

**CORRELATION OF INTERLEUKIN 31 SERUM LEVELS IN PATIENTS
WITH ATOPIC DERMATITIS, ALLERGIC RHINITIS
AND ATOPIC ASTHMA**

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LIST OF ABBREVIATIONS

Abbreviations

AA	Atopic asthma
aa	Amino acid
α	level of significance
α -Helix	Alpha helix
AD	Atopic dermatitis
AIDS	acquired immunodeficiency syndrome
AR	Allergic rhinitis
Arg	Arginine
ARIA	Allergic Rhinitis and Its impact on Asthma
β	Beta
BSA	Bovine serum albumin
(CLA) ⁺	cutaneous lymphocyte antigen
cDNA	complementary deoxyribonucleic acid
CSF	Colony stimulating factors
d	effect size
DC	Dendritic cell
DD	Detectable difference
ECP	eosinophil cationic protein
EIA	enzyme immunoassay
ELISA	Enzyme linked-immunosorbent assay

FcεRI	Fc epsilon receptor I
FcεRII	Fc epsilon receptor II
FEV ₁	Forced expiratory volume in one second
Filaggrin	FLG
γ	Gamma
GINA	Global initiative for asthma
GM-CSF	granulocyte-macrophage colony stimulating factor
gp	glycoprotein
H	Histamine
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
HRPZ II	Hospital Sultanah Perempuan Zainab II
H ₂ SO ₄	Sulfuric acid
HUSM	Hospital Universiti Sains Malaysia
IBD	Intestinal bowel disease
IFN	Interferon
Ig E	Immunoglobulin E
IL	Interleukin
κ-opiate	Kappa opiate
KPP	Klinik Pakar Perubatan/ Outpatient Specialist Clinic
IQR	Interquartile
ISAAC	The International Study of Asthma and Allergies in Childhood
KCl	Potassium chloride
KH ₂ PO ₄	Potassium dihydrogen phosphate
LPS	Lipopolysaccharide

m	ratio between group
MHC	major histocompatibility complex
μ -opiate	micro opiate
μ L	microliter
μ g/mL	microgram permilliliter
mL	milliliter
μ m	micrometer
mM	milimolarity
MMPs	matrix metalloproteinases
mRNA	messenger ribonucleic acid
n	number of sample
Nacl	Sodium chloride
Na ₂ PO ₄	Disodium phosphate
NAPTs	nasal allergen provocation test
ng/mL	nanogram permilliliter
NK	Natural killer
nm	nanometer
NUNC	Nordic Union of Novel Camouflage
ORL-HNS	Otorhinolaryngology- Head and Neck Surgery
PBS	Phosphate buffer solution
PEF	Peak expiratory flow
pg/mL	picogram permilliliter
RA	Receptor antibody
R&D	Research and development
rpm	Rotation perminute

SABA	short-acting β_2 -agonist
SCORAD	Scoring atopic dermatitis
SD	Standard deviation
SEMFs	subepithelial myofibroblasts
sIgE	specific immunoglobulin E
SPSS	Statistical package for the social sciences
TFH	follicular TH
TGF	Transforming growth factor
T _H	T helper
TMB	Tetramethylbenzidine
TNF	Tumor necrosis factors
Treg	regulatory T cell
USA	United States of America
VNTR	variable number of tandem repeats
WHO	World Health Organization
%	Percent
1- β	power of study
°C	degree Celcius

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**KORELASI ANTARA PARAS INTERLEUKIN 31 DI DALAM DARAH
PESAKIT ATOPIC DERMATITIS, ALLERGIC RHINITIS
DAN ATOPIC ASTHMA**

ABSTRAK

Interleukin 31 (IL-31), merupakan salah satu daripada sitokin yang menjadi pengawal atur yang penting terhadap tindak balas T_H2 . Kajian terdahulu telah dilakukan oleh Zhang *et al.* (2008), untuk mengukur paras IL-31 di kalangan atopik dermatitis (AD). Walaubagaimanapun, paras IL-31 di kalangan allergic rhinitis (AR) dan atopik asthma (AA) adalah masih tidak jelas. Objektif-objektif dalam kajian “cross sectional” ini adalah untuk mengukur paras IL-31 di kalangan AD, AR dan AA, untuk mengukur korelasi antara IL-31 dan faktor-faktor yang mempengaruhi penyakit, untuk mengukur IL-31 dan tahap keterukan penyakit dan untuk mengukur paras IL-31 dan pruritus di kalangan pesakit. Kajian ini melibatkan 70 pesakit AD dari Klinik Kulit, Hospital Universiti Sains Malaysia (HUSM) dan Hospital Raja Perempuan Zainab II (HRPZ II), 70 pesakit AR dari Klinik Telinga, Hidung dan Tekak (ENT) di HUSM, 70 pesakit AA dari Klinik Dada dan Klinik Kanak-kanak di HUSM dan 70 subjek sihat (staf dan individu di HUSM). Lima mililiter darah diambil dan diemparkan untuk 5 minit pada 2000 putaran perminit (rpm) untuk mendapatkan serum dan dianalisa untuk paras IL-31 menggunakan kit “enzyme-linked immunosorbent” (ELISA) (Human IL-31 Duoset, R&D System). “Independent t-test” adalah kaedah statistic yang digunakan untuk melihat perbezaan paras IL-31 antara individu sihat dan penyakit alergik begitu juga

