLR1 972 T/C gene in asthmatic patients			
	TC	**TT**	**CC**	**P**
IgE				0.11[NS]
Range	(10.65to 689.60)	(29.31 to 833.30)	(20.52to 278.56)	
Median	221.3300	285.82	33.53	
N	46	42	12	
Mean=	243.09	309.59	111.30	
IL-5				0.29[NS]
Range	(23.58 to 98.31)	(37.98to 121.76)	(39.65 to 66.31)	
Median	52.90	64.87	50.21	
N	46	42	12	
Mean=	56.21	66.82	52.30	
Eosinophil				0.26[NS]
Range	(0.12 to 1.41)	(0.23 to 1.21)	(0.55 to 1.03)	
Median	0.51	0.62	0.59	
N	46	42	12	
Mean=	0.54	0.66	0.71	

Other investigators demonstrated the expression of human CysLT1R can up-regulated by augmented transcriptional activity of priming cells with cytokines produce by Th2 (i.e., IL-5 in eosinophil-differentiated cells) (Thivierge *et al.*, 2000) also IL-13 and IL-4 in monocyte-derived macrophages and human monocytes (Thivierge *et al.*, 2001 and Shirasaki *et al.*, 2007).

Many investigators that recorded the B lymphocytes have ability to increase their expression from CysLT1R after exposure to a combination of activating anti-CD40 antibody and IL-4 . These increase in CysLT1R expression leads to induce an increase in responsiveness to LTD4 in terms of Ca2_ flux and up-regulation of IgG and IgE production. All of that can prevented by the specific CysLT1R antagonist montelukast (Lamoureux *et al.*, 2006), also other investigator Rovati &Capra ,2007 found that IL-4, but not IL-13, have ability to significantly induce mRNA and protein concentrations for CysLT1R from Band T cells .depending on these studies discussed above conclude the elevated levels of these three parameters in asthmatic patients with difference in three cysteine leukotriene receptor 1 (CysLR1 972 T/C) genotypes and also need more investigation in this field .

3.6.2.4 The levels of parameters among asthmatic patients according to difference in each two cysteine leukotriene receptor 1

The table (3.6) show the comparison for the mean serum IgE level , serum IL-5 level and blood eosinophil between each two asthmatic groups according to polymorphism in Cysteine leukotriene receptor 1(CysLR1 972 T/C).

The results of this table recorded statistically non-significant difference in mean levels of these three parameters in each two polymorphic groups. While only the mean level of IL-5 show statically significant difference between the T/C ,TT and C/C groups . Depending on the result previous recorded show increase in these three parameters in asthmatic patients and other show non-significant in these parameters between three genotypes of cysteine leukotriene receptor1.Depending on that and due to the studies and investigation were very

rear about the relation of these parameters and polymorphism in this receptor and the role of CysLR1 972 T/C coded by T/C, T/T genotype .

Figure(3-6)Distribution of three parameters among asthmatic patients according to each two CysLR1 972 T/C genotypes groups .

	CysLR1 positive	N	Mean	Std. Deviation	Std. Error Mean	F-test	P-Value
IgE	T/C	46	243.090	205.9249	42.93832	0.50	0.825 [NS]
	TT	42	309.589	215.8423	47.10066		
IL-5	T/C	46	56.2063	25.46443	5.309701	0.53	0.471 [NS]
	TT	42	66.8150	23.115736	5.044267		
Eosinophil	T/C	46	.5422	.31445	.06557	0.023	0.880 [NS]
	TT	42	.6638	.26183	.05714		
IgE	T/C	46	243.0908	205.92493	42.93832	1.457	0.238 [NS]
	CC	12	111.2955	129.67612	52.94005		
IL-5	T/C	46	56.20630	25.464430	5.309701	4.493	0.043 [S]
	CC	12	52.29950	11.413595	4.659580		
Eosinophil	T/C	46	.5422	.31445	.06557	0.14	0.706 [NS]
	CC	12	.7083	.21665	.08845		
IgE	TT	42	309.5892	215.84235	47.10066	0.68	0.415 [NS]
	CC	12	111.2955	129.67612	52.94005		
IL-5	TT	42	66.81500	23.115736	5.044267	2.43	0.04 [S]
	CC	12	52.29950	11.413595	4.659580		
Eosinophil	TT	42	.6638	.26183	.05714	0.17	0.678 [NS]
	CC	12	.7083	.21665	.08845		

3.6.2.5.The correlation among three parameters depending on CysLR1 972 T/C polymorphism.

Table (3.7) shows correlation among three parameters in asthmatic patients depending on the differences in CysLR1 972 T/C polymorphism .

The results of this table recorded a correlation between serum IgE ,serum interleukin 5 and elevated of blood eosinophil in patients group having T/C ,these correlation reach to highly significant for correlation between these there parameters . The TT genotype give a significant correlation between these three parameters but these correlation consider less than T/C , also there are minimum correlation for IL-5 IgE, blood eosinophil in patients with CC genotype .

The correlation of these three parameters (IgE, IL-5, Eosinophil) reported in this table obviously occur , Oettgen *etal* .,1999 ,Oettgen& Geha, 2016 ,Rivas *et al.,* 2016, Incorvaia *etal.*,2017 recorded the main role of IgE in pathophysiology of patients with allergic disease , especially hypersensitive asthma.

The immunoglobulin E bind on the surface of mast cells and basophil due to cross linked with allergen after that subsequently cause release of inflammatory mediators and finally cause symptoms of asthma.(Holgate ,2012) ,Also the IgE has an important function in activation of T cells through IgE-facilitated allergen presentation T cell-derived cytokines such as IL-5 (Interleukin 5) and IL-13 contribute to mucus secretion and eosinophilic infiltration.

Table (3.7)Correlation of three parameters among asthmatic patients according to difference in cysteine leukotriene receptor 1(CysLR1 972 T/C)genotypes

groups	CysLR1 positive			IL-5	Eosinophil
patients	T/C	IgE	Pearson Correlation	.656	.855
			Sig. (2-tailed)	.001	.000
			Number	46	46
		IL-5	Pearson Correlation	1	.716
			Sig. (2-tailed)		.000
			Number	46	46
	TT	IgE	Pearson Correlation	.669	.317
			Sig. (2-tailed)	.001	.161
			Number	42	21
		IL-5	Pearson Correlation	1	.158
			Sig. (2-tailed)		.493
			Number	42	21
	CC	IgE	Pearson Correlation	.804	.528
			Sig. (2-tailed)	.054	.282
			Number	12	12
		IL-5	Pearson Correlation	1	.915
			Sig. (2-tailed)		.010
			Number	12	12

3.6.3. polymorphism in cysteine leukotriene receptor 2 (CyLTR2 M201V) gene.

The results of figure (3.10) included the polymorphism in CyLTR2 M201V in patients and controls groups. In this results the A/G genotypes frequency represented high present and more frequents (54.0%) ,A/A (24. 0%), and less frequency in genotypes G/G(22.0%), but when camper these results with controls groups found that the genotypes A/G(60.0%) represented high present , while A/A genotypes and the G/G genotypes all of them showed equals percent (20%) frequently in control groups .

There are many independent groups have identified significant associations between the CYSLTR2 gene loci and asthma (Park *et al*., 2005; Thompson *et al*., 2013), also Cysteine receptors type 2 is consider as a candidate gene for atopy and asthma ,that depending of its map to region of chromosome 13q14 that has been related to atopy and asthma (Yokouchi *et al.*, 2000, Ober&Hoffija,2006) .

A study done by Tristan da Cunha identified by atopy associated with Cysteine leukotriene receptor types 2 Met 201 Val (M201V),formerly reported as Met202 val) variant, these receptor different from cysteine leukotriene receptor 1 variant is partially in activated .on the other hand the CYSLTR1 and CYSLTR2 are consider polymorphic in different individuals that represented co-expression of variant receptors may change CysLT signaling .Farther more CYSLTR2 can consider as target for photolytic CYSLTR1 antagonist.(Dong *et al;* 2013)

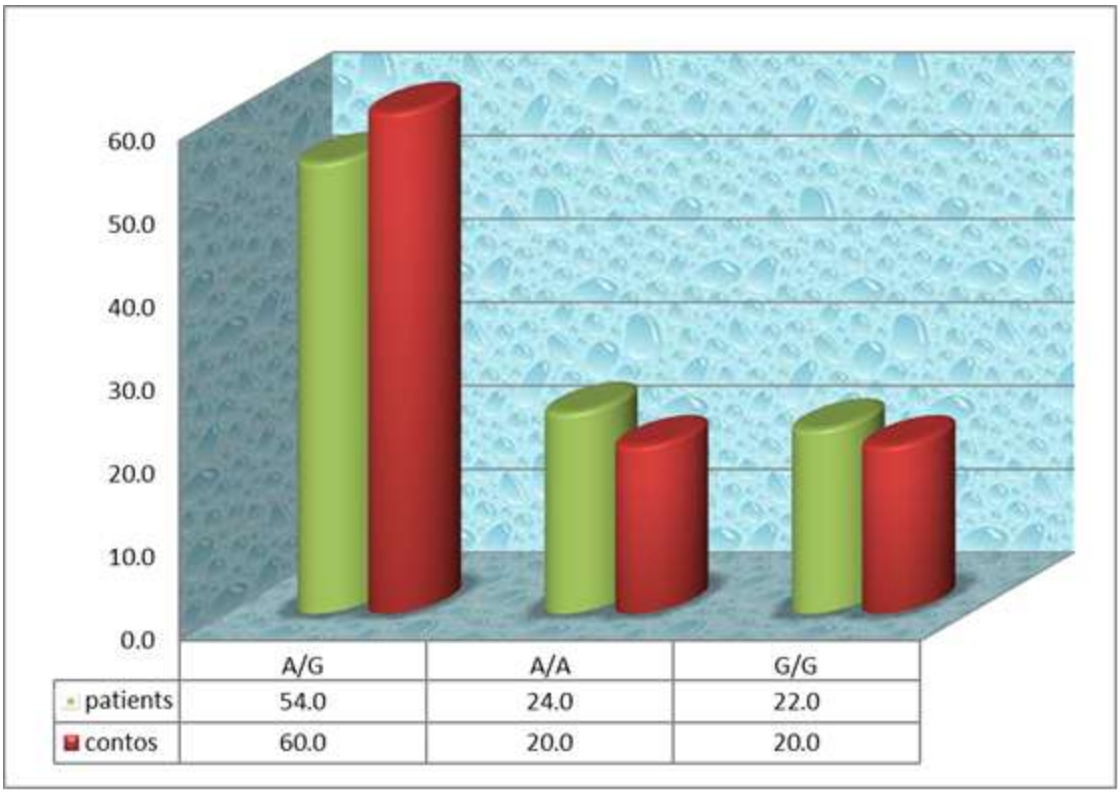


Figure (3-10) Distribution of percent of cysteine leukotriene receptors type 2 gene (CyLTR2 M01 V)polymorphism in patients and controls groups.

In the other genetic variables that associated with asthma , difficult to distinguish these variables considered as protective factor for some populations and risk factors for others peoples. It can be explained by study done by Wysocki 2011an endoganous isolation affected by a high frequency of asthma . that find the CYSLTR2 M201V SNP was related with some of atopy like sensitization to cockroach, Now found a possibility of functional CYSLTR2 M201V variants may associated with asthma in causative or protective manner in different population.

3.6.3.1Association of Cys LTR2(M201V) gene polymorphism and asthma severity.

The results of table (3-8) that show there were significant difference in AA and AG polymorphism and GG gene according to asthma severity. Both of A and G alleles show significant difference in severity groups that may be explain the AA and AG different in gene polymorphism increased in patients with mild and

moderate asthma, but GG gene associated with severe asthma .The A allele associated with mild and moderate, but G allele associated with severe asthma have high expression of M201V CysLTR2 variant associated with GG geneand that exhibits decreased responsiveness to LTD4 suggesting that this hypo-responsiveness underlies its asthma transmission-protecting effect.(Brochu-Bourque *etal.*,2011) . Gene polymorphism variant of CysLTR2 has been associated significantly with development of asthma in a Japanese population; the impact of this polymorphism on the genes expression or product has not been determined.(Fukai *etal.,* 2004) .These results suggest that CYSLTR2 contributes to the etiology and development asthma and that drugs targeting CYSLTR2 may work in a manner that differs from those of CYSLTR1 antagonists.(Fukai *etal.,* 2004)

Table (3.8) Distribution of patients with CysLTR2(M201V)gene polymorphism according to asthma severity.

