## CONTENTS

### CHAPTER ONE: INTRODUCTION, REVIEW OF LITERATURE & OBJECTIVES OF THE STUDY

1.1 INTRODUCTION 1

1.2 REVIEW OF LITERATURE

   1.2.1 Definition of asthma 7
   1.2.2 Childhood Asthma 7
   1.2.3 Symptoms of asthma 7
      1.2.3.1 Wheeze 7
      1.2.3.2 Cough 8
      1.2.3.3 Breathlessness 8
   1.2.4 Risk Factors of Asthma in Children 8
      1.2.4.1 Allergic Environmental Triggers 8
         1.2.4.1.1 House Dust Mites 8
         1.2.4.1.2 Cockroaches 8
         1.2.4.1.3 Companion Animal Allergens 9
         1.2.4.1.4 Fungi 9
      1.2.4.2 Nonallergic Environmental triggers 9
         1.2.4.2.1 Environmental Tobacco Smoke (ETS) 9
         1.2.4.2.2 Viral Infections 9
         1.2.4.2.3 Endotoxin 10
         1.2.4.2.4 Pollutants 10
         1.2.4.2.5 Microbes and their products 10
         1.2.4.2.6 Maternal Diet during Pregnancy and/or Lactation 10
         1.2.4.2.7 Psychosocial Factors 11
   1.2.5 Diagnosis of asthma in children 11
      1.2.5.1 Clinical History 12
      1.2.5.2 Therapeutic trial 12
      1.2.5.3 Test for IgE-mediated Allergy (Atopy) 12
      1.2.5.4 Chest Radiograph (X-ray) 12
      1.2.5.5 Lung Function Testing 13
         1.2.5.5.1 Peak Expiratory Flow 13
         1.2.5.5.2 Spirometry 13
   1.2.6 Treatment of Asthma 13
      1.2.6.1 Reliever medications 14
         1.2.6.1.1 Short-acting β2 agonists 14
1.2.6.1.2 Ipratropium bromide

1.2.6.2 Regular controller therapy
   1.2.6.2.1 Inhaled corticosteroid (ICS)  
   1.2.6.2.2 Leukotriene Receptor Agonist (LTRA)  
   1.2.6.2.3 Long-acting inhaled β2-agonists (LABAs)  
   1.2.6.2.4 Oral theophylline  
   1.2.6.2.5 Cromolyn sodium (nedocromil)  
   1.2.6.2.6 Anti-IgE antibodies  
   1.2.6.2.7 Oral glucocorticosteroids

1.2.7 Pathophysiology of asthma
   1.2.7.1 Airway inflammation  
   1.2.7.2 Bronchial hyperreactivity  
   1.2.7.3 Airflow obstruction  
   1.2.7.4 Airway Remodeling  
   1.2.7.5 Effector Cells of Inflammation and Remodeling in Asthma  
      1.2.7.5.1 Mast Cells  
      1.2.7.5.2 Basophils  
      1.2.7.5.3 Eosinophils  
      1.2.7.5.4 Neutrophils  
      1.2.7.5.5 T lymphocytes  
      1.2.7.5.6 Dendritic Cells  
      1.2.7.5.7 Platelets  
      1.2.7.5.8 Macrophages  
      1.2.7.5.9 Epithelial cells

1.2.8 Prevalence of Childhood Asthma

1.2.9 Economic Burden of Asthma

1.2.10 Social Impact of Asthma

1.2.11 C-Reactive Protein (CRP)
   1.2.11.1 C-reactive protein in asthma

1.2.12 Immunoglobulin E (IgE)
   1.2.12.1 Pathophysiologic Role of IgE in Asthma
   1.2.12.2 IgE in asthma

1.2.13 Cytokines
   1.2.13.1 CD4⁺ T cell subsets and Cytokine profile
   1.2.13.2 Role of cytokines in the pathogenesis of asthma
   1.2.13.3 Role of Th2 Cytokines in Allergic Inflammation
      1.2.13.3.1 IL-4
1.2.13.3.2 IL-5 34
1.2.13.3.3 IL-9 34
1.2.13.3.4 IL-13 34
1.2.13.4 Cytokines in asthma 35

1.2.14 Human Leukocyte Antigen (HLA) 36
1.2.14.1 General Organization of the HLA system 37
1.2.14.2 HLA Class I molecules 38
1.2.14.3 HLA Class II Molecules 39
1.2.14.4 Antigen Processing and Presentation 40
1.2.14.5 Genetics of HLA 41
    1.2.14.5.1 Polymorphism 42
    1.2.14.5.2 Inheritance of HLA 42
    1.2.14.5.3 Linkage disequilibrium 43
    1.2.14.5.4 Cross-reactivity 44
1.2.14.6 HLA and Disease Susceptibility 44
1.2.14.7 Association of HLA with Asthma 45

1.3 OBJECTIVES OF THE STUDY 52

CHAPTER TWO: MATERIALS AND METHODS

2. MATERIALS & METHODS

2.1 Subjects 53
2.2 Collection of the demographic data & clinical history 53
2.3 Estimation of prevalence of asthma in children between 3 to 12 years 54
2.4 Collection of Blood Samples 54
    2.4.1 Separation of serum 54
2.5 Determination of serum CRP level (Latex Agglutination Test) 54
2.6 Determination of total serum IgE level 55
2.7 Determination of serum levels of IL-4 and IFN-γ 55
    2.7.1 Assay Procedure 56
2.8 Extraction of genomic DNA 56
    2.8.1 Quantification of DNA 57
2.9 PCR-SSP Typing of HLA alleles 58
    2.9.1 PCR amplification 58
    2.9.2 Preparation of reaction mixture 60
    2.9.3 Amplification procedure 61
    2.9.4 Amplification check by agarose gel electrophoresis 62
        2.9.4.1 Procedure 63
        2.9.4.2 Documentation and Interpretation 63
2.10 Statistical Analysis 63
CHAPTER THREE: RESULTS AND DISCUSSION

3. RESULTS & DISCUSSION

3.1 ESTIMATION OF PREVALENCE AND ASSESSMENT OF RISK FACTORS OF ASTHMA IN CHILDREN: A HOSPITAL BASED STUDY

3.1.1 Results 64
3.1.2 Discussion 65

3.2 DETERMINATION OF SERUM LEVEL OF C-REACTIVE PROTEIN

3.2.1 Results 68
3.2.2 Discussion 69

3.3 DETERMINATION OF TOTAL SERUM IMMUNOGLOBULIN E

3.3.1 Results 71
3.3.2 Discussion 72

3.4 DETERMINATION OF SERUM LEVEL OF IL-4 AND IFN-\gamma

3.4.1 Results 76
3.4.2 Discussion 78

3.5 PCR-SSP TYPING OF HLA CLASS I & CLASS II ALLELIC GROUPS

3.5.1 Results 82
3.5.2 Discussion 89

CHAPTER FOUR: COMPREHENSIVE DISCUSSION

4. COMPREHENSIVE DISCUSSION 93

4.1 Assessment of prevalence and associated risk factors of asthma 95
4.2 Serum C-reactive protein level 96
4.3 Total serum IgE level 97
4.4 Serum levels of IL-4 and IFN-\gamma 99
4.5 Typing of HLA allelic groups 101

CHAPTER FIVE: SUMMARY AND CONCLUSION

5. SUMMARY & CONCLUSION 104

BIBLIOGRAPHY 110
INDEX 150
APPENDIX - 1 (Questionnaire) 153
APPENDIX - 2 (Chemicals, reagents and Kits) 160
APPENDIX – 3 (Publications) 164