paras IL-31 dan pruritus di kalangan pesakit. “Simple” dan “multiple logistic regressions” digunakan untuk menganalisa korelasi. “Kruskal Wallis test” digunakan untuk mengukur paras IL-31 dan tahap keterukan AD dan AA dan “Mann-Whitney test” digunakan untuk mengukur paras IL-31 dan tahap keterukan AR. Hasil kajian menunjukkan bahawa tiada perbezaan yang jelas dalam min (sisihan piawai) di antara paras IL-31 dalam kalangan pesakit, 5048.79 (24628.27) and individu sihat, 2195.55 (9016.57) ($p=0.344$). Paras IL-31 dan faktor-faktor yang mempengaruhi menunjukkan hubungan yang jelas di dalam status merokok, pendedahan pekerjaan dan kawasan tempat tinggal bagi AD dan AR, walaubagaimanapun dalam AA, hubungan yang jelas hanya didapati di dalam status merokok dan pendedahan pekerjaan. Kajian menunjukkan tiada perbezaan yang jelas di antara paras IL-31 dan tahap keterukan pesakit alergik, AD ($p=0.104$), AR ($p=0.245$) dan AA ($p=0.745$). Keputusan menunjukkan tiada perbezaan yang jelas di antara paras IL-31 dan pruritus di dalam pesakit dengan min (sisihan piawai) di dalam “pruritic”, 6168.25 (30552.07) and “non-pruritic”, 3693.64 (14600.99).

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ABSTRACT

Interleukin 31 (IL-31), is one of the cytokines which appears to be an important regulator of T_H2 responses. Previous study has been done by Zhang *et al.* (2008), to determine IL-31 serums levels in atopic dermatitis (AD). However, the serum levels of IL-31 in allergic rhinitis (AR) and atopic asthma (AA) is still unclear. The objectives of this cross sectional study are to determine IL-31 serum levels in AD, AR and AA, to study the association between IL-31 and predisposing factors with allergic diseases, to correlate IL-31 levels and the severity of allergic diseases and to compare IL-31 levels and pruritus in patients with allergic diseases. This study involved 70 patients with AD from the skin clinic of Hospital Universiti Sains Malaysia (HUSM) and Hospital Raja Perempuan Zainab II (HRPZ II), 70 patients with AR from the Ear, Nose and Throat Clinic (ENT clinic) of HUSM, 70 patients with AA from the Chest Clinic and Pediatrics Clinic of HUSM and 70 healthy controls (staff and people of HUSM). Five milliliters of blood were withdrawn and centrifuged for 5 minutes at 2000 rpm to obtain the serum and analyzed for IL-31 levels by using enzyme-linked immunosorbent (ELISA) kits (Human IL-31 DuoSet, R&D System). Independent t-test was the statistical method used to compare IL-31 levels between healthy controls and allergic diseases as well as IL-31 levels and pruritus in allergy. Simple and multiple logistic regressions were used to

analyze the association. Kruskal Wallis test was used to determine IL-31 levels and the severity of AD and AA and Mann-Whitney test was used to determine IL-31 and the severity of AR. The results showed that there was no statistically significant difference of mean (SD) of IL-31 levels among diseases, 5048.79 (24628.27) and controls, 2195.55 (9016.57) ($p=0.344$). The levels of IL-31 and other predisposing factors showed significant associations in smoking status, occupational exposure and area of living for AD and AR, however in AA, the significant association only found in smoking status and occupational exposure. There was no significant difference between IL-31 levels and the severity of allergic diseases, AD ($p=0.104$), AR ($p=0.245$) and AA ($p=0.745$). The results showed there was no significant differences between IL-31 levels and pruritus in patients with mean (SD) in pruritic, 6168.25 (30552.07) and non-pruritic, 3693.64 (14600.99).

CHAPTER 1

INTRODUCTION

1.1 Allergy and atopy

In 1906, Clemens P. Pirquet introduced the term of “allergy” to describe the reaction of protective immunity and hypersensitivity (Bukantz, 2002). Later, allergy was used to describe the unexpected reactions in the skin and mucosa (Wuthrich, 1999). Allergy usually refers to the clinical expression of atopic IgE-mediated disease. It is often expressed towards a target organ (Durham and Church, 2001).

Atopy refers to the implication to an allergic disease due to IgE response to one or several common environmental allergens (Gusareva *et al.*, 2009). Clinical symptoms may or may not be found in atopic individuals. Individuals that suffer atopy produce abnormally high levels of IgE in response to the allergens and overexpression of IgE receptors than normal people (Corrigan and Kay, 1992; Abbas *et al.*, 1994).

1.2 Atopic dermatitis

Atopic dermatitis (AD) is a chronic inflammation manifested by itchiness of the skin. Patients with AD demonstrate a range of primary pruritic lesions including macular erythema, a fine papular eruption, erythematous urticaria, indurated papules, plaque and diffuse edematous erythema. The scurf is characterized by itchy papules that become excoriated and lichenified (Friedman and Holden, 2004). A study done in Granada, Spain stated that 60.7% of the population were never awakened at night because of the AD, 11.8% said that they had woken less than once per week and 2.2% showed they were awakened once or more per week (Guiote-Dominguez *et al.*, 2008).