Variable	mild	moderate	sever	P value
AA	12	12	*0*	*P*=0.007*
AG	24	26	*4*	*P*<0.001*
GG	2	0	20	*P*<0.001*
Allele				
A	48	50	*4*	*P*<0.001*
G	28	26	44	*P*<0.001*

3.6.3.2.The levels of three Parameters in difference in cysteine leukotriene receptor 2 (CysLR2 M01 V) genotypes.

In table (3.9) the comparison of the results of levels of serum IgE , serum IL-5 and blood eosinophil percent between asthmatic cases and healthy control group depending on Cysteine leukotriene receptor 2 there are three gene morphology(AA,AG,GG).

The results of serum IgE, serum IL-5 and blood eosinophil reported statistically highly significant results difference in people have AG morphology of Cysteine leukotriene receptor 2 M01V gene between asthmatic patients and healthy control group .

Figure (3-9)Distribution of three parameters among asthmatic patients & control group in Each gene of cysteine leukotriene receptor 2 (CysLR2 M01 V) genotypes.

Group Statistics								
CysLR2 positive		groups	N	Mean	Std. Deviation	Std. Error Mean	F-test	P-value
AG	IgE	patients	54	258.2167	177.56415	34.17224	21.172	0.000 [HS]
		controls	36	55.7453	29.77046	7.01696		
	IL-5	patients	54	61.79944	17.275990	3.324766	19.899	0.000 [HS]
		controls	36	5.89033	2.166976	.510761		
	Eosinophil	patients	54	.6248	.25574	.04922	11.682	0.001 [HS]
		controls	36	.2217	.03959	.00933		
AA	IgE	patients	24	113.8490	100.29215	28.95185	6.272	0.023 [S]
		controls	12	32.6327	12.61530	5.15018		
	IL-5	patients	24	37.17775	6.573565	1.897625	8.569	0.010 [S]
		controls	12	4.38800	1.671846	.682528		
	Eosinophil	patients	24	.5408	.32715	.09444	3.036	0.101 [NS]
		controls	12	.1600	.08877	.03624		
GG	IgE	patients	22	402.0177	270.36636	81.51852	8.376	0.011 [S]
		controls	12	59.0087	24.51450	10.00800		
	IL-5	patients	22	81.35809	27.703840	8.353022	9.515	0.008 [HS]
		controls	12	13.11800	1.353983	.552761		
	Eosino	patients	22	.6636	.32141	.09691	8.498	0.011

	phil							**[S]**
		controls	**12**	**.3383**	**.02787**	**.01138**		

In the asthmatic patients and health group having AA Cysteine leukotriene receptor 2 M01V gene polymorphism , There are statistically significant difference in the results of IgE ,IL-5, and statistically non-significant result for blood eosinophil between patients and healthy group.

The people having GG Cysteine leukotriene receptor 2 M01V gene polymorphism, the result of IL-5 consider statistically highly significant between patients and healthy cases .While statistically significant result for IgE and blood eosinophil among patients and healthy group.

Finally in the Cysteine leukotriene receptor 2 M01V gene coded by GG and AG recorded highly effect for production of the parameters ,that may be due to that genes increase expression of coded receptors or the receptors coded by these genes give highly effect on production of IgE,IL-5,eosinophils. That agreed with some investigation related to found the levels of CysLTR subtype expression in peripheral blood leukocytes of the human (eosinophils, T lymphocytes , monocytes, and neutrophils) ,was determinate significantly increase in expression for CysLT2 gene polymorphism . (Mita *et al.*, 2001).

The results of table (3-10) show distribution of three parameters (IgE ,IL-5,Eosinophil) according to patients with cysteine leukotriene receptor type 2 (CysLR M201V gene)polymorphism .

Figure (3-10) Distribution of three parameters among asthmatic patients according to difference in cysteine leukotriene receptor 2(CysLR2 M 201 V)genotypes .

	CysLR2 M201V gene in asthmatic patients			
	A/G	**A/A**	**G/G**	**P**
IgE				**0.003 [HS]**
Range	(16.64to 599.90)	(10.65 to 306.56)	(15.40 to 833.30)	
N	54	24	22	
Mean=	258.22	113.85	402.02	
IL-5				**0.000[HS]**
Range	(28.48 to 98.31)	(23.58 to 46.87)	(24.53 to 121.76)	
N	54	24	22	
Mean=	61.80	37.18	81.36	
Eosinophil				**0.571[NS]**
Range	(0.12 to 1.41)	(0.13 to 1.21)	(0.13 to 1.23)	
N	54	24	22	
Mean=	0.62	0.54	0.66	

The IgE and IL-5 parameters show high significant difference in asthmatic patients according to difference in polymorphism of CysLR2 M 201 V genes while there is increase in level of eosinophil but not reach to give statistically significant difference for eosinophil .

Because this study may be considered as the first one deals with the relation of polymorphism in CysLR2 M 201 V gene and these three parameters ,there for there was not found any studies recorded this relation to compar it with our study

3.6.3.4. Levels of three parameters between each two cysteine leukotriene receptor 2 (CysLR2 M01 V) genotypes groups .

Table (3-11) recorded the difference in mean levels of IgE and IL-5 show statistically significant differences between asthmatic cases having A/G and A/A groups ,but blood eosinophil not show significant difference between these two groups. The difference between AG and GG groups not show any significant difference for mean values of serum IgE ,serum IL-5 and blood eosinophil .The results of the AA and GG groups show the significant difference in mean level of serum IgE ,and not recorded any significant difference in serum IL-5 and Blood eosinophil .

According to the results of this table there is significant difference in serum IgE and IL-5 in asthmatic cases among patients having AA polymorphism of CysLR2 M01 V and patients having GG and GA polymorphism for this receptors .

The previous studies about the correlation of these parameters were very rare ,and present study may be considered as the first study according to our knowledge to find the relation between these parameters. According to the results of the present study previously recorded the concentration of IgE and IL-5 show low levels in patients with AA genotypes when comper with patients AG and GG genotypes ,that explain the significant difference , Investigation done by Mita *et al.,* (2001) recorded increase expression of CysLR2 M01 V in eosinophil ,but AA genotypes not show high levels for these parameters.

Table(3-11)Distribution of three parameters among asthmatic patients according to difference in each two cysteine leukotriene receptor 2 (CysLR2 M01 V) genotypes groups .

	CysLR2 positive	N	Mean	Std. Deviation	Std. Error Mean	F-test	P-Vaue
IgE	A/G	54	258.2167	177.56415	34.17224	3.992	0.047 [S]
	A/A	24	113.8	100.29215	28.95185		
IL-5	A/G	54	61.79944	17.275990	3.324766	5.900	0.020 [S]
	A/A	24	37.177	6.573565	1.897625		
Eosinophil	A/G	54	.6248	.25574	.04922	0.513	0.478 [NS]
	A/A	24	.5408	.32715	.09444		
IgE	A/G	54	258.2167	177.56415	34.17224	2.523	0.121 [NS]
	G/G	22	402.07	270.36636	81.51852		
IL-5	A/G	54	61.79944	17.275990	3.324766	3.505	0.069 [NS]
	G/G	22	81.358	27.703840	8.353022		
Eosinophil	A/G	54	.6248	.25574	.04922	1.187	0.283 [NS]
	G/G	22	.6636	.32141	.09691		
IgE	A/A	24	113.8490	100.29215	28.95185	7.440	0.013 [S]
	G/G	22	402.07	270.36636	81.51852		
IL-5	A/A	24	37.17775	6.573565	1.897625	11.729	0.003 [HS]
	G/G	22	81.358	27.703840	8.353022		

Eosinophi	**AlA**	**24**	**.5408**	**.32715**	**.09444**	**0.06**	**0.7[NS]**
	G/G	**22**	**.6636**	**.32141**	**.09691**		

3.6.3.5.Correlation of three parameters in CysLR2 M01 V gene polymorphism

Table (3.12) show correlation among three (IL-5,IgE,Eosinophil) parameters in asthmatic patients depending on the differences in CysLR2 M01 V polymorphism .the result of this table recorded a correlation between serum IgE ,serum IL- 5 and elevated of blood eosinophil in patients group having G/G ,these correlation reach to highly significant for correlation between these there parameters .

The A/G genotype give a significant correlation between these three parameters but these correlation consider less than G/G. also there are minimum correlation for IL-5 IgE, blood eosinophil in patients with AA genotype .

This table shows the correlation between these three parameters(IgE ,IL-5 an Eosinophil) , there were specific correlation among these three parameters ,that because the important role of IL-5 have ability to plays a fundamental role in the proliferation, maturation of eosinophil in the bone marrow, recruitment and activation at sites of allergic inflammation of eosinophils. The engagement of interleukin-5R through the interaction of IL-5 with interleukin-5aR and results in differentiation and maturation of eosinophils in the bone marrow, enhanced cell migration, release of granule proteins. The IL-5 consider as important mediator in activation of eosinophil which produced by Th-2 cells and mast cells, usually in allergic patients and also occur in infectious diseases, that IL-5 have ability to stimulates B cell growth and development also play a role in increase immunoglobulin secretion there for explain these relation among these three parameters ,and this agreed with (Zaynagetdinov *et al;* 2015).

Table (3.12)Correlation of three parameters among asthmatic patients according to difference in cysteine leukotriene receptor 2(CysLR2 M 201 V)genotypes

groups	CysLR2 positive			IL-5	Eosinophil
patients	A/G	IgE	Pearson Correlation	.229	.452
			Sig. (2-tailed)	.250	.018
			N	54	54
		IL-5	Pearson Correlation	1	.477
			Sig. (2-tailed)		.012
			N	54	54
	A/A	IgE	Pearson Correlation	.300	.116
			Sig. (2-tailed)	.343	.721
			N	24	24
		IL-5	Pearson Correlation	1	.634
			Sig. (2-tailed)		.027
			N	24	24
	G/G	IgE	Pearson Correlation	.776	.922
			Sig. (2-tailed)	.005	.000
			N	22	22
		IL-5	Pearson Correlation	1	.715
			Sig. (2-tailed)		.013
			N	22	22

Conclusions

1-In the CyLTR1 972 T/C) ,the T/C genotypes increased to represent 46% in patients ,CC genotype associated with severe asthma .

2-The CyLTR2 M01 V was tested for the presence of genes polymorphism ,the A/G genotypes increased to represent 54% in patients ,GG genotype associated with severe asthma.

3-The personal correlation for IgE,IL-5 and eosinophli recorded high significant for patients with in association to CyLTR1 972 T/C and CyLTR2 M01 V gene polymorphism .

4- There is statistically significant difference in the levels of total IgE and IL-5 and blood eosinophil in asthmatic patients group when compare to the health control group.

5-Use sequence or real time PCR for detection the quantitative effect of this mutation.

Recommendations

1- The *CyLTR1 972 T/C* and *CyLTR2 M201V* genes polymorphisms should be considered in planning of control strategies against asthma.

2- Studying others polymorphisms in the CyLTR1 and CyLTR2 genes include synonymous polymorphisms and detecting its effect on pathophysiology of asthma.

3-Further studies are in need for investigating the role of polymorphisms in other genes that may affect on pathophysiology of asthma and their effect on susceptibility to different allergen of Iraqi population.

4- Further studies are in need for investigating the role of genetic polymorphisms in CyLTR1 and CyLTR2 in association with specific receptor antagonist .

References

Aaron, Shawn D, Katherine L Vandemheen, Louis-Philippe Boulet, R Andrew McIvor, J Mark FitzGerald, Paul Hernandez, Catherine Lemiere, Sat Sharma, Stephen K Field, and Gonzalo G Alvarez. 2008. "Overdiagnosis of asthma in obese and nonobese adults." *Canadian Medical Association Journal* 179 (11):1121-1131.

American Veterinary Medical Association (AVMA) 2016 [webpage on the Internet]. U.S. Pet Ownership and Demographics Sourcebook. 2012. Available from: https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-pet-ownership.aspx. Accessed November 7 .

Aggarwal, S, YP Moodley, PJ Thompson, and NL Misso. 2010. "Prostaglandin E2 and cysteinyl leukotriene concentrations in sputum: association with asthma severity and eosinophilic inflammation." *Clinical & Experimental Allergy* 40 (1):85-93.

Agha, Farida, A Sadaruddin, Shahid Abbas, and SM Ali. 1997. "Serum IgE levels in patients with allergic problems and healthy subjects." *JOURNAL-PAKISTAN MEDICAL ASSOCIATION* 47:166-168.

Akdis, Mübeccel, Johan Verhagen, Alison Taylor, Fariba Karamloo, Christian Karagiannidis, Reto Crameri, Sarah Thunberg, Günnur Deniz, Rudolf Valenta, and Helmut Fiebig. 2004. "Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells." *Journal of Experimental Medicine* 199 (11):1567-1575.

Akhabir, Loubna, and Andrew J Sandford. 2011. "Genome-wide association studies for discovery of genes involved in asthma." *Respirology* 16 (3):396-406.

