$CD4^+$ T CELL SUBSETS IN ADULT ALLERGIC RHINITIS PATIENTS ATTENDING HOSPITAL UNIVERSITI SAINS MALAYSIA

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by

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Mastura Md Sani

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LIST OF ABBREVIATIONS

AC	allergic conjunctivitis
AD	atopic dermatitis
AIDS	acquired immune deficiency syndrome
ANOVA	analysis of variance
AR	allergic rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
ATRA	all-trans retinoic acid
BMI	body mass index
CCL	chemokine (C-C motif) ligand
CCR	chemokine (C-C motif) receptor
$CD34^+$	hematopoietic progenitor cells
$CD4^+$	helper T cells
CD62L	L-selectin
CLA	cutaneous leucocyte-associated antigen
Der f	*Dermatophagoides farinae*
Der p	*Dermatophagoides pteronyssinus*
DNA	deoxyribonucleic acid
ECP	eosinophil cationic protein
EDN	eosinophil-derived neurotoxin
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence-activated cell sorting
FcεRI	Fc epsilon RI

FEIA	fluoroenzymeimmunoassay
FinEsS	Finland, Estonia and Sweden
FSC	forward-scattered
GATA3	GA-TA binding protein 3
GM-CSF	granulocyte macrophage colony-stimulating factor
HDMs	house dust mites
HIV	human immunodeficiency virus
HUSM	Hospital Universiti Sains Malaysia
ICAM	intercellular adhesion molecule
IDM	Instrument Data Manager
IFN-γ	interferon gamma
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
IQR	interquartile range
IRF4	interferon regulatory factor 4
ISAAC	International Study of Asthma and Allergies in Childhood
ISAC	immuno-solid phase allergen chip
kg	kilogram
KLRG1	killer cell lectin like receptor G1
LFA-1	lymphocyte function-associated antigen 1
LTC4	leukotriene C4
m	meter
MBP	major basic protein
MCP-4	monocyte chemotactic protein-4

MIP	macrophage inflammatory proteins
miR-135a	microRNA-135a
ml	millilitre
mRNA	messenger ribonucleic acid
n	number
NA	not applicable
OLIN	Obstructive Lung Disease in Northern Sweden
ORL-HNS	Otorhinolaryngology-Head and Neck Surgery
PBMCs	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PS	Power and Sample Size Calculation
RANTES	regulated upon activation normal T cell expressed and presumably secreted
SCF	stem cell factor
SCIT	subcutaneous immunotherapy
SD	standard deviation
SIT	specific immunotherapy
SLIT	sublingual immunotherapy
SPSS	Statistical Package for the Social Sciences
SSC	side-scattered
TARC	thymus and activation-regulated chemokine
T_{CM}	central memory T cells
TCR	T cell receptor
T_{EM}	effector memory T cells
T_{EMRA}	terminally differentiated effector memory T cells

TGF-β	transforming growth factor beta
T_H	$CD4^+$ T lymphocytes
Thy	lymphatic endothelial cells
T_N	naïve T cells
TNF-α	tumor necrosis factor alpha
TSA	Trichostatin A
TSLP	thymic stromal lymphopoietin
VAS	visual analog scale
VCAM-1	vascular cell adhesion molecule 1
VLA	very late antigen
%	percentage
^oC	degree Celsius
µl	microlitre
α	alpha
β	beta
γ	gamma

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SUBSET SEL CD4$^+$ T BAGI PESAKIT ALLERGIC RHINITIS DEWASA DI HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Sel T memori mengeluarkan fungsi effector atau menghasilkan sel effector sebagai tindak balas terhadap antigen. Peratusan subset sel CD4$^+$ T terutamanya sel-sel memori dalam pesakit allergic rhinitis (AR; alahan radang hidung) yang sensitif kepada alergen umum termasuklah habuk rumah dan makanan laut tidak dikaji secara meluas. Kajian ini bertujuan untuk membandingkan purata peratusan dan jumlah mutlak subset sel memori CD4$^+$ T antara: (i) individu sihat dan pesakit AR (ii) pesakit AR ringan dan pesakit AR yang sederhana-teruk. Di samping itu, tahap sensitif terhadap alergen, skor tahap keterukan simptom, dan purata jumlah mutlak subset leukosit juga dianalisa. Lima puluh individu sihat dan 100 pesakit AR yang telah didiagnosis oleh doktor pakar telah direkrut dalam kajian ini. Walau bagaimanapun, hanya 33 individu sihat dimasukkan ke dalam analisis kerana individu yang lain adalah sensitif kepada alergen (*Dermatophagoides farinae* (*Der f*), ketam dan udang) berdasarkan ujian spesifik IgE yg dilakukan. Analisis berstrata dilakukan berdasarkan dua definisi pesakit, iaitu pesakit AR berdasarkan (i) bukan pengantaraan IgE; dan (ii) pengantaraan IgE. "Flow cytometry" digunakan untuk melihat peratusan sel CD4$^+$ T "naïve" (T_N; CD45RA$^+$ CCR7$^+$), "central memory" (T_{CM}; CD45RA$^-$ CCR7$^+$), "effector memory" (T_{EM}; CD45RA$^-$ CCR7$^-$) dan "terminally differentiated effector memory" (T_{EMRA}; CD45RA$^+$ CCR7$^-$) dari dalam darah. Jumlah mutlak subset sel CD4$^+$ T diperolehi daripada gabungan dua kaedah iaitu "flow cytometry" dan "hematology analyzer". Didapati bahawa pesakit AR