1.2.1 Prevalence study of atopic dermatitis

Females are more affected to this allergic diseases compared to males as reported by Kapoor *et al.* (2008). A recent study reported that 80.0% of the patients suffered AD in their family. About 1/3 of AD patients also suffered from asthma and allergic rhinitis. Patients with AD are prone to get AR and AA (Asher *et al.*, 2006).

The worldwide prevalence showed an increasing trend of allergic diseases (Cookson *et al.*, 2001; Nomura *et al.*, 2003; Cookson *et al.*, 2004; Marenholz *et al.*, 2006; Morar *et al.*, 2006). The annual prevalence of AD appears to be increased.

A study in Granada found that 29.4% of their schoolchildren had been diagnosed with AD. The prevalence of AD among 6 to 7 years old (36.1%) was higher between 13 and

14 (23.5%). The diagnosis of AD was higher among those living in the urban areas than those living along the coastal region (Guiote-Dominguez *et al.*, 2008).

The previous studies in Nikel, Russia done by Dotterud *et al.* (2004), and Ulsan, Korea done by Kim *et al.* (2000), reported that lower prevalences were found in industrialized areas than in nonindustrialized part. However, the studies done in Jimma, (Ethiopia) by Yemaneberhan *et al.* (2004) and Seoul (Korea) done by Kim *et al.* (2000) showed the higher prevalence of AD was found in urban areas than in rural ones.

A previous study done in Malaysia, found that the overall prevalence of AD symptoms was 12.0%. The prevalence of AD in the 5 to 7 year age group was recorded 13.7% while among 12 to 14 year age group showed 9.9% (Quah *et al.*, 1997).

The results showed no significant changes in the prevalence of symptoms in the previous 12 months (1995 vs 2001) in the same study. These were also no major changes in the prevalence rates of these diseases over a period of 6 years (Quah *et al.*, 2005).

1.2.2 Aetiology of atopic dermatitis

There are various factors that contribute to the expression of AD. They can be environmental, genetic and immunological factors (Gustafsson *et al.*, 2000; Abramovits and Abramovits, 2005; Asher *et al.*, 2006; Fiset *et al.*, 2006). The triggers of atopic dermatitis also included foods, house dust mites, emotional factors as well as skin irritants.

Hygiene hypothesis can be the best explanation for the development of AD (Holt, 2000). This reason was explained with the exposure to pollutants. However, in some way it protects against the development of atopy. The exposure to infectious diseases which were associated with poorer, less developed nations also protects against atopy.

1.2.3 Clinical manifestations of atopic dermatitis

The major symptoms of AD include pruritus, dermatitis affecting flexural surfaces in adults, face and extensors in infants. There is usually also history of chronic or relapsing dermatitis and personal or family history of cutaneous or respiratory atopy. The minor symptoms consist of features of so-called atopic facies : facial pallor or erythema, hypopigmented patches, infraorbital darkening, infraorbital folds or wrinkles, cheilitis (inflammation of the lip), recurrent conjunctivitis and anterior neck folds.

The other symptoms of AD also include dry skin, ichthyosis (excessive amounts of dry surface scales), hyperlinear palms, keratosis pilaris (plugged hair follicles of the proximal extremities), hand and foot dermatitis, nipple eczema, white dermatographism and perifollicular accentuation. AD patients may also have emotional disturbances such as feeling anxious and unable to manage anger in certain circumstances (Ginsburg *et al.*, 1993).

The intensely pruritic, erythematous macules or papules can present initially in acute lesions of AD. With the scratching of the primary lesions, secondary lesions may occur as excoriated papules with serum exudates and crust. In young children, lesions tend to

appear on the scalp, face and extensor surface of the legs and arms. In older children, lesion occurs within the flexural areas of the extremities.

In both children and adult, skin of AD is dry and this reflects loss of cutaneous barrier function (Blauvelt *et al.*, 2003). AD skin has increased permeability to microbes as well as other allergens. Chronic AD appeared to have lichenification (thickening) of the skin. With intense scratching or rubbing, the epidermis may result to prurigo nodules.

1.2.4 Scoring system of atopic dermatitis (Objective SCORAD)

Objective SCORAD was used to determine the severity of AD. In the Objective SCORAD, mild eczema is defined as a score less than 15, for moderate eczema a score is more than 15 but less than 40 and for severe AD, the score is greater than 40.

The severity of AD is classified by using Objective SCORAD which include the extension of the disease (A) and the intensity of the disease (B) with mean calculation (1 for mild, 2 for moderate and 3 for severe). The distribution of the score is achieved using the formula $A/5 + 7B/2$.

The interpretation of disorder are (A: according to the rule of nines; 20% of the score), the intensity composed of six items (B: erythema, oedema/papules, effect of scratching, oozing /crust formation, lichenification and dryness; 60% of the score; each item has four grades: 0, 1, 2, 3). The maximum score can reach 93.

In this study, Objective SCORAD system is used based on a European consensus. This system is representative and well evaluated but shows, as with other systems, intra- and

interobserver disagreements. The Objective SCORAD gives more information about extent and intensity of eczema. Therefore the Objective SCORAD should be used in clinical comparative trials.