Aldington, Sarah, and Richard Beasley. 2007. "Asthma exacerbations· 5: Assessment and management of severe asthma in adults in hospital." *Thorax* 62 (5):447-458.

Al-Habboo, Dhiher Jameel, Khalid Omar Sultan, Zainab Najim, and Hellme Najim. 2017. "Psychiatric Manifestation of Patients with Bronchial Asthma in Mosul, Iraq." *Psychiatria Danubina* 29 (Suppl 3):649-651.

Allen, BW. 1980. "Inhibitory effect of rubber on the growth of M. tuberculosis." *Tubercle* 61 (2):91-95.

Almqvist, Catarina, Henrik Olsson, Vilhelmina Ullemar, Brian M D'onofrio, Emma Frans, and Cecilia Lundholm. 2015. "Association between parental age and asthma in a population-based register study." *Journal of Allergy and Clinical Immunology* 136 (4):1103-1105. e2.

Anandan, C, Ulugbek Nurmatov, OCP Van Schayck, and A Sheikh. 2010. "Is the prevalence of asthma declining? Systematic review of epidemiological studies." *Allergy* 65 (2):152-167.

Anderson, Gary P. 2008. "Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease." *The Lancet* 372 (9643):1107-1119.

Anwar, Yasir, Jamal SM Sabir, Muhammad I Qureshi, and Kulvinder S Saini. 2014. "5-lipoxygenase: a promising drug target against inflammatory diseases-biochemical and pharmacological regulation." *Current drug targets* 15 (4):410-422.

Arriba-Méndez, S, C Sanz, M Isidoro-García, M Pascual, C Avila, I Dávila, and F Lorente. 2008. "Analysis of 927T> C CYSLTR1 and–444A> C LTC4S polymorphisms in children with asthma." *Allergologia et immunopathologia* 36 (5):259-263.

Arriba-Mendez, Sonia, Catalina Sanz, Maria Isidoro-Garcia, Ignacio Davild, Elena Laffond, Esther Horeno, Carmelo Avila, and Felix Lorente. 2006. "927T> C polymorphism of the cysteinyl-leukotriene type-1 receptor (CYSLTR1) gene in children with asthma and atopic dermatitis." *Pediatric allergy and immunology* 17 (5):323-328.

Asher, I, and N Pearce. 2014. "Global burden of asthma among children." *The international journal of tuberculosis and lung disease* 18 (11):1269-1278.

Asher, M Innes, Stephen Montefort, Bengt Björkstén, Christopher KW Lai, David P Strachan, Stephan K Weiland, Hywel Williams, and ISAAC Phase Three Study Group. 2006. "Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys." *The Lancet* 368 (9537):733-743.

Austen, K Frank, Akiko Maekawa, Yoshihide Kanaoka, and Joshua A Boyce. 2009. "The leukotriene E4 puzzle: finding the missing pieces and revealing the pathobiologic implications." *Journal of Allergy and Clinical Immunology* 124 (3):406-414.

Bandeira-Melo, Christianne, and Peter F Weller. 2003. "Eosinophils and cysteinyl leukotrienes." *Prostaglandins, leukotrienes and essential fatty acids* 69 (2-3):135-143.

Bandeira-Melo, Christianne, John C Hall, John F Penrose, and Peter F Weller. 2002. "Cysteinyl leukotrienes induce IL-4 release from cord blood–derived human eosinophils." *Journal of Allergy and Clinical Immunology* 109 (6):975-979.

Bankova, Lora G, Juying Lai, Eri Yoshimoto, Joshua A Boyce, K Frank Austen, Yoshihide Kanaoka, and Nora A Barrett. 2016. "Leukotriene E4 elicits respiratory epithelial cell mucin release through the G-protein–coupled receptor, GPR99." *Proceedings of the National Academy of Sciences* 113 (22):6242-6247.

Barnish, Maxwell S, Nara Tagiyeva, Graham Devereux, Lorna Aucott, and Steve Turner. 2017. "Changes in the relationship between asthma and associated risk factors over fifty years." *Pediatric Allergy and Immunology* 28 (2):162-169.

Barrenäs, Fredrik, Bengt Andersson, Lars Olaf Cardell, Michael Langston, Reza Mobini, Andy Perkins, Juhani Soini, Arne Ståhl, and Mikael Benson. 2008. "Gender differences in inflammatory proteins and pathways in seasonal allergic rhinitis." *Cytokine* 42 (3):325-329.

Bautz, Frank, Claudio Denzlinger, Lothar Kanz, and Robert Möhle. 2001. "Chemotaxis and transendothelial migration of CD34+ hematopoietic progenitor cells induced by the inflammatory mediator leukotriene D4 are mediated by the 7-transmembrane receptor CysLT1." *Blood* 97 (11):3433-3440.

Beasley, Richard, Alex Semprini, and Edwin A Mitchell. 2015. "Risk factors for asthma: is prevention possible?" *The Lancet* 386 (9998):1075-1085.

Belda, Jose, Krishnan Parameswaran, Catherine Lemière, Dennis Kamada, Paul M O'Byrne, and Frederick E Hargreave. 2006. "Predictors of loss of asthma control induced by corticosteroid withdrawal." *Canadian respiratory journal* 13 (3):129-133.

Bergeron, Celine, and Louis-Philippe Boulet. 2006. "Structural changes in airway diseases: characteristics, mechanisms, consequences, and pharmacologic modulation." *Chest* 129 (4):1068-1087.

Bessa, Vasiliki, Eleni Tseliou, Petros Bakakos, and Stelios Loukides. 2008. "Noninvasive evaluation of airway inflammation in asthmatic patients who smoke: implications for application in clinical practice." *Annals of Allergy, Asthma & Immunology* 101 (3):226-232.

Beuther, David A. 2010. "Recent insight into obesity and asthma." *Current opinion in pulmonary medicine* 16 (1):64-70.

Bhosle, Vikrant K, José Carlos Rivera, and Sylvain Chemtob. 2017. "New insights into mechanisms of nuclear translocation of G-protein coupled receptors." *Small GTPases*:1-10.

Bisgaard, H. 2001. "Pathophysiology of the cysteinyl leukotrienes and effects of leukotriene receptor antagonists in asthma." *Allergy* 56 (s 66):7-11.

Bisgaard, Hans, and Klaus Bønnelykke. 2010. "Long-term studies of the natural history of asthma in childhood." *Journal of Allergy and Clinical Immunology* 126 (2):187-197.

Bjerg, Anders, Thomas Sandström, B Lundbäck, and E Rönmark. 2010. "Time trends in asthma and wheeze in Swedish children 1996–2006: prevalence and risk factors by sex." *Allergy* 65 (1):48-55.

Bleecker, Eugene R, J Mark FitzGerald, Pascal Chanez, Alberto Papi, Steven F Weinstein, Peter Barker, Stephanie Sproule, Geoffrey Gilmartin, Magnus Aurivillius, and Viktoria Werkström. 2016. "Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial." *The Lancet* 388 (10056):2115-2127.

Boehmler, Andreas M, Adriana Drost, Lena Jaggy, Gabriele Seitz, Tina Wiesner, Claudio Denzlinger, Lothar Kanz, and Robert Möhle. 2009. "The CysLT1 ligand leukotriene D4 supports α4β1-and α5β1-mediated adhesion and proliferation of CD34+ hematopoietic progenitor cells." *The journal of Immunology* 182 (11):6789-6798.

Borish, Larry, Bradley Chipps, Yamo Deniz, Sheila Gujrathi, Beiyao Zheng, and Chantal M Dolan. 2005. "Total serum IgE levels in a large cohort of patients with severe or difficult-to-treat asthma." *Annals of Allergy, Asthma & Immunology* 95 (3):247-253.

Braccioni, Fausto, Sandra C Dorman, Paul M O'byrne, Mark D Inman, Judah A Denburg, Krishnan Parameswaran, Adrian J Baatjes, Ronan Foley, and Gail M Gauvreau. 2002. "The effect of cysteinyl leukotrienes on growth of eosinophil progenitors from peripheral blood and bone marrow of atopic subjects." *Journal of allergy and clinical immunology* 110 (1):96-101.

Brakhas, SA, MR Atia, YJ Aziz, and SAH Al-Sharqi. 2015. "Study of total IgE levels and eosinophil count according to age and gender in patients with allergic rhinitis." *World J Pharm Res* 4 (1):295-303.

Brochu-Bourque, Ariane, Steeve Véronneau, Marek Rola-Pleszczynski, and Jana Stankova. 2011. "Differential signaling defects associated with the M201V

polymorphism in the cysteinyl leukotriene type 2 receptor." *Journal of Pharmacology and Experimental Therapeutics* 336 (2):431-439.

Brocker, Chad, David Thompson, Akiko Matsumoto, Daniel W Nebert, and Vasilis Vasiliou. 2010. "Evolutionary divergence and functions of the human interleukin (IL) gene family." *Human genomics* 5 (1):30.

Broughton, Sophie E, Tracy L Nero, Urmi Dhagat, Winnie L Kan, Timothy R Hercus, Denis Tvorogov, Angel F Lopez, and Michael W Parker. 2015. "The βc receptor family–Structural insights and their functional implications." *Cytokine* 74 (2):247-258.

Brusselle, Guy G, Tania Maes, and Ken R Bracke. 2013. "Eosinophils in the spotlight: eosinophilic airway inflammation in nonallergic asthma." *Nature medicine* 19 (8):977.

Burrows, Benjamin, Fernando D Martinez, Martha G Cline, and Michael D Lebowitz. 1995. "The relationship between parental and children's serum IgE and asthma." *American journal of respiratory and critical care medicine* 152 (5):1497-1500.

Busse, William W, Robert F Lemanske Jr, and James E Gern. 2010. "Role of viral respiratory infections in asthma and asthma exacerbations." *The Lancet* 376 (9743):826-834.

Busse, William, and Monica Kraft. 2005. "Cysteinyl leukotrienes in allergic inflammation: strategic target for therapy." *Chest* 127 (4):1312-1326.

Campbell, Quincy McKenzie. 2016. "Patterns Of Asthma Exacerbation Related To Climate And Weather In The Northeast Kingdom Of Vermont."

Choy, David F, Kevin M Hart, Lee A Borthwick, Aarti Shikotra, Deepti R Nagarkar, Salman Siddiqui, Guiquan Jia, Chandra M Ohri, Emma Doran, and Kevin M Vannella. 2015. "TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma." *Science translational medicine* 7 (301):301ra129-301ra129.

Cattaneo M (2015). "P2Y12 receptors: structure and function". Journal of Thrombosis and Haemostasis : JTH. 13 Suppl 1: S10–6. PMID 26149010. doi:10.1111/jth.12952.

Centers for Disease Control and Prevention (CDC) , 2016. National Center for Environmental Health. Asthma Stats: Percentage of People with Asthma Who Smoke. 2010. Available from: https://www.cdc.gov/asthma/ asthma_stats/asthma_stats_factsheet.pdf. Accessed November 7.

Cecchi, L, G d'Amato, JG Ayres, C Galan, F Forastiere, Bertil Forsberg, J Gerritsen, C Nunes, H Behrendt, and C Akdis. 2010. "Projections of the

effects of climate change on allergic asthma: the contribution of aerobiology." Allergy 65 (9):1073-1081.

Cohn, Lauren, Jeffrey S Tepper, and Kim Bottomly. 1998. "Cutting edge: IL-4-independent induction of airway hyperresponsiveness by Th2, but not Th1, cells." *The Journal of Immunology* 161 (8):3813-3816.

Corren, Jonathan, Steven Weinstein, Lindsay Janka, James Zangrilli, and Margaret Garin. 2016. "Phase 3 study of reslizumab in patients with poorly controlled asthma: effects across a broad range of eosinophil counts." *Chest* 150 (4):799-810.

Couto, Thaís Amarante Peres de Paula, Nelson Falsarella, Cinara de Cássia Brandão de Mattos, and Luiz Carlos de Mattos. 2014. "Total IgE plasma levels vary according to gender and age in Brazilian patients with allergic rhinitis." *Clinics* 69 (11):740-744.

Cox-Ganser, JM. 2015. "Indoor dampness and mould health effects–ongoing questions on microbial exposures and allergic versus nonallergic mechanisms." *Clinical & Experimental Allergy* 45 (10):1478-1482.

Cuvelier, Susan L, and Kamala D Patel. 2001. "Shear-dependent eosinophil transmigration on interleukin 4–stimulated endothelial cells: a role for endothelium-associated eotaxin-3." *Journal of Experimental Medicine* 194 (12):1699-1709.