yang sensitif kepada alergen (*Dermatophagoides pteronyssinus*, *Der f*, ketam and udang) kebanyakannya sensitif kepada habuk rumah berbanding dengan makanan laut. Pesakit AR sederhana-teruk mempunyai skor simptom nasal dan bukan nasal yang tinggi serta kualiti hidup yang lebih terjejas berbanding dengan pesakit AR ringan. Tambahan pula, kiraan eosinofil adalah lebih tinggi pada pesakit AR berdasarkan pengantaraan IgE berbanding dengan individu sihat. Tidak terdapat perbezaan yang signifikan dalam purata peratusan dan jumlah mutlak sel memori $CD4^+$ T antara individu sihat dan pesakit AR berdasarkan pengantaraan IgE. Walau bagaimanapun, pengurangan yang ketara dalam purata peratusan (p = 0.0287) dan jumlah mutlak (p = 0.0298) sel $CD4^+$ T_{EMRA} telah dilihat pada pesakit AR sederhana-teruk berdasarkan pengantaraan IgE berbanding dengan pesakit AR ringan berdasarkan pengantaraan IgE dan 14/25 (56.0%) pesakit AR sederhana-teruk berdasarkan pengantaraan IgE mempunyai simptom yang berterusan. Kesimpulannya, purata peratusan dan jumlah mutlak sel $CD4^+$ $CD45RA^+$ $CCR7^-$ T_{EMRA} dilihat berkurang pada pesakit AR sederhana-teruk berdasarkan pengantaraan IgE berbanding dengan pesakit AR ringan berdasarkan pengantaraan IgE dalam populasi pesakit AR yang kebanyakannya sensitif kepada habuk rumah.

$CD4^+$ T CELL SUBSETS IN ADULT ALLERGIC RHINITIS PATIENTS ATTENDING HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRACT

Memory T cells exert effector function or generate effector cells in response to antigen. The proportions of $CD4^+$ T cell subsets especially memory cells in allergic rhinitis (AR) patients sensitized to common allergens of house dust mites (HDMs) and shellfish have not been extensively studied. This study aimed to compare the mean percentages and absolute counts of $CD4^+$ memory T cell subsets between: (i) non-allergic controls and AR patients; (ii) mild AR patients and moderate-severe AR patients. In addition, sensitization to common allergens, symptom severity scores, and mean absolute counts of leukocyte subsets were also determined. Fifty non-allergic controls and 100 AR patients diagnosed by physicians were recruited in this study. However, only 33 non-allergic controls were included in the analyses as others were excluded due to sensitization to the common allergens (*Dermatophagoides farinae* (*Der f*), crab and shrimp) as measured by plasma specific IgE tests. Stratified analyses were done based on two different definitions of AR patients , i.e. (i) non IgE-mediated AR patients; and (ii) IgE-mediated AR patients. Flow cytometry was used to determine the percentage of $CD4^+$ naïve (T_N; $CD45RA^+$ $CCR7^+$), central memory (T_{CM}; $CD45RA^-$ $CCR7^+$), effector memory (T_{EM}; $CD45RA^-$ $CCR7^-$) and terminally differentiated effector memory (T_{EMRA}; $CD45RA^+$ $CCR7^-$) T cells from the peripheral blood. The absolute counts of $CD4^+$ T cell subsets were obtained by dual platform methods from flow cytometer and hematology analyzer. It was observed that AR patients sensitized to common

allergens (*Dermatophagoides pteronyssinus*, *Der f*, crab and shrimp) measured were predominantly sensitized to HDMs as compared to shellfish allergens. Moderate-severe AR patients had higher nasal and non-nasal symptom scores and reduced quality of life as compared to mild AR patients. Furthermore, the eosinophil count was significantly higher in IgE-mediated AR patients as compared to non-allergic controls. There were no significant differences in the mean percentages and absolute counts of $CD4^+$ T cell subsets between non-allergic controls and IgE-mediated AR patients. However, significant reduction in the mean percentage (p = 0.0287) and absolute count (p = 0.0298) of $CD4^+$ T_{EMRA} cells were found in IgE-mediated moderate-severe AR patients as compared to IgE-mediated mild AR patients and 14/25 (56.0%) IgE-mediated moderate-severe AR patients had persistent symptoms. In conclusion, the mean percentage and absolute count of $CD4^+$ $CD45RA^+$ $CCR7^-$ T_{EMRA} cells were siginificantly reduced in IgE-mediated moderate-severe AR patients as compared to IgE-mediated mild AR patients in our population of AR patients predominantly sensitized to HDMs.

CHAPTER 1

INTRODUCTION

This chapter provides the overview of this study. The background of study, research problems, theoretical framework, research questions, purpose of study, rationale of study, objectives and hypothesis of this study are described in detailed in this chapter.

1.1 Background of study

The immune system in human body is essential for a human to stay healthy. It protects the human body against microorganisms such as bacteria, viruses and fungi by destroying these infectious microorganisms out of the body. The immune system is incredibly complex as it is made up of vital network of cells and organs that protect the body from infections and other diseases. Allergic disease is one of the diseases that results from the immune system's response to a harmless substance. The immune system may over react by producing antibodies towards the harmless substance which results in the clinical symptoms of allergic disease.

The immune system has the ability to immediately and specifically recognize an antigen that the body has encountered before. This is known as immunological memory where the immune system will immediately initiate an immune response after the recognition of previously encountered antigen. This secondary immune response towards the same antigen is the main component of the adaptive immune

system. Memory B and T cells are the cells involved in the development of immunological memory. Memory B cells are plasma cells that produce antibodies while memory T cells are $CD4^+$ and $CD8^+$ T cells that are capable in recognizing antigen specifically.

Allergic rhinitis (AR) is one of the most common allergic diseases, affecting about 400 million people worldwide. AR is a major risk factor for poor asthma control and markedly impair the quality of life, sleep, social life, school, and work performance, leading to a huge socioeconomic burden, with medical costs greater than those of diabetes, coronary heart disease and asthma. Improved understanding of the underlying immune mechanisms is central to developing precision medicine or therapies and to prevent worsening of symptoms.