1.2.5 Pathophysiology of atopic dermatitis

AD resulted from dysregulated Th2-biased immune responses to the environmental stimuli (Leung and Soter, 2001). An increasing number of eosinophils is found in the blood with a corresponding elevated IgE levels in AD. Allergen-specific T cells that produce cytokines also appear to be increased in the peripheral blood of AD patients. In addition to promote the antibody responses, the cytokines also inhibit the ability of the T cells to generate T_{H1} cytokines.

Blood mononuclear cells from AD patients have reduced capacity to generate this critical T_{H1} cytokine. The loss of IFN- γ (interferon gamma) may be a critical pathophysiologic mechanism in atopic disease as AD patients respond well to treatment with IFN- γ (Stevens *et al.*, 1998).

T_{H1} supportive cytokine that up-regulate IFN- γ , is also down-regulated in AD subjects (Ong *et al.*, 2002). Previous evidence showed that chemokines and their proteins could play an important role in the recruitment of specific immune populations into the skin in AD and other inflammatory skin conditions (Locati and Murphy, 1999).

The shift in balance between cytokines produced by T_{H1} and T_{H2} towards T_{H2} predominance might trigger allergic inflammation (Scavuzzo *et al.*, 2003; Ciprandi *et*

al., 2009). T_H2 lymphocyte proliferation is induced by the allergen with the release of characteristic combination of cytokines and granulocyte-macrophage colony stimulating factor (GM-CSF) (Scavuzzo *et al.*, 2003). Cytokines like IL-18 appear to be an essential requirement for IgE production.

Filaggrin (FLG) is a filament-associated protein that binds to keratin fibers in epithelial cells. It plays an important role in skin barrier. AD tends to develop in individuals that carrying the FLG null allele variants (Palmer *et al.*, 2006). FLG protein is located in the granular layers of the epidermis. FLG monomers are degraded into natural moisturizing factors by bleomycin hydrolase or caspase 14 which are important hydration maintenance and ensuring skin pH low. The FLG gene within intragenic copy number variation (20-24 copies in one person) can contribute to AD with a dose dependent effect (Brown *et al.*, 2012).

Treg (regulatory T) cell, exists in the skin which is one of the non-lymphoid tissues. It composes of a high proportion of Treg. Treg in the skin are CD44 and CD103 (Sather *et al.*, 2007; Dudda *et al.*, 2008; Tomura *et al.*, 2010). Chemokine receptors which included of CCR4, CCR5, CCR6 and CCR7 are expressed by Treg. A complete loss of CCR4 on Treg might contribute to spontaneous lymphocytic infiltration and severe inflammation in the skin and lungs. The development of AD lesion is related to Treg (Ochs *et al.*, 2005). The number of Treg in the blood can be altered by using the treatment for AD such as cyclosporine and glucocorticoid (Brandt *et al.*, 2009; Hijnen *et al.*, 2009; Loser & Beissert, 2009; Baumgrass *et al.*, 2010; Stary *et al.*, 2011).

Polymorphism is resulted in elevated expression at the protein level. Overexpression of IL-1 by microglial cells correlates with the formation of neuritic beta-amyloid plaques in AD (Mrak *et al.*, 1995). The genotypes that overexpress IL-1 confer risk for AD through this mechanism. An additional polymorphism in the C allele of a variable number of tandem repeats (VNTR) in the 3' region of the IL-6 gene is also associated with AD. It has been shown to confer delayed onset and reduced risk of disease.

Polymorphism may be in linkage disequilibrium with yet another polymorphism leading to reduced expression of IL-6 (Papassotiropoulos *et al.*, 1999). Lack of IL-6 may protect neural tissue directly. Alternatively it may do so by ameliorating an inflammatory reaction involved in the pathogenesis of AD.

This latter concept is supported by the finding that individuals exposed to anti-inflammatory agents such as non-steroidal anti-inflammatory drugs have a lower probability of developing AD (Breitner *et al.*, 1994).

1.2.6 Diagnosis of atopic dermatitis

The diagnosis of AD is basically from the clinical history and physical examination. The diagnosis of AD is based on the clinical features such as pruritus, a chronic relapsing course and also family history of the triad diseases (AD, AR and AA). The diagnosis of AD requires the presence of at least three major symptoms and at least three minor symptoms. Exposure to possible exacerbating factors, such as aeroallergens, irritating chemicals, foods and emotional stress, should be investigated.

Unfortunately, there is no specific laboratory finding or histologic features to define AD. Although elevated IgE levels are found in up to 80.0% of affected patients, IgE levels are also elevated in patients with other atopic diseases (Leung, 1995).

IgE levels can be checked by using serum IgE immunoassay with ImmunoCAP machine. The technology is based on an extremely high total binding capacity, achieved through a high binding capacity per mg cellulose in combination with an optimal amount of cellulose in each solid phase. The allergen of interest, covalently coupled to the solid phase, reacts with the specific IgE in the patient sample. After washing away non-specific IgE, enzyme-labelled antibodies against IgE are added to form a complex. After incubation, unbound enzyme-labelled anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the fluorescence, the more specific IgE is present in the sample (Thermo Fisher Scientific, 2011).