Dahinden, Clemens A, Sylvia Rihs, and Brigitte Ochsensberger. 1997. "Regulation of cytokine expression by human blood basophils." *International archives of allergy and immunology* 113 (1-3):134-137.

Dahlén, Sven-Erik. 2006. "Treatment of asthma with antileukotrienes: first line or last resort therapy?" *European journal of pharmacology* 533 (1-3):40-56.

Davila, I, A Valero, LM Entrenas, N Valveny, and L Herráez. 2015. "Relationship between serum total IgE and disease severity in patients with allergic asthma in Spain." *Journal of investigational allergology & clinical immunology* 25 (2):120-127.

de Marco, Roberto, Alessandro Marcon, Deborah Jarvis, Simone Accordini, Enrique Almar, Massimiliano Bugiani, Adriana Carolei, Lucia Cazzoletti, Angelo Corsico, and David Gislason. 2006. "Prognostic factors of asthma severity: a 9-year international prospective cohort study." *Journal of Allergy and Clinical Immunology* 117 (6):1249-1256.

DeKruyff, Rosemarie H, Sanhong Yu, Hye Young Kim, and Dale T Umetsu. 2014. "Innate immunity in the lung regulates the development of asthma." *Immunological reviews* 260 (1):235-248.

Detoraki, Aikaterini, Lorena Di Capua, Gilda Varricchi, Arturo Genovese, Gianni Marone, and Giuseppe Spadaro. 2016. "Omalizumab in patients with eosinophilic granulomatosis with polyangiitis: a 36-month follow-up study." *Journal of Asthma* 53 (2):201-206.

Dixon, Anne E, Fernando Holguin, Akshay Sood, Cheryl M Salome, Richard E Pratley, David A Beuther, Juan C Celedón, and Stephanie A Shore. 2010. "An official American Thoracic Society Workshop report: obesity and asthma." *Proceedings of the American Thoracic Society* 7 (5):325-335.

Dong, Xiaowu, Yanmei Zhao, Xueqin Huang, Kana Lin, Jianzhong Chen, Erqing Wei, Tao Liu, and Yongzhou Hu. 2013. "Structure-based drug design using GPCR homology modeling: toward the discovery of novel selective CysLT2 antagonists." *European journal of medicinal chemistry* 62:754-763.

Durack, Juliana, Susan V Lynch, Snehal Nariya, Nirav R Bhakta, Avraham Beigelman, Mario Castro, Anne-Marie Dyer, Elliot Israel, Monica Kraft, and Richard J Martin. 2017. "Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment." *Journal of Allergy and Clinical Immunology* 140 (1):63-75.

Duroudier, Nathalie P, David P Strachan, John D Blakey, and Ian P Hall. 2009. "Association of the cysteinyl leukotriene receptor 1 gene with atopy in the British 1958 birth cohort." *Journal of Allergy and Clinical Immunology* 124 (3):566-572.

Duroudier, NP, AS Tulah, and I Sayers. 2009. "Leukotriene pathway genetics and pharmacogenetics in allergy." *Allergy* 64 (6):823-839.

Eder, W, MJ Ege, and E von Mutius. 2006. "The asthma epidemic N Engl J Med 355 (21): 2226–2235." *Find this article online*.

Erbas, B, M Akram, SC Dharmage, R Tham, M Dennekamp, E Newbigin, Philip Taylor, MLK Tang, and MJ Abramson. 2012. "The role of seasonal grass pollen on childhood asthma emergency department presentations." *Clinical & Experimental Allergy* 42 (5):799-805.

Fahy, John V. 2015. "Type 2 inflammation in asthma—present in most, absent in many." *Nature Reviews Immunology* 15 (1):57.

Faith, A, MH Fernandez, J Caulfield, T-K Loke, C Corrigan, B O'Connor, TH Lee, and CM Hawrylowicz. 2008. "Role of cysteinyl leukotrienes in human allergen-specific Th2 responses induced by granulocyte macrophage-colony stimulating factor." *Allergy* 63 (2):168-175.

Fallon, Padraic G, Sarah J Ballantyne, Niamh E Mangan, Jillian L Barlow, Ayan Dasvarma, Duncan R Hewett, Ann McIlgorm, Helen E Jolin, and Andrew NJ McKenzie. 2006. "Identification of an interleukin (IL)-25–dependent cell

population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion." *Journal of Experimental Medicine* 203 (4):1105-1116.

Fernandes, Silvia de Sousa Campos, Cláudia Ribeiro de Andrade, Alessandra Pinheiro Caminhas, Paulo Augusto Moreira Camargos, and Cássio da Cunha Ibiapina. 2016. "Prevalence of self-reported smoking experimentation in adolescents with asthma or allergic rhinitis." *Jornal Brasileiro de Pneumologia* 42 (2):84-87.

Figueroa, David J, Richard M Breyer, Stephanie K DeFoe, Stacia Kargman, Bruce L Daugherty, Kristine Waldburger, Qingyun Liu, Michelle Clements, Zhizen Zeng, and GARY P O'NEILL. 2001. "Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes." *American Journal of Respiratory and Critical Care Medicine* 163 (1):226-233.

FitzGerald, J Mark, Eugene R Bleecker, Parameswaran Nair, Stephanie Korn, Ken Ohta, Marek Lommatzsch, Gary T Ferguson, William W Busse, Peter Barker, and Stephanie Sproule. 2016. "Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial." *The Lancet* 388 (10056):2128-2141.

Fregonese, L, M Silvestri, F Sabatini, and GA Rossi. 2002. "Cysteinyl leukotrienes induce human eosinophil locomotion and adhesion molecule expression via a CysLT1 receptor-mediated mechanism." *Clinical & Experimental Allergy* 32 (5):745-750.

Fereidounia M, Hossinia RF, Azada FJ, Assarezadegan MA, and Varasteha A(2009). Skin prick test reactivity to common aeroallergens among allergic rhinitis patients in IranAllergol et Immunopathol.;37(2):73-9.

Frieri, Marianne, Jaya Therattil, Soo Fang Wang, Chien-Yiu Huang, and Yung-Chih Wang. 2003. "Montelukast inhibits interleukin-5 mRNA expression and cysteinyl leukotriene production in ragweed and mite-stimulated peripheral blood mononuclear cells from patients with asthma." Allergy and asthma proceedings.

Fukai, Hiromi, Yoshino Ogasawara, Ohsuke Migita, Minori Koga, Kunio Ichikawa, Masanao Shibasaki, Tadao Arinami, and Emiko Noguchi. 2004. "Association between a polymorphism in cysteinyl leukotriene receptor 2 on chromosome 13q14 and atopic asthma." *Pharmacogenetics and Genomics* 14 (10):683-690.

.

Fukutomi Yuma, and Masami Taniguchi. 2015. "Sensitization to fungal allergens: resolved and unresolved issues." *Allergology International* 64 (4):321-331.

Furuta, Glenn T, F Dan Atkins, Nancy A Lee, and James J Lee. 2014. "Changing roles of eosinophils in health and disease." *Annals of allergy, asthma & immunology* 113 (1):3-8.

Gauvreau, Gail M, Krishnan N Parameswaran, Richard M Watson, and PAUL M O'BYRNE. 2001. "Inhaled leukotriene E4, but not leukotriene D4, increased airway inflammatory cells in subjects with atopic asthma." *American Journal of Respiratory and Critical Care Medicine* 164 (8):1495-1500.

George, Leena, and Christopher E Brightling. 2016. "Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease." *Therapeutic advances in chronic disease* 7 (1):34-51.

Ghosal, Kavita, Bodhisattwa Saha, and Swati Gupta Bhattacharya. 2016. "Clinical and immuno-proteomic approach on Lantana camara pollen allergy—a major health hazard." *Allergy, Asthma & Clinical Immunology* 12 (1):33.

Green, RH, CE Brightling, G Woltmann, D Parker, AJ Wardlaw, and ID Pavord. 2002. "Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids." *Thorax* 57 (10):875-879.

Guilbert, Theresa W, Wayne J Morgan, Robert S Zeiger, David T Mauger, Susan J Boehmer, Stanley J Szefler, Leonard B Bacharier, Robert F Lemanske Jr, Robert C Strunk, and David B Allen. 2006. "Long-term inhaled corticosteroids in preschool children at high risk for asthma." *New England Journal of Medicine* 354 (19):1985-1997.

Gupta, Mukesh Kumar, Jyoti Patodia, Pawan Chaudhary, and Munish Kakkar. 2018. "The rising trend of asthma prevalence in urban school children of Jaipur: A questionnaire based study." *Indian Journal of Allergy, Asthma and Immunology* 32 (1):10.

Haeggström, Jesper Z, and Colin D Funk. 2011. "Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease." *Chemical reviews* 111 (10):5866-5898.

Hallstrand, Teal S, and William R Henderson Jr. 2010. "An update on the role of leukotrienes in asthma." *Current opinion in allergy and clinical immunology* 10 (1):60.

Hamano, Nobuhisa Terada, Ken-Ichi Maesako, Gen Hohki, Tatsuhiko Ito, Tetsuji Yamashita, Akiyoshi Konno, Nanako. 1998. "Effect of female hormones on the production of IL-4 and IL-13 from peripheral blood mononuclear cells." *Acta Oto-Laryngologica* 118 (537):27-31.

Hamid, Q, M Azzawi, Sun Ying, R Moqbel, AJ Wardlaw, CJ Corrigan, B Bradley, SR Durham, JV Collins, and PK Jeffery. 1991. "Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma." *The Journal of clinical investigation* 87 (5):1541-1546.

Hao, L, I Sayers, JA Cakebread, SJ Barton, B Beghe, ST Holgate, AP Sampson, and JW Holloway. 2006. "The cysteinyl-leukotriene type 1 receptor polymorphism 927T/C is associated with atopy severity but not with asthma." *Clinical & Experimental Allergy* 36 (6):735-741.

Hoffmann, David. 2017. *Easy Breathing: Natural Treatments for Asthma, Colds, Flu, Coughs, Allergies, and Sinusitis*: Storey Publishing.

Hogan, Simon P, Helene F Rosenberg, Redwan Moqbel, Simon Phipps, Paul S Foster, Paige Lacy, A Barry Kay, and Marc E Rothenberg. 2008. "Eosinophils: biological properties and role in health and disease." *Clinical & Experimental Allergy* 38 (5):709-750.

Holgate, Stephen T, and Mike Thomas. 2017. "Asthma." In *Middleton's Allergy Essentials*, 151-204. Elsevier.

Holgate, Stephen T, Marc Peters-Golden, Reynold A Panettieri, and William R Henderson. 2003. "Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling." *Journal of Allergy and Clinical Immunology* 111 (1):S18-S36.

Holgate, Stephen T. 2012. "Innate and adaptive immune responses in asthma." *Nature medicine* 18 (5):673.

Holgate, Stephen T. 2014. "New strategies with anti-IgE in allergic diseases." *World Allergy Organization Journal* 7 (1):1.

Holguin, Fernando, and Anne Fitzpatrick. 2009. "Obesity, asthma, and oxidative stress." *Journal of Applied Physiology* 108 (3):754-759.

Hong, X, H Zhou, HJ Tsai, X Wang, X Liu, B Wang, and X Xu. 2009. "Cysteinyl leukotriene receptor 1 gene variation and risk of asthma." *European Respiratory Journal* 33 (1):42-48.

Humbert, Marc, Christopher J Corrigan, Patrick Kimmitt, Stephen J Till, A BARRY KAY, and Stephen R Durham. 1997. "Relationship between IL-4 and IL-5 mRNA expression and disease severity in atopic asthma." *American journal of respiratory and critical care medicine* 156 (3):704-708.

Ihaku, Daizo, Lisa Cameron, Masaru Suzuki, Sophie Molet, James Martin, and Qutayba Hamid. 1999. "Montelukast, a leukotriene receptor antagonist, inhibits the late airway response to antigen, airway eosinophilia, and IL-5–expressing cells in Brown Norway rats." *Journal of allergy and clinical immunology* 104 (6):1147-1154.

Incorvaia, Cristoforo, Gian Galeazzo Riario-Sforza, and Erminia Ridolo. 2017. "IgE depletion in severe asthma: what we have and what could be added in the near future." *EBioMedicine* 17:16-17.

Jacobsen, Lars, Ulrich Wahn, and M Beatrice Bilo. 2012. "Allergen-specific immunotherapy provides immediate, long-term and preventive clinical effects in children and adults: the effects of immunotherapy can be categorised by level of benefit-the centenary of allergen specific subcutaneous immunotherapy." *Clinical and translational allergy* 2 (1):8.

Janssens Thomas, and Thomas Ritz. 2013. "Perceived triggers of asthma: key to symptom perception and management." *Clinical & Experimental Allergy* 43 (9):1000-1008.