Recently, it has been observed that the proportions of $CD4^+$ memory T cell populations differ in seasonal AR patients compared to non-allergic controls, in parallel to the difference seen in epigenetics in terms of DNA methylation patterns, which separated the allergic patients from healthy controls (Nestor *et al.*, 2014). This finding represents an important advancement in the understanding of the underlying mechanisms, highlighting the potential importance of changes in both epigenomics and $CD4^+$ memory T cells in complex immune disease like AR. To date, there are only very few studies on $CD4^+$ memory T cells in allergic diseases as described in Chapter 2: Literature review (section 2.5). However, these studies collectively pointed towards a potential involvement of memory T cell subsets in the immunopathogenesis of allergic diseases.

These studies focused on seasonal AR patients in which birch and grass pollen were the main allergens patients were sensitized to, while in Malaysia we have mostly perennial AR patients sensitized to HDMs of *Dermatophagoides* species as the most common allergens. There is a lack of literature on the role of $CD4^+$ memory T cells in perennial AR patients which needs to be addressed. In addition, no study has investigated the differences in proportion of $CD4^+$ memory T cell subsets in different severity of AR, which may shed light on their roles in the development of AR from mild to moderate-severe.

Furthermore, the observations from previous studies were from very small sample sizes, so we aimed to look at a bigger sample size to obtain a more reliable data on memory T cells in perennial AR. Also, since differences in genetic ancestry may influence the observation seen in complex diseases including allergic diseases, there is a need to investigate the differences in the proportion of memory T cells in our local population, as previous observations were from caucasian populations.

Targeting the specific $CD4^+$ memory T cell subset in AR patients may represent an interesting and novel approach for personalised treatment, e.g. for moderate-severe perennial AR patients. Thus, our study is undertaken to investigate the proportions of $CD4^+$ memory T cell subsets in perennial AR patients of a Malaysian population, in comparison to the healthy controls and in association with disease severity. This study is also aimed to provide preliminary data for $CD4^+$ memory T cell subsets in AR patients of a Malaysian population.

1.2 Problem statement

This study focuses on the determination of the proportions of $CD4^+$ memory T cell subsets in adult AR patients attending Hospital Universiti Sains Malaysia (HUSM) in Kelantan. Previously, several studies have been done in AR patients attending HUSM (Asha'ari *et al.*, 2010; Ashari, 2009; Wan Majdiah *et al.*, 2011). However, these studies focused on the clinical features and the sensitization patterns of the AR patients. It is strongly believed that the proportions of $CD4^+$ memory T cells and epigenetic changes are the underlying causes of AR.

Thus, this study was conducted to investigate the proportions of $CD4^+$ memory T cells in AR patients compared to healthy controls. First, we identified the sensitization patterns of AR patients towards common inhalant and food allergens that become the immediate causes of AR. Secondly, the predisposing factors such as demographic and environmental factors that may be involved in the development of AR are were examined in this study. Finally, the proportions of $CD4^+$ memory T cell subsets were determined to identify the changes at cellular level that may become the underlying causes of AR.

1.3 Theoretical framework

This study focuses on determining the immediate and underlying determinants that reflect the causes and severity of AR. Firstly, the immediate determinant namely sensitization to common allergens i.e. inhalant and food allergens were determined to provide reasons for determining the underlying determinants. Secondly, predisposing factors such as demographic (age, gender, body mass index, smoking status) and environmental factor (home location) were examined as these factors may be the underlying determinants that cause AR.

Finally, changes at cellular level i.e. proportions of leukocyte subsets and $CD4^+$ memory T cell subsets were measured as their changes may be the predominant underlying determinants that caused the impairment of the immune system which subsequently lead to AR.