Instead of ImmunoCAP, the latest test used to determine specific IgE is allergodip. This is an enzyme immunoassay (EIA) for the semiquantitative determination of specific IgE in serum or plasma. It does not require any laboratory equipment as it comes with ready to use reagents. Specific IgE from the sample binds with the allergen with allergen coupled to the solid phase. The unbound material is removed in a washing phase. Enzyme labelled anti-IgE is added which binds to any specific IgE. The unbound anti-IgE is removed in washing phase. Substrate is added and the solution is incubated. The intensity of the blue colour is compared with colour chart. The amount of specific IgE is estimated using class scoring system (Thermo Fisher Scientific, 2011).

In skin testing, the positive and negative controls must be used to ensure the validity of the results. Skin testing can be affected by patient age, quality of extract, sensitivity towards allergens which included antihistamines and antidepressants, volume and antigen's potency, reactivity of the skin, race, area of body tested and distance between injections (Rosenwasser, 2002)

Serum IgE immunoassay is less traumatic compared to skin prick test. However, it is less sensitive and the results are not released promptly (Nguyen and Close, 2005). The results can be obtained within two days.

1.2.7 Treatment of atopic dermatitis

The successful treatment of AD requires relief of pruritus, having barrier function of the skin and elimination of environmental characteristics that contribute to AD disease and repairment of inflammation (Tofte and Hanifin, 2001). For severe pruritus, doxepin hydrochloride blocks both H1 and H2 histamine receptors. The relationship between dry skin and AD can be avoided by avoiding hot shower, occlusive dressings on traumatized skin and other avoidance techniques. Topical corticosteroid is still the treatment for AD. For moderate to severe AD, ointment-based corticosteroids and higher potential agents should be used to control the disease promptly.

Tacrolimus and pimecrolimus bind to the binding protein and block T-cell function through inhibition of calcineurin-dependent transcription of genes (Paller *et al.*, 2001). In severe AD, ultraviolet light therapy and other devices have immunosuppressive

effects secondary to its ability to induce apoptosis and able to stimulate keratinocyte production of cytokines (Reynolds *et al.*, 2001).

T_H1 cytokine had shown to be safe and long term daily treatment of AD patients is able to reduce other atopic symptoms (Stevens *et al.*, 1998). AD-specific chemokines that attract T_H2 cells and eosinophils can be explored in order to treat this disease as far as therapeutics target is concerned.

1.3 Allergic rhinitis

Allergic rhinitis (AR) is defined as an IgE-mediated inflammation of nasal mucosa, characterized by one or multiple of the major symptoms like nasal obstruction, rhinorrhea, sneezing and nose itchness (International Rhinitis Management Working Group, 1994). AR has been recognized in recent years as a disease requiring attention. Classically, simple rating diagnostic of severity were recorded which included of mild AR and moderate to severe AR (Juniper *et al.*, 2005).

1.3.1 Prevalence study of allergic rhinitis

Ten to twenty five percent (10.0% to 25.0%) of the population in the world were affected with allergic rhinitis (AR) (Lynch, 2004; Noriaki *et al.*, 2006). The most common of all atopic diseases is AR which has been estimated to affect up to 40.0% of the children in the United States (Rosenwasser, 2002) and 20.0% of the adult population in the world (Corren, 2000).

Most of the patients report the onset of the symptoms before 30 years of the age or during some of the most productive years of life although AR can develop at any age (Corren, 2000). The sex proportion is equal for males and females (Parslow *et al.*, 2001).

Over the past 40 years, there is a substantial increased in the prevalence of AR worldwide (Noriaki *et al.*, 2006) with a higher increase reported in an industrialization part and in developing countries as well as rural populations (Rosenwasser, 2002). The prevalence of allergic diseases had increased predominantly in urban areas and developing countries.

Seasonal AR is found in approximately 10.0% to 20.0% in the perennial rhinitis of the population and 10.0% of the general population (Meltzer, 1997). From 1971 to 1981, an increased prevalence was shown in a Swiss population. The prevalence of AR since this time increased from 4.4% to 8.4% (Spector, 1997).

The previous study showed that the families with a bilateral family history of allergy generally tend to have children having symptoms of allergy before puberty. Those with a unilateral family history tend to have symptoms later in life or not at all. In 1 of 5 children by 2 to 3 years of age, the children tend to have development symptoms of AR and approximately 40.0% by age of 6. During adolescence, the development of symptoms will turn in approximately 30.0%.

The study done in Singapore estimated general population prevalence of chronic rhinitis was 10.8%. Higher prevalences were recorded in males, in younger adults, in Indians and Chinese and those with higher socio-economic status. AR was highly significantly

associated with asthma as reported in the previous study (Ng and Tan, 1994). The study done in Kelantan, Malaysia stated that there is no significant difference between gender and AR in the prevalence observed (Quah *et al.*, 1997).

1.3.2 Aetiology of allergic rhinitis

Lifestyle and environmental risk factors (The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee, 1998; Sly, 1999) as well as heredity (Porsbjerg *et al.*, 2002) seem to be major determinants of allergic diseases. There are many factors triggering this disease such as family history of allergic, parental smoking background, sex and early exposure to allergens or pollutions (Tamay *et al.*, 2007). The other factors such as age, gender and place of resident also associated with allergic sensitization.