Javelle, Emilie, Anne Ribera, Isabelle Degasne, Bernard-Alex Gaüzère, Catherine Marimoutou, and Fabrice Simon. 2015. "Specific management of post-chikungunya rheumatic disorders: a retrospective study of 159 cases in Reunion Island from 2006-2012." *PLoS neglected tropical diseases* 9 (3):e0003603.

Jennette, John C, RJ Falk, PA Bacon, N Basu, MC Cid, F Ferrario, LF Flores-Suarez, WL Gross, L Guillevin, and EC Hagen. 2013. "2012 revised international chapel hill consensus conference nomenclature of vasculitides." *Arthritis & Rheumatology* 65 (1):1-11.

Jiang, Yongfeng, Laura A Borrelli, Yoshihide Kanaoka, Brian J Bacskai, and Joshua A Boyce. 2007. "CysLT2 receptors interact with CysLT1 receptors and down-modulate cysteinyl leukotriene–dependent mitogenic responses of mast cells." *Blood* 110 (9):3263-3270.

Jindal, SK. 2017. *Textbook of Pulmonary and Critical Care Medicine: Two Volume Set*: JP Medical Ltd.

Kadry, Heba M, Amal H Atta, Mostafa K Sultan, and Nagwa A El-Baz. 2014. "Leukotriene C4 synthase and leukotriene receptor-1 genes polymorphism among atopic asthmatic patients." *Am. J. Immunol* 10:63-72.

Kalpaklĩoğlu, AF, and A Baççıoğlu. 2008. "Evaluation of quality of life: impact of allergic rhinitis on asthma." *J Investig Allergol Clin Immunol* 18 (3):168-173.

Kamble, Shital, and Murtuza Bharmal. 2009. "Incremental direct expenditure of treating asthma in the United States." *Journal of Asthma* 46 (1):73-80.

Kaminuma, Osamu, Akio Mori, Noriko Kitamura, Tomomi Hashimoto, Fujiko Kitamura, Shigeko Inokuma, and Shoichiro Miyatake. 2005. "Role of

GATA-3 in IL-5 gene transcription by CD4+ T cells of asthmatic patients." *International archives of allergy and immunology* 137 (Suppl. 1):55-59.

Kanaoka, Yoshihide, Akiko Maekawa, and K Frank Austen. 2013. "Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand." *Journal of Biological Chemistry* 288 (16):10967-10972.

Kanaoka, Yoshihide, and Joshua A Boyce. 2014. "Cysteinyl leukotrienes and their receptors; emerging concepts." *Allergy, asthma & immunology research* 6 (4):288-295.

Kar, Murat, Niyazi Altıntoprak, Nuray Bayar Muluk, Seckin Ulusoy, Sameer Ali Bafaqeeh, and Cemal Cingi. 2016. "Antileukotrienes in adenotonsillar hypertrophy: a review of the literature." *European Archives of Oto-Rhino-Laryngology* 273 (12):4111-4117.

Kim, Jayeun, Younhee Lim, and Ho Kim. 2014. "Outdoor temperature changes and emergency department visits for asthma in Seoul, Korea: a time-series study." *Environmental research* 135:15-20.

Kolbeck, Roland, Alexander Kozhich, Masamichi Koike, Li Peng, Cecilia K Andersson, Melissa M Damschroder, Jennifer L Reed, Robert Woods, William W Dall'Acqua, and Geoffrey L Stephens. 2010. "MEDI-563, a humanized anti–IL-5 receptor α mAb with enhanced antibody-dependent cell-mediated cytotoxicity function." *Journal of Allergy and Clinical Immunology* 125 (6):1344-1353. e2.

Konradsen, Jon R, Takao Fujisawa, Marianne Van Hage, Gunilla Hedlin, Christiane Hilger, Jörg Kleine-Tebbe, Elizabeth C Matsui, Graham Roberts, Eva Rönmark, and Thomas AE Platts-Mills. 2015. "Allergy to furry animals: new insights, diagnostic approaches, and challenges." *Journal of Allergy and Clinical Immunology* 135 (3):616-625.

Kopřiva, František, Jarmila Szotkowská, Marta Ordeltová, and Martin Zápalka. 2006. "Percentage of eosinophils with surface CD44 and levels of eosinophil cationic protein in the peripheral blood in children with bronchial asthma and their changes after montelukast therapy." Allergy and asthma proceedings.

Kormann, Michael SD, David Carr, Norman Klopp, Thomas Illig, Wolfgang Leupold, Christian Fritzsch, Stephan K Weiland, Erika von Mutius, and Michael Kabesch. 2005. "G-protein–coupled receptor polymorphisms are associated with asthma in a large German population." *American journal of respiratory and critical care medicine* 171 (12):1358-1362.

Korn, Stephanie, Ina Haasler, Florian Fliedner, Gunther Becher, Pavel Strohner, Antonia Staatz, Christian Taube, and Roland Buhl. 2012. "Monitoring free serum IgE in severe asthma patients treated with omalizumab." *Respiratory medicine* 106 (11):1494-1500.

Kovalszki, Anna, and Peter F Weller. 2016. "Eosinophilia." *Primary Care: Clinics in Office Practice* 43 (4):607-617.

Kumar, Roshan M, R Pajanivel, G Koteeswaran, Surendra K Menon, and Pravin MV Charles. 2017. "Correlation of total serum immunoglobulin E level, sputum, and peripheral eosinophil count in assessing the clinical severity in bronchial asthma." *Lung India: official organ of Indian Chest Society* 34 (3):256.

Kurt, Emel, Ayse Aktas, Zafer Gulbas, Sinan Erginel, and Sertac Arslan. 2010. "The effects of natural pollen exposure on inflammatory cytokines and their relationship with nonspecific bronchial hyperresponsiveness in seasonal allergic rhinitis." Allergy and asthma proceedings.

Kushiya, Mariko, Keiko Saito, Izumi Kikuchi, Takehito Kobayashi, Koichi Hagiwara, Minoru Kanazawa, and Makoto Nagata. 2006. "Differential effects of salbutamol and montelukast on eosinophil adhesion and superoxide anion generation." *International archives of allergy and immunology* 140 (Suppl. 1):17-22.

Kynyk, Jessica A, John G Mastronarde, and Jennifer W McCallister. 2011. "Asthma, the sex difference." *Current Opinion in Pulmonary Medicine* 17 (1):6-11.

Lai, Tianwen, Mindan Wu, Juan Liu, Man Luo, Lulu He, Xiaohui Wang, Bin Wu, Songmin Ying, Zhihua Chen, and Wen Li. 2018. "Acid-Suppressive Drug Use During Pregnancy and the Risk of Childhood Asthma: A Meta-analysis." *Pediatrics*:e20170889.

Laidlaw, Tanya M, and Joshua A Boyce. 2016. "Aspirin-exacerbated respiratory disease—new prime suspects." *New England Journal of Medicine* 374 (5):484-488.

Laitinen, Annika, Ari Lindqvist, Maija Halme, Alan Altraja, and Lauri A Laitinen. 2005. "Leukotriene E4–induced persistent eosinophilia and airway obstruction are reversed by zafirlukast in patients with asthma." *Journal of allergy and clinical immunology* 115 (2):259-265.

Lamoureux, Josée, Jana Stankova, and Marek Rola-Pleszczynski. 2006. "Leukotriene D4 enhances immunoglobulin production in CD40-activated human B lymphocytes." *Journal of Allergy and Clinical Immunology* 117 (4):924-930.

Lazic, N, G Roberts, A Custovic, D Belgrave, CM Bishop, J Winn, JA Curtin, S Hasan Arshad, and A Simpson. 2013. "Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts." *Allergy* 68 (6):764-770.

Leckie, MJ, GR Jenkins, J Khan, SJ Smith, C Walker, PJ Barnes, and TT Hansel. 2003. "Sputum T lymphocytes in asthma, COPD and healthy subjects have the phenotype of activated intraepithelial T cells (CD69+ CD103+)." *Thorax* 58 (1):23-29.

Lee, Janet S, Hugh D Campbell, Christine A Kozak, and Ian G Young. 1989. "TheIl-4 andIl-5 genes are closely linked and are part of a cytokine gene cluster on mouse chromosome 11." *Somatic cell and molecular genetics* 15 (2):143-152.

Lee, Yong Chul, Kyung Hun Lee, Heung Bum Lee, and Yang Keun Rhee. 2001. "Serum levels of interleukins (IL)-4, IL-5, IL-13, and interferon-γ in acute asthma." *Journal of Asthma* 38 (8):665-671.

Li, Shanshan, Gail Williams, Bin Jalaludin, and Peter Baker. 2012. "Panel studies of air pollution on children's lung function and respiratory symptoms: a literature review." *Journal of Asthma* 49 (9):895-910.

Lindén, Anders. 2001. "Role of interleukin-17 and the neutrophil in asthma." *International archives of allergy and immunology* 126 (3):179-184.

Lipszyc, Joshua C, Simeon Gotzev, Jack Scarborough, Gary M Liss, Samir Gupta, and Susan M Tarlo. 2016. "Evaluation of the efficacy of a web-based work-related asthma educational tool." *Journal of Asthma* 53 (10):1071-1075.

Liu, Min, and Takehiko Yokomizo. 2015. "The role of leukotrienes in allergic diseases." *Allergology International* 64 (1):17-26.

Lynch, Kevin R, Gary P O'neill, Qingyun Liu, Dong-Soon Im, Nicole Sawyer, Kathleen M Metters, Nathalie Coulombe, Mark Abramovitz, David J Figueroa, and Zhizhen Zeng. 1999. "Characterization of the human cysteinyl leukotriene CysLT 1 receptor." *Nature* 399 (6738):789.

Maekawa, Akiko, K Frank Austen, and Yoshihide Kanaoka. 2002. "Targeted gene disruption reveals the role of cysteinyl leukotriene 1 receptor in the enhanced vascular permeability of mice undergoing acute inflammatory responses." *Journal of Biological Chemistry* 277 (23):20820-20824.

Manohar, S, and R Selvakumaran. 2012. "Estimation of serum immunoglobulin E (IgE) level in allergic asthma and allergic rhinitis patients before and after treatment." *Eur J Exp Bio* 2:2199-2205.

Matera, Maria Gabriella, Paola Rogliani, Luigino Calzetta, and Mario Cazzola. 2018. "Pharmacokinetic/pharmacodynamic profile of reslizumab in asthma." *Expert opinion on drug metabolism & toxicology*.

Mattern, Michael R, Seymour Mong, Shau-Ming Mong, J O'L Bartus, HM Sarau, MA Clark, JJ Foley, and ST Crooke. 1990. "Transient activation of topoisomerase I in leukotriene D4 signal transduction in human cells." *Biochemical Journal* 265 (1):101-107.

Mehrotra, Anjuli K, Jr Henderson, and R William. 2009. "The role of leukotrienes in airway remodeling." *Current molecular medicine* 9 (3):384-391.

Melgert, Barbro N, Anuradha Ray, Machteld N Hylkema, Wim Timens, and Dirkje S Postma. 2007. "Are there reasons why adult asthma is more common in females?" *Current allergy and asthma reports* 7 (2):143-150.

Meliton, Angelo Y, Nilda M Munoz, and Alan R Leff. 2007. "Blockade of avidity and focal clustering of β2-integrin by cysteinyl leukotriene antagonism attenuates eosinophil adhesion." *Journal of Allergy and Clinical Immunology* 120 (6):1316-1323.

Menachem-Zidon, Ofra Ben, Avi Avital, Yair Ben-Menahem, Inbal Goshen, Tirzah Kreisel, Eli M Shmueli, Menahem Segal, Tamir Ben Hur, and Raz Yirmiya. 2011. "Astrocytes support hippocampal-dependent memory and long-term potentiation via interleukin-1 signaling." *Brain, behavior, and immunity* 25 (5):1008-1016.

.

Mirzaei, Masoud, Mehran Karimi, Shima Beheshti, and Masoud Mohammadi. 2017. "Prevalence of asthma among Middle Eastern children: A systematic review." *Medical journal of the Islamic Republic of Iran* 31:9.

Mita, H, M Hasegawa, H Saito, and K Akiyama. 2001. "Levels of cysteinyl leukotriene receptor mRNA in human peripheral leucocytes: significantly higher expression of cysteinyl leukotriene receptor 2 mRNA in eosinophils." *Clinical & Experimental Allergy* 31 (11):1714-1723.

Miyazaki, Yasuhiro, Takahiro Satoh, Kiyoshi Nishioka, and Hiroo Yokozeki. 2006. "STAT-6-mediated control of P-selectin by substance P and interleukin-4 in human dermal endothelial cells." *The American journal of pathology* 169 (2):697-707.