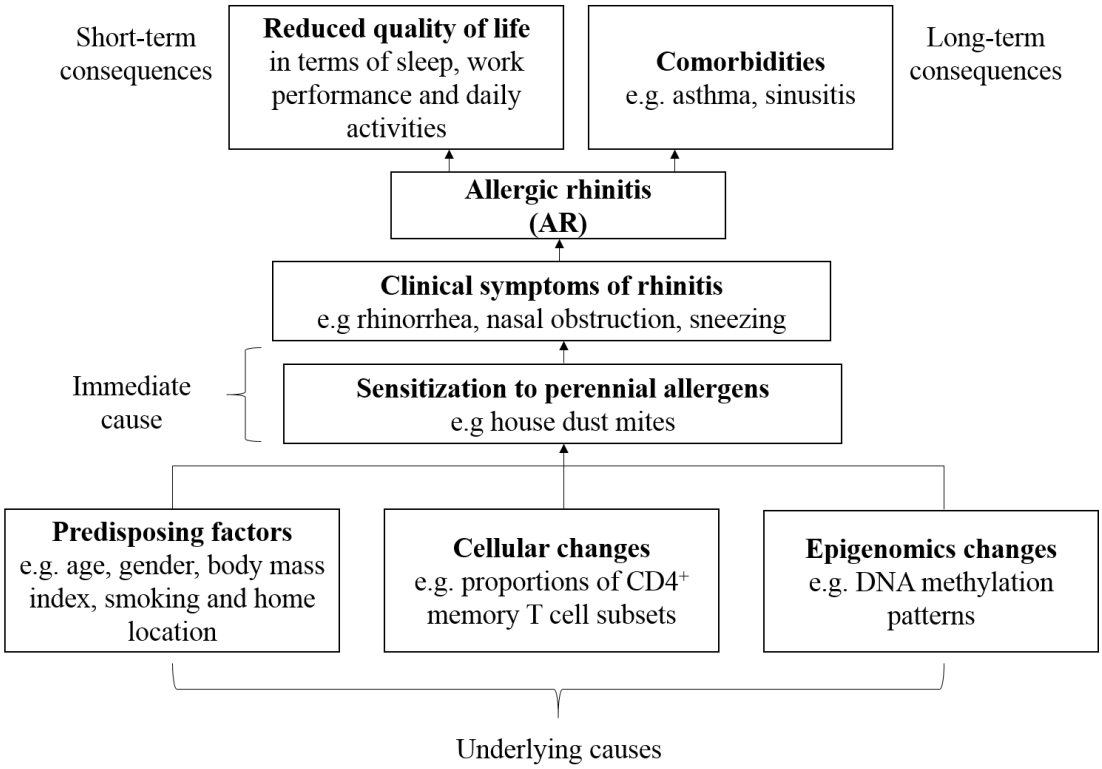

Figure 1.1: Theoretical framework of determinants of perennial allergic rhinitis

1.4 Research questions

1. Which allergens are mild and moderate-severe AR patients predominantly sensitized to?
2. Which predisposing factors cause AR in mild and moderate-severe AR patients?
3. What cellular changes may contribute to the immunopathogenesis of AR in mild and moderate-severe AR patients?

1.5 Purpose of study

This study was conducted in AR patients attending HUSM in Kelantan which is located in the North East of Peninsular Malaysia. AR patients from different regions of Malaysia may have different clinical features, sensitization patterns, predisposing factors and underlying causes i.e. changes at cellular level that contribute to the immunopathogenesis of AR. Thus, this study was conducted to identify which of these factors may play a role in the immunopathogenesis of AR in adult AR patients.

This study also adds to the literature regarding the proportions of $CD4^+$ memory T cell subsets in AR patients from North East of Peninsular Malaysia. We hope that the findings of this study can be used to guide other similar perennial AR studies in other parts of Malaysia and South East Asia countries.

1.6 Rationale of study population

The reason for choosing AR patients attending HUSM as the study population was mainly because the AR patients were diagnosed by ear, nose and throat (ENT) specialists from Otorhinolaryngology-Head and Neck Surgery (ORL-HNS) clinic in HUSM. They were given proper diagnosis of AR as nasal endoscopic examination was done to AR patients and this examination may not be done in other clinics in Kelantan. This examination confirms the inflammation of the membranes lining the nose suffered by AR patients. Furthermore, HUSM is a tertiary referral hospital in Kelantan where AR patients can be easily recruited with the help of ENT specialists and nurses from ORL-HNS clinic.

1.7 Objectives

1.7.1 General objective

To determine the mean percentages and mean absolute counts of $CD4^+$ T cell subsets (naïve, central memory, effector memory and terminally differentiated effector memory) in AR patients attending Hospital Universiti Sains Malaysia and non-allergic controls.

1.7.2 Specific objectives

1. To determine the demographic and clinical characteristics of AR patients and non-allergic controls.
2. To determine the sensitization to common allergens among physician-diagnosed AR patients.
3. To compare AR symptom severity scores and quality of life scores between mild and moderate-severe AR patients.
4. To compare mean absolute counts of leukocyte subsets between:
    a. AR patients and non-allergic controls.
    b. Mild and moderate-severe AR patients.
5. To compare mean percentages and absolute counts of $CD4^+$ T cell subsets (naïve, central memory, effector memory and terminally differentiated effector memory) between:
    a. AR patients and non-allergic controls.
    b. Mild and moderate-severe AR patients.

1.8 Hypothesis

1. There is a difference in the demographic and clinical characteristics between AR patients and non-allergic controls.
2. There is a difference in sensitization to common allergens between mild and moderate-severe AR patients in physician-diagnosed AR patients.
3. There is a difference in AR symptom severity scores and quality of life scores between mild and moderate-severe AR patients.
4. There is a difference in mean absolute counts of leukocyte subsets between:
    a. AR patients and non-allergic controls.
    b. Mild and moderate-severe AR patients.
5. There is a difference in mean percentages and absolute counts of $CD4^+$ T cell subsets (naïve, central memory, effector memory and terminally differentiated effector memory) between:
    a. AR patients and non-allergic controls.
    b. Mild and moderate-severe AR patients.

CHAPTER 2

LITERATURE REVIEW

Previous literature is reviewed in this chapter to provide detailed explanation about the important topics addressed in this study. This chapter consists of six major topics that describe the overall study. Firstly, allergy and allergic rhinitis (AR) are explained in detail. Secondly, the immunopathogenesis and immune cells that are involved in allergic inflammation of AR are elaborated. Finally, the role of $CD4^+$ memory T cells in allergy as well as $CD4^+$ memory T cell subsets and their markers investigated in this study are clearly explained.

2.1 Allergy

Allergy is an antibody and cell mediated hypersensitivity reaction in response to a normally harmless substance known as allergen (Johansson *et al.*, 2001). Allergic immune response involves the production of specific immunoglobulin E (IgE) antibody by plasma cells towards the allergen (Sircar *et al.*, 2014) and the binding of these specific antibodies to FcεRI on mast cells, basophils and eosinophils, leading to the release of mediators like histamines. The involvement of $CD4^+$ T lymphocytes is central in the allergic response as they secrete T_H2 cytokines in response to activation by allergens, with the long-lived human memory T_H2 cells playing an important role as they are allergen-specific (Woodfolk, 2007). The development of allergic diseases is frequently associated with atopy, which refers to

the personal or familial tendency in producing IgE antibodies in response to sensitized allergens (Tanno *et al.*, 2016).

Allergy can be categorized into respiratory, skin and gastrointestinal allergies where the symptoms commonly manifest in allergic individuals. Allergic rhinitis (AR) and allergic asthma are the respiratory allergies manifested in the upper respiratory tract and lower respiratory tract of the respiratory system respectively (Brooks *et al.*, 2017). The most common skin allergies are atopic dermatitis (AD) and urticaria (Schlapbach and Simon, 2014) while food allergies such as cow's milk allergy are usually manifested as gastrointestinal allergy (Wuthrich, 2014), although the symptoms can be observed in skin and respiratory system as well. These allergic diseases commonly co-exist, typically following the atopic march (Figure 2.1), where the development of AD in infancy precedes the development of AR and asthma at later stages in life (Bantz *et al.*, 2014; Spergel, 2010). Allergic inflammation can be divided into early-phase reaction where the symptoms start to appear within minutes of allergen exposure, and late-phase reaction where the symptoms develop in a few hours after allergen exposure (Galli *et al.*, 2008).