The other risk as well as predisposing factors include elevated serum IgE levels (>100 IU/ml) before 6 years of age, introduction to food formula early in infancy, exposure to indoor allergens, primary or secondary heavy exposure to cigarette smoke and higher socioeconomic status (Rosenwasser, 2002). The increasing trend of allergic diseases prevalence has been partly explained by “hygiene hypothesis”, suggesting that reducing microbial exposure in early childhood skewing the reciprocal balance from T_H1 to T_H2 towards T_H2-mediated allergic disorders (Tamay *et al.*, 2007).

In Malaysia, the most common allergens that trigger the symptoms of AR are house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farina* (Wan Majdiah *et*

al., 2011). The other allergens that precipitate the symptoms of AR include cat fur, milk, shrimp and weed (Choon-Kook and Teck-Soong, 1995). Cat fur was found the commonest animal allergen among Kelantanese (Elango *et al.*, 1989). The most common allergens found in US are animal allergens, grass pollen, trees, weeds, fungi and dust mites (Naclerio and Solomon, 1997).

1.3.3 Clinical manifestations of allergic rhinitis

The symptoms of AR includes nasal itchiness, rhinorrhoea, nasal obstruction, sneezing, ear fullness, nasal congestion, post nasal drip, itchy, puffy red and watery eyes (Rutkowski, 2005; Lehman and Lieberman, 2007; Masuda and Schmitz, 2007). This is also normally associated with ocular symptoms (Rondon *et al.*, 2007; Rondon *et al.*, 2008; Rondon *et al.*, 2009).

The symptoms which involving the ears, eyes and throat including postnasal drainage were frequently accompanied (Parslow *et al.*, 2001; Skoner, 2001). Postnasal drainage can lead to sore throat, clearing of the throat and cough. Severe attacks of AR are usually followed by systemic malaise, fatigue and weakness. The headache is due to obstruction of the ostium of the paranasal sinuses occurred as a result of swelling of the nasal mucosa overlying it (Parslow *et al.*, 2001).

AR has significant effect on the quality of life of patients such as sleep, daily activities, work and school performance (ARIA, 2001; Rutkowski, 2005; Valero *et al.*, 2007). Even though it is not life threatening, it is now causing substantial medical care

expenditure as well as impairment both physical and cognitive functions in adult (Corren, 2000). AR is also associated with the patient's complaint, like impairments function at home, work and school. Patients might be interrupted by sleep disorders, impairment in activities, emotional problems and social functioning (ARIA, 2001; Bousquet *et al.*, 2006).

In chronic or severe AR, a transverse nasal salute is often seen across the bridge of the nose. The palpebral conjunctivae may be injected with a watery discharge and puffiness of the eyelids (Lehman and Lieberman, 2007). There are systemic manifestations of the allergic inflammation at these sites (Bousquet *et al.*, 2008).

1.3.4 Scoring system of allergic rhinitis (ARIA classification)

In the ARIA classification, allergic rhinitis can be classified as mild or moderate to severe depending on the severity of the symptoms and their impact on social life, school and work. It has also been proposed to classify the severity as mild, moderate or severe (Van Hoecke *et al.*, 2006a; 2006b; Valero *et al.*, 2007). Traditionally, AR has been classified as seasonal or perennial. It was depending on the symptoms occurred. Currently, a new classification for AR based on the duration and severity of symptoms had been proposed (ARIA, 2001; Lehman and Lieberman, 2007).

Mild AR is defined according to symptoms such as normal sleep, no impairment of daily activities, sport or leisure, normal work and school and no troublesome symptoms. Moderate to severe AR is defined when more items occurring such as abnormal sleep,

impairment of daily activities, sport or leisure, impairment of work or school and troublesome symptoms.

Based on duration, AR is divided into persistent and intermittent disease and for severity, it is divided into mild or moderate to severe (Table 1.1). The intensity was depending on symptoms and quality of life parameters (ARIA, 2001; Lynch, 2004). Majority of patients with AR reported to have persistent rhinitis with moderate-to-severe symptoms (Rondon *et al.*, 2011) frequently associated with conjunctivitis (25% to 57%) and asthma (33% to 47%) (Rondon *et al.*, 2007; Rondon *et al.*, 2008).

Table 1.1 : Classification of allergic rhinitis by using ARIA classification

	Intermittent	Persistent
Duration	Symptoms occur on less than 4 days a week, or less than 28 days at a time	Symptoms occur on the majority of days of the week and for more than 28 days
	Mild AR	Moderate to severe AR
Severity	No disturbance in sleep, leisure, school or work activities	Disturbance to sleep, leisure school or work activities

1.3.5 Pathophysiology of allergic rhinitis

A characterization of pathophysiologic mechanisms (endotypes) and clinical manifestations (phenotypes) is needed for a better understanding of AR. Several authors had studied a concept of local production of IgE in nasal mucosa of patients with AR. The previous study has found the expression of ϵ germline gene transcripts and mRNA for the ϵ heavy chain of IgE in nasal B cells (Durham *et al.*, 1997; Coker *et al.*, 2003).