Moffatt, Miriam F, Ivo G Gut, Florence Demenais, David P Strachan, Emmanuelle Bouzigon, Simon Heath, Erika Von Mutius, Martin Farrall, Mark Lathrop, and William OCM Cookson. 2010. "A large-scale, consortium-based genomewide association study of asthma." *New England Journal of Medicine* 363 (13):1211-1221.

Moffatt, Miriam F, Michael Kabesch, Liming Liang, Anna L Dixon, David Strachan, Simon Heath, Martin Depner, Andrea von Berg, Albrecht Bufe, and Ernst Rietschel. 2007. "Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma." *Nature* 448 (7152):470.

MÖhle, Robert, Andreas M Boehmler, Claudio Denzlinger, and Lothar Kanz. 2003. "Nonpeptide mediators in the hematopoietic microenvironment." *Annals of the New York Academy of Sciences* 996 (1):61-66.

Mondino, Chiara, Giovanni Ciabattoni, Pierluigi Koch, Riccardo Pistelli, Andrea Trové, Peter J Barnes, and Paolo Montuschi. 2004. "Effects of inhaled corticosteroids on exhaled leukotrienes and prostanoids in asthmatic children." *Journal of allergy and clinical immunology* 114 (4):761-767.

Montuschi, Paolo, Angelo Sala, Sven-Erik Dahlen, and Giancarlo Folco. 2007. "Pharmacological modulation of the leukotriene pathway in allergic airway disease." *Drug discovery today* 12 (9-10):404-412.

Montuschi, Paolo, Chiara Mondino, Pierluigi Koch, Peter J Barnes, and Giovanni Ciabattoni. 2006. "Effects of a leukotriene receptor antagonist on exhaled leukotriene E4 and prostanoids in children with asthma." *Journal of allergy and clinical immunology* 118 (2):347-353.

Moore, Wendy C, Eugene R Bleecker, Douglas Curran-Everett, Serpil C Erzurum, Bill T Ameredes, Leonard Bacharier, William J Calhoun, Mario Castro, Kian Fan Chung, and Melissa P Clark. 2007. "Characterization of the severe asthma phenotype by the national heart, lung, and blood institute's severe asthma research program." *Journal of Allergy and Clinical Immunology* 119 (2):405-413.

Muhammed, Shakir M, Kassim M Sultan, and Mohammad Y Abdulrazaq. 2012. "Asthma in adults; epidemiology, risk factor and patterns of presentation: a cross sectional, questionnaire based study in Baghdad Teaching." *Kerbala Jorunal of Medicine* 5 (11):1255-1261.

Mukherjee, Manali, Sarah Svenningsen, and Parameswaran Nair. 2017. "Glucocortiosteroid subsensitivity and asthma severity." *Current opinion in pulmonary medicine* 23 (1):78-88.

Murray, Clare S, Gina Poletti, Tatiana Kebadze, Julie Morris, Ashley Woodcock, SL Johnston, and Adnan Custovic. 2006. "Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children." *Thorax* 61 (5):376-382.

Nabe, T, K Yamashita, M Miura, T Kawai, and S Kohno. 2002. "Cysteinyl leukotriene-dependent interleukin-5 production leading to eosinophilia

during late asthmatic response in guinea-pigs." *Clinical & Experimental Allergy* 32 (4):633-640.

Nagata, Makoto, and Keiko Saito. 2003. "The roles of cysteinyl leukotrienes in eosinophilic inflammation of asthmatic airways." *International archives of allergy and immunology* 131 (Suppl. 1):7-10.

Nagata, Makoto, Keiko Saito, Izumi Kikuchi, Koichi Hagiwara, and Minoru Kanazawa. 2005. "Effect of the cysteinyl leukotriene antagonist pranlukast on transendothelial migration of eosinophils." *International archives of allergy and immunology* 137 (Suppl. 1):2-6.

Nagata, Makoto, Keiko Saito, Koji Tsuchiya, and Yoshio Sakamoto. 2002. "Leukotriene D4 upregulates eosinophil adhesion via the cysteinyl leukotriene 1 receptor." *Journal of allergy and clinical immunology* 109 (4):676-680.

National Heart, Lung, and Blood Institute. 2007. "National Asthma Education and Prevention Program. Expert panel report 3: guidelines for the diagnosis and management of asthma: full report 2007." *http://www. nhlbi. nih. gov/guidelines/asthma/asthgdln. pdf*.

Nevalainen, Aino, Martin Täubel, and Anne Hyvärinen. 2015. "Indoor fungi: companions and contaminants." *Indoor air* 25 (2):125-156.

Neville, E, H Gribbin, and BDW Harrison. 1991. "Acute severe asthma." *Respiratory medicine* 85 (6):463-474.

Nussbaum, Jesse C, Steven J Van Dyken, Jakob von Moltke, Laurence E Cheng, Alexander Mohapatra, Ari B Molofsky, Emily E Thornton, Matthew F Krummel, Ajay Chawla, and Hong-Erh Liang. 2013. "Type 2 innate lymphoid cells control eosinophil homeostasis." *Nature* 502 (7470):245.

Ober, C, and S Hoffjan. 2006. "Asthma genetics 2006: the long and winding road to gene discovery." *Genes and immunity* 7 (2):95.

Ochiai, K, M Kagami, R Matsumura, and H Tomioka. 1997. "IL-5 but not interferon-gamma (IFN-γ) inhibits eosinophil apoptosis by up-regulation of bcl-2 expression." *Clinical & Experimental Immunology* 107 (1):198-204.

Oettgen, Hans C, and Raif S Geha. 1999. "IgE in asthma and atopy: cellular and molecular connections." *The Journal of clinical investigation* 104 (7):829-835.

Oettgen, Hans C. 2016. "Fifty years later: emerging functions of IgE antibodies in host defense, immune regulation, and allergic diseases." *Journal of Allergy and Clinical Immunology* 137 (6):1631-1645.

Ohshima, Nobuharu, Hiroyuki Nagase, Takeshi Koshino, Misato Miyamasu, Masao Yamaguchi, Koichi Hirai, Kazuhiko Yamamoto, Takao Fujisawa,

Naoki Nakagawa, and Katsuya Kishikawa. 2002. "A functional study on CysLT1 receptors in human eosinophils." *International archives of allergy and immunology* 129 (1):67-75.

Okano, M, AR Satoskar, M Abe, DA Harn, K Nishizaki, Y Takeda, T Yoshino, F Brombacher, and AA Satoskar. 2000. "Interleukin-4-independent production of Th2 cytokines by nasal lymphocytes and nasal eosinophilia in murine allergic rhinitis." *Allergy* 55 (8):723-731.

Örtqvist, Anne K, Cecilia Lundholm, Helle Kieler, Jonas F Ludvigsson, Tove Fall, Weimin Ye, and Catarina Almqvist. 2014. "Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis." *Bmj* 349:g6979.

Osisi, K, KR Iseh, ND Ndodo, MI Inoh, SS Yikawe, JH Solomon, and N Aliyu.(2014) "Serum IgE of Allergic Rhinitis (AR) with or without Asthma in Sokoto, North Western Nigeria."

Oussalah, A, C Mayorga, M Blanca, A Barbaud, A Nakonechna, J Cernadas, M Gotua, K Brockow, J-C Caubet, and A Bircher. 2016. "Genetic variants associated with drugs-induced immediate hypersensitivity reactions: a PRISMA-compliant systematic review." *Allergy* 71 (4):443-462.

Palange, Paolo, Pierantonio Laveneziana, J Alberto Neder, and Susan A Ward. 2018. Clinical exercise testing. Vol. 80: European Respiratory Society.

Palomino, Addy LM, Maria Helena CF Bussamra, Beatriz M Saraiva-Romanholo, Milton A Martins, Maria do Patrocínio T Nunes, and Joaquim C Rodrigues. 2005. "Induced sputum in children and adolescents with asthma: safety, clinical applicability and inflammatory cells aspects in stable patients and during exacerbation." *Jornal de pediatria* 81 (3):216-224.

Parameswaran, Krishnan, Richard Watson, Gail M Gauvreau, Roma Sehmi, and Paul M O'byrne. 2004. "The effect of pranlukast on allergen-induced bone marrow eosinophilopoiesis in subjects with asthma." *American journal of respiratory and critical care medicine* 169 (8):915-920.

Park, Jong Sook, Hun Soo Chang, Choon-Sik Park, June-Hyuk Lee, Yong Mok Lee, Jung Hee Choi, Hae-Sim Park, Lyoung Hyo Kim, Byung Lae Park, and Yoo Hyun Choi. 2005. "Association analysis of cysteinyl-leukotriene receptor 2 (CYSLTR2) polymorphisms with aspirin intolerance in asthmatics." *Pharmacogenetics and genomics* 15 (7):483-492.

Pawankar, Ruby, Giorgio W Canonica, Stephen T Holgate, and Richard F Lockey. 2012. "Allergic diseases and asthma: a major global health concern." *Current opinion in allergy and clinical immunology* 12 (1):39-41.

Pearce, Neil, Nadia Aït-Khaled, Richard Beasley, Javier Mallol, Ulrich Keil, Ed Mitchell, and Colin Robertson. 2007. "Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC)." *Thorax* 62 (9):758-766.

Pergola, Carlo, and Oliver Werz. 2010. "5-Lipoxygenase inhibitors: a review of recent developments and patents." *Expert opinion on therapeutic patents* 20 (3):355-375.

Peters, Michael C, Zesemayat K Mekonnen, Shaopeng Yuan, Nirav R Bhakta, Prescott G Woodruff, and John V Fahy. 2014. "Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma." *Journal of Allergy and Clinical Immunology* 133 (2):388-394. e5.

Peters-Golden, Marc, and William R Henderson Jr. 2007. "Leukotrienes." *New England Journal of Medicine* 357 (18):1841-1854.

Phillips, Clair, William R Coward, David I Pritchard, and Colin RA Hewitt. 2003. "Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites." *Journal of leukocyte biology* 73 (1):165-171.

Platts-Mills, Thomas AE. 2015. "The allergy epidemics: 1870-2010." *Journal of Allergy and Clinical Immunology* 136 (1):3-13.

Poff, Candace D, and Michael Balazy. 2004. "Drugs that target lipoxygenases and leukotrienes as emerging therapies for asthma and cancer." *Current Drug Targets-Inflammation & Allergy* 3 (1):19-33.

Pretolani, Marina, Anders Bergqvist, Gabriel Thabut, Marie-Christine Dombret, Dominique Knapp, Fatima Hamidi, Loubna Alavoine, Camille Taillé, Pascal Chanez, and Jonas S Erjefält. 2017. "Effectiveness of bronchial thermoplasty in patients with severe refractory asthma: Clinical and histopathologic correlations." *Journal of Allergy and Clinical Immunology* 139 (4):1176-1185.

Price, David, Andrew M Wilson, Alison Chisholm, Anna Rigazio, Anne Burden, Michael Thomas, and Christine King. 2016. "Predicting frequent asthma exacerbations using blood eosinophil count and other patient data routinely available in clinical practice." *Journal of asthma and allergy* 9:1.

Profita, Mirella, Angelo Sala, Anna Bonanno, Liboria Siena, Maria Ferraro, Rossana Di Giorgi, Angela M Montalbano, Giusy D Albano, Rosalia Gagliardo, and Mark Gjomarkaj. 2008. "Cysteinyl leukotriene-1 receptor activation in a human bronchial epithelial cell line leads to signal transducer and activator of transcription 1-mediated eosinophil adhesion." *Journal of Pharmacology and Experimental Therapeutics* 325 (3):1024-1030.

Pyrhönen, Kaisa, Simo Näyhä, and Esa Läärä. 2015. "Dog and cat exposure and respective pet allergy in early childhood." *Pediatric Allergy and Immunology* 26 (3):247-255.

Queto, Tulio, Maria I Gaspar-Elsas, Daniela Masid-de-Brito, Zilton FM Vasconcelos, Fausto K Ferraris, Carmen Penido, Fernando Q Cunha, Yoshihide Kanaoka, Bing K Lam, and Pedro Xavier-Elsas. 2010. "Cysteinyl-leukotriene type 1 receptors transduce a critical signal for the up-regulation of eosinophilopoiesis by interleukin-13 and eotaxin in murine bone marrow." *Journal of Leukocyte Biology* 87 (5):885-893.

Radwan M, Jamil H, Hamdan Th, Alnoor T and Alnoor N ;2017 Impact of chemical and non-chemical agents on respiratory system including asthma among Iraqi people; J Allergy Ther, Volume 8, Issue 3,P49

Rapini, Ronald P, Jean L Bolognia, and Joseph L Jorizzo. 2007. "Dermatology: 2-Volume Set. St. Louis: Mosby."