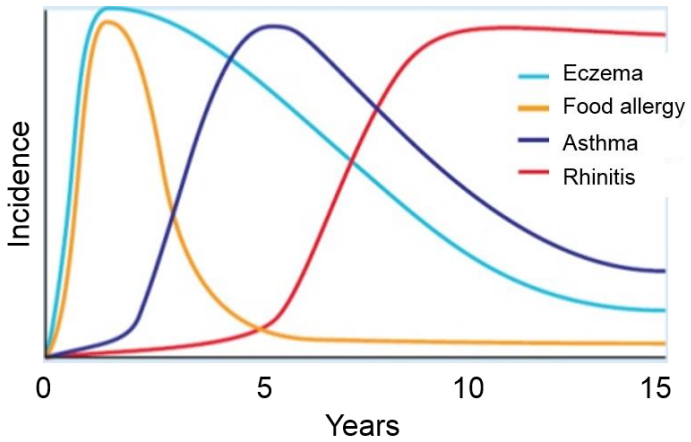

Figure 2.1: The incidence of atopic march. Adapted from (Spergel, 2010).

2.2 Allergic rhinitis (AR)

AR is clinically defined as IgE-mediated inflammation of membranes lining the nose after allergen exposure associated with nasal symptoms including rhinorrhea, sneezing, nasal obstruction and nasal itchiness (Bousquet *et al.*, 2008). The symptoms of AR usually impact the quality of life by causing sleep disturbances, reduced work or school performance and abnormal daily activities. In addition, comorbidities such as asthma, sinusitis, AD and otitis media are commonly associated with AR (Bousquet *et al.*, 2008). Although AR is not a very serious medical condition that causes severe morbidity and mortality but it has become a frequent reason for the sufferers to seek treatment from the physician (Brozek *et al.*, 2017). This leads to a huge negative impact to the economy, with the total costs related to AR estimated to be up to US$20.9 billion in the United States (Pawankar, 2014). In societies with emerging economies like the Asia Pacific region, indirect losses e.g. due to loss of productivity caused by AR further impact the financial outcome, resulting in annual per-patient costs that ranged from US$ 184 to US$ 1,189. Therefore, the cost of allergic rhinitis should not be underestimated as it can be enormous (Kushnir *et al.*, 2015).

2.2.1 Prevalence of AR

Currently AR is affecting 10% to 40% of the population worldwide (Brozek *et al.*, 2017). It has been estimated that 40% of adults and 25% of children worldwide suffer from AR. This disease is also very common in many countries in South East Asia region (Katelaris *et al.*, 2012). The prevalence of AR in different regions of the world is shown in Figure 2.2 and its prevalence in South East Asia countries is described in Table 2.1.

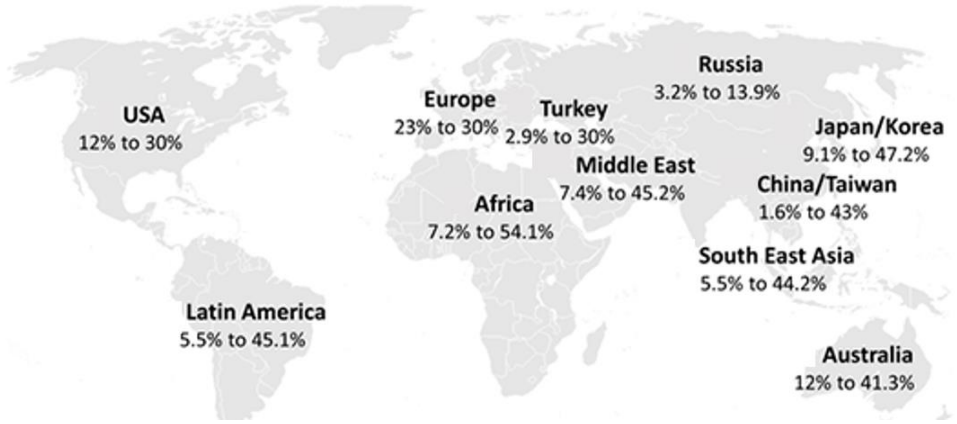

Figure 2.2: Prevalence of allergic rhinitis in different regions of the world. Adapted from (Katelaris *et al.*, 2012).

Table 2.1: Prevalence of allergic rhinitis in South East Asia Countries. Adapted from (Katelaris *et al.*, 2012).

Country	Population characteristics	Study location type	Study design / method for assessing prevalence	Prevalence of AR (%) (lifetime, unless stated otherwise)	Reference
Malaysia	409 children aged 12 – 20 years (Chinese ethnic only)	Kota Kinabalu (urban)	Questionnaire and skin prick tests	11.2% AR	(Leung and Ho, 1994)
Singapore	9636 children aged 6 – 15 years	Singapore (urban)	ISAAC study questionnaires	25.5 – 42.1% AR within past 12 months	(Wang *et al.*, 2004)
	2868 adults aged 20 – 74 years	Singapore (urban)	Standardized questionnaire	5.5% AR	(Ng and Tan, 1994)
Thailand	7341 children aged 6 – 14 years	Bangkok and its vicinity	ISAAC study questionnaires	17.9 – 44.2% AR	(Bunnag *et al.*, 2009)
Vietnam	7008 adults aged 21 – 70 years	Hoankiem (urban) and Bavi (rural) in Hanoi	FinEsS questionnaire modified from Swedish OLIN study questionnaire	50.2% AR	(Lam *et al.*, 2011)

Abbreviations: AR, allergic rhinitis; FinEsS, Finland, Estonia and Sweden; ISAAC, International Study of Asthma and Allergies in Childhood; OLIN, Obstructive Lung Disease in Northern Sweden.