Further research has demonstrated the existence of class-switch recombination to IgE in nasal mucosa of patients with AR (Durham *et al.*, 1997; Coker *et al.*, 2003). More evidence supporting the local synthesis of sIgE (specific immunoglobulin E) in the nasal mucosa of patients with AR has recently been reported (Powe *et al.*, 2010).

The activation of mast cells and eosinophils and IgE production were induced after nasal stimulation with aeroallergens in reported study. Patients had an immediate or dual response to NAPT (nasal allergen provocation test) accompanied by release of tryptase, ECP (eosinophil cationic protein) and sIgE in nasal secretions. The kinetics study of tryptase showed a strong correlation with nasal itching and sneezing and a pattern of release that varied with the type of response.

This rapid secretion of sIgE after challenge, with basal detection of sIgE in some patients, supports the existence of a persistent local production of sIgE in nasal mucosa that rapidly increases after allergen stimulation. These findings have led researchers in considering the need to evaluate whether local production of sIgE in patients with other apparently nonallergic respiratory diseases, such as chronic rhinosinusitis with or

without nasal polyps (van Zele *et al.*, 2007; Sabirov *et al.*, 2008), asthma (Humbert *et al.*, 1996; Takhar *et al.*, 2007; Campo *et al.*, 2011) or conjunctivitis, could exist.

1.3.6 Diagnosis of allergic rhinitis

There are several tests done in the medical line to diagnose AR. It includes physical examination (history of medical problems and family history of allergies). The demonstration of skin testing and allergen-specific IgE are used to confirm the diagnosis of AR as well as the guidance due to treatment process (Rijn *et al.*, 1998). The previous study demonstrated that total IgE has poor clinical correlation and low predictive value than antigen-specific IgE antibodies in diagnosing of inhalant allergy (ARIA, 2001; Nguyen and Close, 2005). Specific IgE measurement (specific IgE serum immunoassay) or skin prick test is known to be useful (Ahlstedt and Murray, 2006).

The important markers of the possible allergic causes of disorders of the upper respiratory tract are skin prick test and measurement of specific IgE (Droste *et al.*, 1996). However, skin testing is the standard method in diagnosing of AR (Chapnik and Hakemi, 2003). Skin prick test is used to identify specific allergen and to determine the sensitivity of the patients towards the allergen.

Nasal cytology is also one of the methods to diagnose AR. Nasal secretions are stained with hematoxylin and eosin. Generally, the presence of eosinophils and goblet cells is suggestive of allergy while the presence of neutrophils and bacteria is referred to infection.

1.3.7 Treatment of allergic rhinitis

The management of AR involves allergen avoidance, education, immunotherapy and pharmacologic treatment (Corren, 2000; ARIA Pocket Guide, 2001; Rutkowski, 2005; Lehman and Lieberman, 2007; Bousquet *et al.*, 2008). Patients should report the response of medications like corticosteroids and antihistamine (Rondon *et al.*, 2007; 2008).

The allergen avoidance is an effective treatment strategy (Lehman and Lieberman, 2007). However, an avoidance of known allergic triggers always non-practical (Dykewicz, 1998; Rosenwasser, 2002; ARIA, 2004; Plaut and Valentine, 2005). An improvement to a level of regular medications in some patients might be resulted from decreased exposure to indoor allergens (Bousquet *et al.*, 2001).

The allergic management is important in order to control allergic symptoms and maintain the healthy lifestyle. These interventions can control the symptoms with minimal side effects in most patients. The medications such as anti-IgE therapy and cytokine antagonist may provide some alternative relief to patients (Corren, 2000).

Antihistamines can control the symptoms of itching, sneezing, rhinorrhoea and eye irritation but not nasal congestion (Spector, 1997; Rutkowski, 2005). Intranasal steroids are used for patients with more severe symptoms (Rosenwasser, 2002).

Montelukast, which is one leukotriene antagonist has been approved for the seasonal AR treatment. Those not controlled with antihistamine and nasal corticosteroids had an option to use it. It works on the same pathway of corticosteroids. It is helpful to relieve

the symptoms for those with mild allergies and mild asthma (Rutkowski, 2005). A mast cell stabilizer, cromolyn sodium prevents the mast cells from releasing chemical mediators thus preventing the allergic response. It reduces nasal itching, sneezing, runny nose and nasal congestion (Spector, 1997; Rutkowski, 2005).

Specific immunotherapy, anti-IgE therapy also helps in treating AR and other allergic diseases. The reduction of IgE included systemic administration of humanized monoclonal anti-IgE antibodies (Corren, 2000; Abbas & Lichtman, 2003). A recombinant humanized monoclonal anti-IgE antibody, omalizumab can blocks the interaction of IgE with its receptors (Owen, 2007).

Cytokine-based therapies may lead in AR treatment due to recognition that allergy is associated with imbalanced T_H1/T_H2 activity. Cytokines themselves or pharmacological agents can be the treatment agents that can modulate specific cytokines profiles. Recombinant cytokines are being studied currently in a variety of allergic diseases. However, high pharmacological doses of recombinant cytokines may create other imbalances in the host that may lead to other disease problems (Hannigan and Pallister, 2000).