Reddel, Helen K, and Mark L Levy. 2015. "The GINA asthma strategy report: what's new for primary care?" *NPJ primary care respiratory medicine* 25:15050.

Ricciardolo, Fabio LM, Gert Folkerts, Anna Folino, and Barbara Mognetti. 2018. "Bradykinin in asthma: modulation of airway inflammation and remodelling." *European journal of pharmacology*.

Rivas, Magali Noval, Oliver T Burton, Hans C Oettgen, and Talal Chatila. 2016. "IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function." *Journal of Allergy and Clinical Immunology* 138 (3):801-811.

Robinson, Douglas, Qutayba Hamid, Andrew Bentley, Sun Ying, A Barry Kay, and Stephen R Durham. 1993. "Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma." *Journal of Allergy and Clinical Immunology* 92 (2):313-324.

Rosenbaum, MICHAEL, MARGERY Nicolson, JULES Hirsch, STEVEN B Heymsfield, DYMPNA Gallagher, FLORENCE Chu, and RUDOLPH L Leibel. 1996. "Effects of gender, body composition, and menopause on plasma concentrations of leptin." *The Journal of Clinical Endocrinology & Metabolism* 81 (9):3424-3427.

Rosenstreich, David L, Peyton Eggleston, Meyer Kattan, Dean Baker, Raymond G Slavin, Peter Gergen, Herman Mitchell, Kathleen McNiff-Mortimer, Henry Lynn, and Dennis Ownby. 1997. "The role of cockroach allergy and

exposure to cockroach allergen in causing morbidity among inner-city children with asthma." *New England Journal of Medicine* 336 (19):1356-1363.

Roufosse, Florence. 2018. "Targeting the interleukin-5 Pathway for Treatment of eosinophilic Conditions Other than Asthma." *Frontiers in medicine* 5:49.

Rovati, G Enrico, and Valérie Capra. 2007. "Cysteinyl-leukotriene receptors and cellular signals." *The Scientific World Journal* 7:1375-1392.

Saito, Keiko, Makoto Nagata, Izumi Kikuchi, and Yoshio Sakamoto. 2004. "Leukotriene D4 and eosinophil transendothelial migration, superoxide generation, and degranulation via β2 integrin." *Annals of allergy, asthma & immunology* 93 (6):594-600.

Sanz, C, M Isidro-Garcia, I Davila, E Moreno, E Laffond, and F Lorente. 2006. "Patients with Asthma." *J Investig Allergol Clin Immunol* 16 (6):331-337.

Schäppi, George F, Cenk Suphioglu, Philip E Taylor, and R Bruce Knox. 1997. "Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere." *Journal of Allergy and Clinical Immunology* 100 (5):656-661.

Schneider, JA Spence, AJ Bircher, and K Hofmeier Scherer. 2014. "The significance of an allergological examination in asthma and COPD." *Therapeutische Umschau. Revue therapeutique* 71 (5):267-274.

Scirica, Christina V, and Juan C Celedón. 2007. "Genetics of asthma: potential implications for reducing asthma disparities." *Chest* 132 (5):770S-781S.

Sehmi, R, SG Smith, M Kjarsgaard, K Radford, L-P Boulet, C Lemiere, CM Prazma, H Ortega, JG Martin, and P Nair. 2016. "Role of local eosinophilopoietic processes in the development of airway eosinophilia in prednisone-dependent severe asthma." *Clinical & Experimental Allergy* 46 (6):793-802.

Severien, C, A Artlich, S Jonas, and G Becher. 2000. "Urinary excretion of leukotriene E4 and eosinophil protein X in children with atopic asthma." *European respiratory journal* 16 (4):588-592.

Shahabuddin, Syed, Paul Ponath, and Robert P Schleimer. 2000. "Migration of eosinophils across endothelial cell monolayers: interactions among IL-5, endothelial-activating cytokines, and CC chemokines." *The Journal of Immunology* 164 (7):3847-3854.

Shaver, Joseph R, James G Zangrilli, Seung-Kil Cho, Rosemary A Cirelli, Mary Pollice, Annette T Hastie, James E Fish, and Stephen P Peters. 1997. "Kinetics of the development and recovery of the lung from IgE-mediated inflammation: dissociation of pulmonary eosinophilia, lung injury, and

eosinophil-active cytokines." *American journal of respiratory and critical care medicine* 155 (2):442-448.

Sheehan, William J, Pitud A Rangsithienchai, Michael L Muilenberg, Christine A Rogers, Jeffrey P Lane, Jalal Ghaemghami, Donald V Rivard, Kanao Otsu, Elaine B Hoffman, and Elliot Israel. 2009. "Mouse allergens in urban elementary schools and homes of children with asthma." *Annals of Allergy, Asthma & Immunology* 102 (2):125-130.

Shirasaki, Hideaki, Nobuhiko Seki, Manabu Fujita, Megumi Kikuchi, Etsuko Kanaizumi, Kazumasa Watanabe, and Tetsuo Himi. 2007. "Agonist-and TH2 cytokine-induced up-regulation of cysteinyl leukotriene receptor messenger RNA in human monocytes." *Annals of Allergy, Asthma & Immunology* 99 (4):340-347.

Simpson, Angela, Nevena Lazic, Danielle CM Belgrave, Phil Johnson, Christopher Bishop, Clare Mills, and Adnan Custovic. 2015. "Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years." *Journal of Allergy and Clinical Immunology* 136 (5):1224-1231.

Singh, Rakesh Kumar, Ruchi Tandon, Sunanda Ghosh Dastidar, and Abhijit Ray. 2013. "A review on leukotrienes and their receptors with reference to asthma." *Journal of Asthma* 50 (9):922-931.

Skobeloff, Emil M, William H Spivey, SS St. Clair, and John M Schoffstall. 1992. "The influence of age and sex on asthma admissions." *JOURNAL-AMERICAN MEDICAL ASSOCIATION* 268:3437-3437.

Smit, LAM, Emmanuelle Bouzigon, Isabelle Pin, Valérie Siroux, F Monier, Hugues Aschard, Jean Bousquet, Frédéric Gormand, Jocelyne Just, and Nicole Le Moual. 2010. "17q21 variants modify the association between early respiratory infections and asthma." *European Respiratory Journal* 36 (1):57-64.

Söderström, L, G Lilja, MP Borres, and C Nilsson. 2011. "An explorative study of low levels of allergen-specific IgE and clinical allergy symptoms during early childhood." *Allergy* 66 (8):1058-1064.

Sokolowska, Milena, Karolina Wodz-Naskiewicz, Malgorzata Cieslak, Karolina Seta, Andrzej K Bednarek, and Rafal Pawliczak. 2009. "Variable expression of cysteinyl leukotriene type I receptor splice variants in asthmatic females with different promoter haplotypes." *BMC immunology* 10 (1):63.

Song, Woo-Jung, Hyun Jung Kim, Ji-Su Shim, Ha-Kyeong Won, Sung-Yoon Kang, Kyoung-Hee Sohn, Byung-Keun Kim, Eun-Jung Jo, Min-Hye Kim, and Sang-Heon Kim. 2017. "Diagnostic accuracy of fractional exhaled nitric oxide measurement in predicting cough-variant asthma and eosinophilic

bronchitis in adults with chronic cough: a systematic review and meta-analysis." *Journal of Allergy and Clinical Immunology* 140 (3):701-709.

Sordillo, Joanne E, Christina V Scirica, Sheryl L Rifas-Shiman, Matthew W Gillman, Supinda Bunyavanich, Carlos A Camargo, Scott T Weiss, Diane R Gold, and Augusto A Litonjua. 2015. "Prenatal and infant exposure to acetaminophen and ibuprofen and the risk for wheeze and asthma in children." *Journal of Allergy and Clinical Immunology* 135 (2):441-448.

Spector, Sheldon Laurence, and Ricardo Antonio Tan. 2012. "Is a single blood eosinophil count a reliable marker for “eosinophilic asthma?”." *Journal of asthma* 49 (8):807-810.

Stadhouders, Ralph, Bobby WS Li, Marjolein JW de Bruijn, Antonio Gomez, Tata Nageswara Rao, Hans Jörg Fehling, Wilfred FJ van IJcken, Ai Ing Lim, James P Di Santo, and Thomas Graf. 2018. "Epigenome analysis links gene regulatory elements in group 2 innate lymphocytes to asthma susceptibility." *Journal of Allergy and Clinical Immunology*.

Stafford, Susan, Clifford Lowell, Sanjiv Sur, and Rafeul Alam. 2002. "Lyn tyrosine kinase is important for IL-5-stimulated eosinophil differentiation." *The Journal of Immunology* 168 (4):1978-1983.

Stefanowicz, Dorota, Tillie-Louise Hackett, Farshid S Garmaroudi, Oliver P Günther, Sarah Neumann, Erika N Sutanto, Kak-Ming Ling, Michael S Kobor, Anthony Kicic, and Stephen M Stick. 2012. "DNA methylation profiles of airway epithelial cells and PBMCs from healthy, atopic and asthmatic children." *PLoS One* 7 (9):e44213.

Sterk, Peter J. 2016. Chronic diseases like asthma and COPD: do they truly exist? : Eur Respiratory Soc.

Stevens, Whitney W, Christopher J Ocampo, Sergejs Berdnikovs, Masafumi Sakashita, Mahboobeh Mahdavinia, Lydia Suh, Tetsuji Takabayashi, James E Norton, Kathryn E Hulse, and David B Conley. 2015. "Cytokines in chronic rhinosinusitis. Role in eosinophilia and aspirin-exacerbated respiratory disease." *American journal of respiratory and critical care medicine* 192 (6):682-694.

Strachan, MWJ, and BR Walker. 2006. "Endocrine disease." *Davidson Principles and Practice of Medicine, 22nd ed.; Walker, BR, Ed*:738-740.

Suaad A. Brakhas and Amna N. Jassim 2016. "Study of total Immunoglobulin E and Eosinophil count in allergic disease." *Baghdad Science Journal* 13 304-298:(2).

Suzuki, Masato, Masahiko Kato, Hirokazu Kimura, Toru Fujiu, and Akihiro Morikawa. 2003. "Inhibition of human eosinophil activation by a cysteinyl

leukotriene receptor antagonist (pranlukast; ONO-1078)." *Journal of Asthma* 40 (4):395-404.

Szefler, Stanley J, Brenda R Phillips, Fernando D Martinez, Vernon M Chinchilli, Robert F Lemanske, Robert C Strunk, Robert S Zeiger, Gary Larsen, Joseph D Spahn, and Leonard B Bacharier. 2005. "Characterization of within-subject responses to fluticasone and montelukast in childhood asthma." *Journal of Allergy and Clinical Immunology* 115 (2):233-242.

Takasaki, Jun, Masazumi Kamohara, Mitsuyuki Matsumoto, Tetsu Saito, Toru Sugimoto, Takahide Ohishi, Hiroyuki Ishii, Toshio Ota, Tetsuo Nishikawa, and Yuri Kawai. 2000. "The molecular characterization and tissue distribution of the human cysteinyl leukotriene CysLT 2 receptor." *Biochemical and biophysical research communications* 274 (2):316-322.

Tanaka, Nobuyuki, Tsuneo Matsumoto, Gouji Miura, Takuya Emoto, Naofumi Matsunaga, Katsuhiko Ueda, and David A Lynch. 2003. "Air trapping at CT: high prevalence in asymptomatic subjects with normal pulmonary function." *Radiology* 227 (3):776-785.

Tarlo, Susan M. 2016. "Update on work-exacerbated asthma." *Int J Occup Med Environ Health* 29 (3):369-374.

Thivierge, Maryse, Jana Staňková, and Marek Rola-Pleszczynski. 2001. "IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages." *The Journal of Immunology* 167 (5):2855-2860.

The European Pet Food Industry Federation (FEDIAF) , 2016. Facts and Figures. 2012. Available from: http://www.fediaf.org/fileadmin/ user_upload/facts_and_figures_2010.pdf. Accessed November 7.

Thivierge, Maryse, Micah Doty, Jeff Johnson, Jana Staňková, and Marek Rola-Pleszczynski. 2000. "IL-5 up-regulates cysteinyl leukotriene 1 receptor expression in HL-60 cells differentiated into eosinophils." *The Journal of Immunology* 165 (9):5221-5226.

Thomas, M, and T Wilkinson. 2014. "Asthma diagnosis in the community–time for a change?" *Clinical & Experimental Allergy* 44 (10):1206-1209.

Thompson, Miles D, Jun Takasaki, Valérie Capra, G Enrico Rovati, Kathy A Siminovitch, W McIntyre Burnham, Thomas J Hudson, Yohan Bossé, and David EC Cole. 2006. "G-Protein-Coupled Receptors and Asthma Endophenotypes." *Molecular diagnosis & therapy* 10 (6):353-366.