2.2.2 Aetiology of AR

AR is usually caused by aeroallergens inhaled by allergic individuals. Based on the timing of exposure, AR can be classified into perennial AR which can occur at any time throughout the year or seasonal AR which usually occur in certain seasons in a year based on the presence of the aeroallergens exposed yearly (May and Dolen, 2017). The most common aeroallergens associated with perennial AR are house dust mites (HDMs) and animal dander. The global major HDMs species are *Dermatophagoides pteronyssinus* (*Der p*), *Dermatophagoides farinae* (*Der f*), *Euroglyphus maynei* and *Blomia tropicalis* (Calderon *et al.*, 2015). Cat (*Felix domesticus*) and dog (*Canis familiaris*) danders are the common aeroallergens from pets that cause allergic reactions in perennial AR (Passali *et al.*, 2018). Meanwhile, pollens from birch (Betulaceae family) and grass (Poaceae family) are the main aeroallergens causing seasonal AR (Asam *et al.*, 2015; Garcia-Mozo, 2017).

Although AR is frequently associated with allergic sensitization to aeroallergens, sensitization to foods may also induce AR (Cingi *et al.*, 2010). The true prevalence of food-induced AR is difficult to identify as it frequently occurs in association with other food allergy symptoms such as asthma, eczema, oral allergic manifestations, urticaria and gastrointestinal symptoms. Cross reactivity between a pollen allergen and a homologous protein allergen in raw fruits or vegetables (e.g. birch pollen protein Bet v 1 and the homologous Mal d 1 protein in apple or Dau d 1 in carrot) may result in allergic sensitization in AR patients (Breiteneder and Mills, 2005; Malik *et al.*, 2007).

HDMs are the most common allergens causing allergic sensitization among AR patients in Malaysia (Asha'ari *et al.*, 2010; Ashari, 2009; Gendeh *et al.*, 2004). They are commonly found as indoor allergens in human habitats (Calderon *et al.*,

2015). The critical factor for the HDMs prevalence both inside and outside the home is humidity as their concentrations were found to be higher in damp homes, and Malaysia has continuous warm and humid environment throughout the year which enable the growth and proliferation of HDMs. HDMs also are more frequently found in beds than carpets at home because the relative humidity start to increase quickly after a bed is occupied (Calderon *et al.*, 2015).

Studies found that AR patients in Malaysia were mostly sensitized to HDMs of *Dermatophagoides* species such as *Der f* and *Der p* (Leung and Ho, 1994; Liam *et al.*, 2002; Wan Majdiah *et al.*, 2011). Furthermore, it has been observed that cat dander was another major aeroallergen that caused AR among adults and children in Malaysia (Asha'ari *et al.*, 2010; Gendeh *et al.*, 2004). Several studies in Malaysia found that food allergens also induced allergic sensitization in AR patients, especially shellfish such as shrimp and crab (Gendeh *et al.*, 2004; Gendeh *et al.*, 2000; Wan Majdiah *et al.*, 2016).

2.2.3 Predisposing factors of AR

AR is a disease that is commonly found in adults and children (Brozek *et al.*, 2017). It can be found across all age groups from childhood to adolescence to late adulthood (Blomme *et al.*, 2013; Eriksson *et al.*, 2011). The prevalence of AR has been found to be significantly decreased in 65 – 84 years age class compared to 20 – 44 years age class in both men and women (Cazzoletti *et al.*, 2015). Other studies also found that the prevalence of AR peaks at the age of 16 – 24 years old and it decreases in the following years up to the age of 65 – 70 years old (Droste *et al.*, 1996; Olivieri *et al.*, 2002). The decrease of AR prevalence in late adulthood could

be due to the decrease in the specific IgE level that occurs with aging in atopic individuals (Slavin, 2006).

AR is commonly present in both male and female gender. A systemic review and meta-analysis on AR found that the prevalence of AR in adults was not sex-specifc (Pinart *et al.*, 2017). However, a consistent male predominance of AR was found in children aged of 3 – 13 years old (Keil *et al.*, 2010). Findings from the Isle of Wight Birth Cohort showed that AR was predominantly found in male at the age of 1 – 2 years old but significant difference in gender was not found in the prevalence of AR in the following first 18 years of life (Kurukulaaratchy *et al.*, 2011). Cross-sectional surveys in the north-east of England found that AR prevalence are higher in pre-pubertal males aged 6 – 7 years old and adolescent girls age 13 – 14 years old (Shamssain and Shamsian, 1999; Shamssain and Shamsian, 2001). The higher prevalence of AR in adolescent girls could be due to the role of estrogens as female hormones have been found to play a role in allergic diseases (Bonds and Midoro-Horiuti, 2013). These findings indicate that gender may influence the occurance of AR at different age groups.

It is known that the increasing prevalence of asthma and allergy in recent years has been associated with the increase prevalence of obesity (Noal *et al.*, 2011). Obesity has been associated with asthma in several populations in childhood and adolescence (Baruwa and Sarmah, 2013). A cross-sectional study in 5,218 adults in United States showed that being overweight or obese was associated with increased risk of having AR (Gogna *et al.*, 2015). However, similar association was not found in children in the study. Another study in 3,327 allergic children in Wuhan City of China observed that obesity increased the prevalence of AR and AD in children (Lei *et al.*, 2016). These findings indicate obesity increase the risk of AR in both adults

and children. Studies have demonstrated that leptin which is adipokines was associated with allergen exposure and severity of AR in AR patients (Ciprandi *et al.*, 2009; Hsueh *et al.*, 2010). Thus, adipokines which is fat related hormones could be involved in the association of obese and AR.