1.4 Atopic asthma

Asthma is one of the common diseases of childhood and causing substantial morbidity (Asher *et al.*, 1995; The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee, 1998). Atopic asthma (AA) is also linked with

the immune system just like the other allergic reactions (Barnes, 2000). AA is a chronic disease that affects approximately 4.0% to 5.0% of the members in the certain country (McFadden and Gilbert, 1992). This disease is characterized by reversible airway obstruction, chronic inflammation of bronchial mucosa and bronchial hyperresponsiveness. Identification of AA has the potential to improve the knowledge and functional status, thus can cut down the cost and expenditure on health care (Nicklas, 1997; Kuda, 1998; Serra-Batles *et al.*, 1998).

1.4.1 Prevalence study of atopic asthma

The previous study reported the increasing prevalence of AA due to the improvement of the treatment, diagnosis and understanding of the disease itself (Strachan *et al.*, 1997; Beasley *et al.*, 1998; Linneberg *et al.*, 2000; Ulrik *et al.*, 2000). Asthma, has become increasingly common in industrialized nations. It affects about 15.0% of children and 10.0% of adults in the affluent countries. The previous study recorded, a smaller proportion of AA was recorded as death in children (Panickar *et al.*, 2005).

An estimated 300 million people are affected by asthma as recorded in worldwide (Beasley, 2004; Masoli *et al.*, 2004). The prevalence of asthma ranges around 1.0%-18.0% of the population in different countries regarding to the application of standardized methods in measuring of the prevalence of asthma in children and adults (Beasley, 2004; Urrutia *et al.*, 2007). Its prevalence has increased in some countries

(Carvajal-Uruena *et al.*, 2005; Yan *et al.*, 2005). However, it has stabilized and begun to decline in other countries (Garcia-Marcos *et al.*, 2004; Asher *et al.*, 2006).

World Health Organization (WHO), has estimated that asthma has affected 15 million disability-adjusted life-years annually lost which representing 1.0% of total global disease burden (Pachter *et al.*, 2002). The cost of controlling asthma is high. However, the cost of not treating asthma correctly is even higher (Accordini *et al.*, 2006; Briggs *et al.*, 2006; Sullivan *et al.*, 2007).

The prevalence of asthma in Malaysia was likely to increase. Previous study done in Malaysia reported 45.0% out of 404 patients enrolled in the study showed daytime asthma symptoms, 42.0% reported sleep disturbances due to asthma and 40.0% recorded getting asthma during exercises (Lai *et al.*, 2003). AA has significant impact on quality of life.

1.4.2 Aetiology of atopic asthma

The aetiology of asthma is multifactorial. Genetic factors may control individual predispositions to asthma. Variation in the beta-adrenergic receptor gene of the (arginine) Arg-Arg type has been associated with adverse responses to inhaled, short-acting beta-agonist inhalers (Barnes, 2000).

Asthma attacks have been linked to exercise and respiratory infections. The exposure to environmental factors such as allergens, tobacco smoke, indoor and outdoor air

pollution are the reasons of asthmatic attacks. However, it can be reduced by avoiding the exposure to known triggers and taking medications (Barnes, 2000).

There are associations between air pollution exposures and asthma in the previous reported studies. The researchers have found an association between increased hospital admissions for asthma with particulate matter and outdoor air pollutant. The phenotype of the familial aggregation associated with AA has been described in the several studies (Davis and Bulpitt, 1981; Jenkins *et al.*, 1993; Abdullrazzaq *et al.*, 1994).

1.4.3 Clinical manifestations of atopic asthma

The clinical manifestations of asthma includes recurrent wheezing, difficulty in breathing, chest tightness, sleep disturbances, limitations of daily activity, impairment of lung function and rescue medications usage (Bateman *et al.*, 2008). The other symptoms of asthma are nasal congestion, runny nose and eye irritation. The wheezing sound usually accompanies exhalation. Asthma can also occur without wheezing (Global Initiative for Asthma, 2006).

Symptoms may be triggered by exercise. Some asthma attacks are triggered by cold, heat or stress. This often causes worsening of symptoms. The symptoms occur or worsen at night and awakening the patient. Reversibility airflow obstruction is determined by an increase in FEV₁ of >200 mL and $\geq 12\%$ from baseline measure after inhalation of short-acting β_2 -agonist (SABA).

1.4.4 Scoring system of atopic asthma (GINA classification)

Global Initiative for Asthma (GINA) was implemented to develop a network of individuals, organizations and public health officials for the dissemination of information regarding the care of patients with asthma, while at the same time assuring a mechanism to incorporate the results of scientific investigations into asthma care (Global Initiative for Asthma, 2002). The classifications of AA are listed in Table 1.2.

Table 1.2 : Classification of atopic asthma by using GINA classification

Characteristic	Controlled (All of the following)	Partly controlled (Any measure present in any week)	Uncontrolled
Daytime symptoms	None (twice or less/week)	More than twice / week	
Limitations of activities	None	Any	Three or more features of partly controlled asthma present in any week
Nocturnal symptoms / awakening	None	Any	
Need for reliever / rescue treatment	None (twice or less/week)	More than twice / week	
Lung function (PEF or FEV1) ⁺⁺	Normal	<80% predicted or personal best (if known)	
Exacerbation	None	One or more/year	One in any week