Thompson, Miles D, Valerie Capra, Jun Takasaki, Giovanna Maresca, G Enrico Rovati, Aurther S Slutsky, Craig Lilly, Noe Zamel, W McIntyre Burnham, and David E Cole. 2007. "A functional G300S variant of the cysteinyl

leukotriene 1 receptor is associated with atopy in a Tristan da Cunha isolate." *Pharmacogenetics and genomics* 17 (7):539-549.

Thomsen, Simon F. 2015. "The contribution of twin studies to the understanding of the aetiology of asthma and atopic diseases." *European clinical respiratory journal* 2 (1):27803.

Thomsen, Simon Francis, S Van Der Sluis, KO Kyvik, A Skytthe, and Vibeke Backer. 2010. "Estimates of asthma heritability in a large twin sample." *Clinical & Experimental Allergy* 40 (7):1054-1061.

Toelle, Brett G, Jennifer K Peat, Cheryl M Salome, Craig M Mellis, and ANNJ WOOLCOCK. 1992. "Toward a Definition of Asthma for Epidemiology1-3." *Am Rev Respir Dis* 146:633-637.

Tornhamre, Susanne, Leif Stenke, Anna Granzelius, Mikael Sjölinder, Barbro Näsman-Glaser, Cecilia Roos, Susanne Widell, and Jan Åke Lindgren. 2003. "Inverse relationship between myeloid maturation and leukotriene C4 synthase expression in normal and leukemic myelopoiesis—consistent overexpression of the enzyme in myeloid cells from patients with chronic myeloid leukemia." *Experimental hematology* 31 (2):122-130.

Thompson, M.D. ,Capra,V., and Stankova,J.(2013). Cysteinyl leukotriene1and cysteinyl leukotriene2receptorsareassociatedwithatopicasthmainafounder population. Eur. J.Hum.Genet. 21(Suppl. 2),405(P16.114).

Tornhamre S, Stenke L, Granzelius A, Sjo¨linder M, Na¨sman-Glaser B, Roos C, Widell S, and Lindgren JA (2003) Inverse relationship between myeloid maturation and leukotriene C4 synthase expression in normal and leukemic myelopoiesis consistent overexpression of the enzyme in myeloid cells from patients with chronic myeloid leukemia. Exp Hematol 31:122–130.

Tovey, Euan R, Damien Liu-Brennan, Frances L Garden, Brian G Oliver, Matthew S Perzanowski, and Guy B Marks. 2016. "Time-based measurement of personal mite allergen bioaerosol exposure over 24 hour periods." *PloS one* 11 (5).

Troisi, Rebecca J, Frank E Speizer, Walter C Willett, D Trichopoulos, and Bernard Rosner. 1995. "Menopause, postmenopausal estrogen preparations, and the risk of adult-onset asthma. A prospective cohort study." *American journal of respiratory and critical care medicine* 152 (4):1183-1188.

Tsai, Yi-Hsuan, Joel S Parker, Ivana V Yang, and Samir NP Kelada. 2018. "Meta-Analysis of Airway Epithelia Gene Expression in Asthma." *European Respiratory Journal*.

Turner, Mark O, Kuku Noertjojo, Sverre Vedal, Tony Bai, Susan Crump, and J MARK FITZGERALD. 1998. "Risk factors for near-fatal asthma: a case–

control study in hospitalized patients with asthma." *American journal of respiratory and critical care medicine* 157 (6):1804-1809.

Vaglio, A, C Buzio, and J Zwerina. 2013. "Eosinophilic granulomatosis with polyangiitis (Churg–Strauss): state of the art." *Allergy* 68 (3):261-273.

Van Keer, C, K Kersters, and J De Ley. 1976. "L-Sorbose metabolism in Agrobacterium tumefaciens." *Antonie van Leeuwenhoek* 42 (1-2):13-24.

VanGarsse, Anne, Richard D Magie, and Aubree Bruhnding. 2015. "Pediatric Asthma for the Primary Care Practitioner." *Primary Care: Clinics in Office Practice* 42 (1):129-142.

Varricchi, Gilda, Diego Bagnasco, Francesco Borriello, Enrico Heffler, and Giorgio W Canonica. 2016. "Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs." *Current opinion in allergy and clinical immunology* 16 (2):186.

Vercelli, Donata. 2010. "Gene-environment interactions in asthma and allergy: the end of the beginning?" *Current opinion in allergy and clinical immunology* 10 (2):145.

Vernon, Margaret K, Ingela Wiklund, Jill A Bell, Peter Dale, and Kenneth R Chapman. 2012. "What do we know about asthma triggers? A review of the literature." *Journal of Asthma* 49 (10):991-998.

Versini, Mathilde, Pierre-Yves Jeandel, Tomer Bashi, Giorgia Bizzaro, Miri Blank, and Yehuda Shoenfeld. 2015. "Unraveling the hygiene hypothesis of helminthes and autoimmunity: origins, pathophysiology, and clinical applications." *BMC medicine* 13 (1):81.

Virchow Jr, JC, S Faehndrich, C Nassenstein, S Bock, H Matthys, and W Luttmann. 2001. "Effect of a specific cysteinyl leukotriene-receptor 1-antagonist (montelukast) on the transmigration of eosinophils across human umbilical vein endothelial cells." *Clinical & Experimental Allergy* 31 (6):836-844.

Vogelmeier, Claus F, Gerard J Criner, Fernando J Martinez, Antonio Anzueto, Peter J Barnes, Jean Bourbeau, Bartolome R Celli, Rongchang Chen, Marc Decramer, and Leonardo M Fabbri. 2017. "Global strategy for the diagnosis, management and prevention of chronic obstructive lung disease 2017 report." *Respirology* 22 (3):575-601.

Wala'a, E Jasim, Batool A Al-Haidary, and Yacoob Y Al-Rubaeyi. 2006. "Possible Role for Interleukin-5 in Asthma." *Journal of the Faculty of Medicine* مجلة كلية الطب. 48 (4):425-430.

Weinmayr, Gudrun, Ulrike Gehring, Jon Genuneit, Gisela Büchele, Andrea Kleiner, Robert Siebers, Kristin Wickens, Julian Crane, Bert Brunekreef,

and David P Strachan. 2013. "Dampness and moulds in relation to respiratory and allergic symptoms in children: results from Phase Two of the International Study of Asthma and Allergies in Childhood (ISAAC Phase Two)." *Clinical & Experimental Allergy* 43 (7):762-774.

Wenzel, Sally E, John B Trudeau, David A Kaminsky, Judith Cohn, Richard J Martin, and Jay Y Westcott. 1995. "Effect of 5-lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma." *American journal of respiratory and critical care medicine* 152 (3):897-905.

Wenzel, Sally E. 2012. "Asthma phenotypes: the evolution from clinical to molecular approaches." *Nature medicine* 18 (5):716.

Wenzel, Sally, Darren Wilbraham, Rick Fuller, Elise Burmeister Getz, and Malinda Longphre. 2007. "Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies." *The Lancet* 370 (9596):1422-1431.

Woodruff, Prescott G, Barmak Modrek, David F Choy, Guiquan Jia, Alexander R Abbas, Almut Ellwanger, Joseph R Arron, Laura L Koth, and John V Fahy. 2009. "T-helper type 2–driven inflammation defines major subphenotypes of asthma." *American journal of respiratory and critical care medicine* 180 (5):388-395.

Wysocki, Kenneth James. 2011. *Leukotriene receptor gene variation and atopic asthma*: The University of Arizona.

Yaddaden, Louiza, Steeve Véronneau, Miles D Thompson, Marek Rola-Pleszczynski, and Jana Stankova. 2016. "Cellular signalling of cysteinyl leukotriene type 1 receptor variants CysLT1-G300S and CysLT1-I206S." *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)* 105:1-8.

Yaddaden, Louiza. 2015. "Caractérisation de variants du récepteur des cystéinyl leucotriènes de type 1, CysLT1-G300S et CysLT1-I206S."

Yamatomo, T, M Okano, T Ono, E Nakayama, T Yoshino, AR Satoskar, DA Harn Jr, and K Nishizaki. 2001. "Sex-related differences in the initiation of allergic rhinitis in mice." *Allergy* 56 (6):525-531.

Yokouchi, Yukako, Yumi Nukaga, Masanao Shibasaki, Emiko Noguchi, Kazumi Kimura, Seiko Ito, Mizuki Nishihara, Kimiko Yamakawa-Kobayashi, Kazunori Takeda, and Natsuki Imoto. 2000. "Significant evidence for linkage of mite-sensitive childhood asthma to chromosome 5q31–q33 near the interleukin 12 B locus by a genome-wide search in Japanese families." *Genomics* 66 (2):152-160.

Zaynagetdinov, Rinat, Taylor P Sherrill, Linda A Gleaves, Allyson G McLoed, Jamie A Saxon, Arun C Habermann, Linda Connelly, Daniel Dulek, R

Stokes Peebles, and Barbara Fingleton. 2015. "Interleukin-5 facilitates lung metastasis by modulating the immune microenvironment." *Cancer research* 75 (8):1624-1634.

Zaynagetdinov, Rinat, Taylor P Sherrill, Linda A Gleaves, Allyson G McLoed, Jamie A Saxon, Arun C Habermann, Linda Connelly, Daniel Dulek, R Stokes Peebles, and Barbara Fingleton. 2015. "Interleukin-5 facilitates lung metastasis by modulating the immune microenvironment." *Cancer research* 75 (8):1624-1634.

Zhang, Jian, Ohsuke Migita, Minori Koga, Masanao Shibasaki, Tadao Arinami, and Emiko Noguchi. 2006. "Determination of structure and transcriptional regulation of CYSLTR1 and an association study with asthma and rhinitis." *Pediatric allergy and immunology* 17 (4):242-249.

Zhang, June Y, and Sally E Wenzel. 2007. "Tissue and BAL based biomarkers in asthma." *Immunology and Allergy Clinics* 27 (4):623-632.

Zhang, Xingyou, Teresa Morrison-Carpenter, James B Holt, and David B Callahan. 2013. "Trends in adult current asthma prevalence and contributing risk factors in the United States by state: 2000–2009." *BMC Public Health* 13 (1):1156.

Zhang, Yue, Li Peng, Haidong Kan, Jianming Xu, Renjie Chen, Yuan Liu, and Weibing Wang. 2014. "Effects of meteorological factors on daily hospital admissions for asthma in adults: a time-series analysis." *PLoS One* 9 (7).

Zheng, Xue-yan, Hong Ding, Li-na Jiang, Shao-wei Chen, Jin-ping Zheng, Min Qiu, Ying-xue Zhou, Qing Chen, and Wei-jie Guan. 2015. "Association between air pollutants and asthma emergency room visits and hospital admissions in time series studies: a systematic review and meta-analysis." *PloS one* 10 (9).

Zhu, Jie, Yu-Sheng Qiu, David J Figueroa, Venkata Bandi, Helen Galczenski, Kaoru Hamada, Kalpalatha K Guntupalli, Jilly F Evans, and Peter K Jeffery. 2005. "Localization and upregulation of cysteinyl leukotriene-1 receptor in asthmatic bronchial mucosa." *American journal of respiratory cell and molecular biology* 33 (6):531-540.

Thompson MD,Gravesande KSV ,Galezenski H etal .A cysteinyl leukotriene 2 receptor variants is associated with atopy in the population of Tristan da cunha.pharmacogenetics 2003;13:641-9.

Global Initiative for Asthma. Global strategy for asthma management and prevention. 2017.

World Allergy Organization. White Book on Allergy [internet]. Milwaukee, Wisconsin: World Allergy Organization; 2011. [cited 2017 Mar 21]. Available from: http://www.worldallergy.org/UserFiles/file/WAO-White-Book-on-Allergy_web.pdf.

((Droste, J.H.J. ; Kerkhof, M. ; de Monchy, J.G.R. ; Schouten, J.P. and Rijcken, B. (2014) : Association of skin test reactivity, specific IgE, total IgE, and eosinophils with nasal symptoms in a community-based population study. J. Allergy Clin. Immunol. 97:922-32.))

Shakir MM, Kassim MS, Mohammad YA. Asthma in adults;epidemiology, risk factor and patterns of presentation: a crosssectional, questionnaire based study in Baghdad Teaching. Kar-bala J Med. 2012;5(11):1255–1261.))

Buttaro, T. M., Trybulski, J., Polgar-Bailey, P., & Sandberg-Cook, J. (2012). Primary Care: A Collaborative Practice (Kindle ed.). St. Louis, MO: Elsevier.

Husain AN, Kumar V. The lung. In: Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran Pathologic Basis of Disease. 7th ed. Philadelphia: W.B. Saunders; 2007. pp. 711–72.