A recent systematic review and meta-analysis on association of smoking and allergic diseases has observed very modest associations between smoking and some allergic diseases in adults (Saulyte *et al.*, 2014). A study on the effect of smoking on symptoms of AR found that smoking was not associated with the severity of nasal symptoms in AR patients (Bousquet *et al.*, 2009). Recently, smoking has been observed to be associated with the significant increase in the occurance of chronic rhinitis but not AR in self-reported or physician diagnosed AR patients (Hisinger-Molkanen *et al.*, 2018). These findings suggest that the association between nasal symptoms and tobacco smoke exposure may be independent of allergy.

Prevalence of AR is higher in the urban areas compared to rural areas based on several studies (Nicolaou *et al.*, 2005). The prevalence of chronic nasal symptoms in self-reported AR patients in West Sweden has been found to be associated with the increasing degree of urbanization (Eriksson *et al.*, 2011). Urban area is frequently associated with heavy traffic. Several studies have described the relation between traffic density and AR (Montnemery *et al.*, 2003; Weiland *et al.*, 1994). It was found that traffic density was positively correlated with the prevalence of wheezing and AR in children (Weiland *et al.*, 1994). Other study suggested that living on busy roads is associated with higher risk of sensitization to pollen allergens in allergic children (Kramer *et al.*, 2000). Collectively, these findings suggest that urbanization and heavy traffic density are associated with the increased prevalence of AR in urban areas.

2.2.4 Diagnosis of AR

AR is diagnosed mainly by the history of nasal symptoms, nasal endoscopic examination by the physician and history of allergy (Bousquet *et al.*, 2008). Patients are suggested to have AR if they have two or more of the symptoms assessed such as watery anterior rhinorrhea, sneezing especially paroxysmal, nasal obstruction, nasal pruritus and conjunctivitis for more than one hour on most days (Bousquet *et al.*, 2008). Although AR patients frequently have non-nasal symptoms such as eye symptoms, throat symptoms, chronic cough, ear symptoms, headache and mental function (cognitive) impairment (Spector *et al.*, 2003) but the assessment of these non-nasal symptoms were not included in diagnosis of AR. The assessment of these non-nasal symptoms can be used to further support the severity of AR (Wallace *et al.*, 2008).

The parameters examined for nasal endoscopic examination are presence of nasal secretions, erythematous or pale of nasal mucosa, nasal septum deviation, inferior turbinate hypertrophy, narrow internal nasal valve and nasal polyps (Ziade *et al.*, 2016). These parameters are examined to exclude other sinonasal diseases (Y and Gupta, 2016). Nasal features including transverse crease of the nose (Ramot *et al.*, 2010) and dark circle under the eyes, also known as allergic shiners (Chen *et al.*, 2009), can further support the diagnosis of AR.

There are several diagnostic tests that can be done to determine the allergens causing allergic sensitization in AR patients. Phadiatop enzyme-linked immunosorbent assay (ELISA) test is one of the assays used to determine the degree of sensitization to aeroallergens based on the presence of specific IgE to a mixture of common aeroallergens (Vidal *et al.*, 2005). However, this assay is unable to individually identify the specific aeroallergens that cause sensitization in AR

patients. Skin prick test (SPT) (Nevis *et al.*, 2016) and allergen specific IgE immunoassay using blood samples (Posa *et al.*, 2017) are respectively, the clinical and laboratory gold standard allergic tests used to confirm the underlying allergic sensitization that causes AR. Both tests can be used to determine the aeroallergens and food allergens AR patients are sensitized to. Studies have shown that there were good correlations between *in vivo* SPT and *in vitro* specific IgE immunoassay (Cho *et al.*, 2014a; Wongpiyabovorn *et al.*, 2017). Therefore, either SPT or specific IgE immunoassay can be used as a diagnostic test to determine the culprit allergens. However, both tests have their own advantages and disadvantages. SPT is commonly used by allergist as primary tool to detect the culprit allergens because of its high sensitivity, rapidity and inexpensiveness (Cox *et al.*, 2008; Wongpiyabovorn *et al.*, 2017). It is convenient to perform this test because no machine is required. Meanwhile, specific IgE immunoassay usually becomes the alternative tool in detection of culprit allergens because it is machine requirement test. It is expensive and lack of rapidity in giving results compared to SPT. However, the results obtained through this test are lessly affected by skin condition and medication. Most importantly, this *in vitro* immunoassay has no risk of severe allergic reaction that will occur to the allergic patients in comparison to the *in vivo* SPT (Wongpiyabovorn *et al.*, 2017).

Another diagnostic test that can determine the allergens AR patients are sensitized to is intradermal skin test. A study showed that AR patients with negative SPT results to HDMs appeared to be positive to HDMs after intradermal skin test was done (Erel *et al.*, 2017). Therefore, intradermal skin test can be considered as an alternative *in vivo* diagnostic test to determine allergens causing sensitization in AR patients if *in vitro* diagnostic tests are not available. Multiple allergen components of

specific IgE antibodies can be detected simultaneously by using immuno-solid phase allergen chip (ISAC) (Griffiths *et al.*, 2017; van Hage *et al.*, 2017). This multiplex *in vitro* diagnostic tool is able to provide the allergen specific IgE antibody profile of AR patients. A study showed that ISAC can be used as a diagnostic tool to determine the allergen components that have cross-reactivity with HDMs in polysensitized AR patients (Mohamad Yadzir *et al.*, 2014). The measurement of the eosinophil (Makihara *et al.*, 2014; Peric *et al.*, 2017), tryptase (Di Cara *et al.*, 2017; Kim *et al.*, 2016) and eosinophilic cationic protein (Di Cara *et al.*, 2017; Kim *et al.*, 2016) are other diagnostic parameters that can further support the diagnosis of AR as these parameters are commonly associated with the allergic inflammation in AR patients.

The severity of AR is classified into mild and moderate-severe based on the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines (Bousquet *et al.*, 2008). Sleep abnormality, impairment of daily activities, impairment of work or school performance and troublesome symptoms are the four items measured to determine the severity of AR. An AR patient is diagnosed as having a mild AR if he/she has none of the four items, while the diagnosis of moderate-severe AR is made if an AR patient has at least one of the four items measured. The ARIA guidelines also classified the symptoms of AR into intermittent and persistent symptoms based on the duration of the symptoms presented in AR patients. Intermittent symptoms are associated with the presence of symptoms for less than four days per week or less than four consecutive weeks; while persistent symptoms are associated with the presence of symptoms for more than four days per week and more than four consecutive weeks. The summary of AR severity and classification of symptoms is as shown in Figure 2.3.

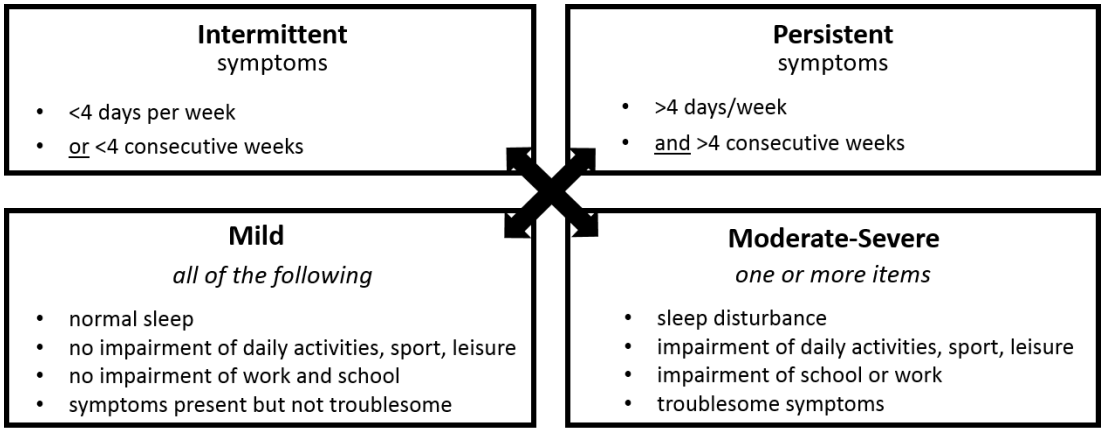

Figure 2.3: Summary of allergic rhinitis severity and classification of symptoms based on Allergic Rhinitis and its Impact on Asthma guidelines (Bousquet *et al.*, 2008).

2.2.5 Treatment of AR

AR can be managed through allergen avoidance (Platts-Mills, 2004), pharmacotherapy (May and Dolen, 2017) and immunotherapy (Rajakulasingam *et al.*, 2018). Currently, pharmacotherapy is considered to be the cornerstone in managing most cases of AR (Ridolo *et al.*, 2014) because the standard allergen avoidance alone does not give positive results in the management of AR (Solelhac and Charpin, 2014). Allergen avoidance by using mite-proof bedding covers have been shown to reduce the HDMs exposure but no significant improvement in the clinical symptoms of AR patients were observed (Terreehorst *et al.*, 2003). This observation suggested that the measured HDMs on the mattress surface did not reflect the allergens inhaled by AR patients (Tovey *et al.*, 2003). Several studies showed that the major cat allergen, Fel d 1, was found in the floor dust from homes (Custis *et al.*, 2003; Fahlbusch *et al.*, 2002) and hospital (Custovic *et al.*, 1998) even with the absence of cat in both places. These observations suggest that not having a

pet was not effective in managing AR. Therefore, standard allergen avoidance alone was no longer considered to be the cornerstone in the management of most cases of AR (Solelhac and Charpin, 2014).

The common pharmacotherapy for AR patients is intranasal corticosteroids (Trangsrud *et al.*, 2002) and antihistamines (Hernandez-Trujillo, 2009). Although AR is a systemic disease (Blanca *et al.*, 2015; Campo *et al.*, 2013), systemic steroids are not recommended in treating AR due to increased risk of diabetes and osteoporosis (Aasbjerg *et al.*, 2013). Intranasal corticosteroids that are commercially available to treat AR in adults and children are beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate, mometasone furoate and triamcinolone acetonide (Braido *et al.*, 2008). The intranasal corticosteroids have been proven to improve the symptoms of AR by reducing the nasal mucosa hyperreactivity through the anti-inflammatory action exerted by these medications (Bousquet *et al.*, 2008). Antihistamines has been classified into first-generation and second-generation drugs (Hoyte and Katial, 2011) used to treat AR. The usage of first-generation antihistamines should be avoided as these drugs penetrate into the brain and caused sedation, drowsiness and fatigue which may impair the ability to work and drive (Church and Church, 2013). Currently, second-generation antihistamines such as loratadine, levocetirizine, fexofenadine, bilastine, rupatadine and desloratadine are widely used in treating AR (Recto *et al.*, 2017). These drugs have been shown to rapidly reduce the nasal and ocular symptoms of AR with the improvement in the quality of life in most AR patients (Demoly *et al.*, 2014).

Allergen-specific immunotherapy is the only current treatment that may potentially decrease or resolve the underlying allergic inflammation in AR (Mortuaire *et al.*, 2017). Currently, subcutaneous immunotherapy (SCIT) and

sublingual immunotherapy (SLIT) have been used to treat AR patients (Durham and Penagos, 2016). AR patients with uncontrolled symptoms although on pharmacotherapy treatment should consider allergen-specific immunotherapy as an alternative treatment to manage the disease (Bousquet *et al.*, 2008). SCIT has been shown to be highly effective to treat seasonal AR as significant reduction in the symptom scores have been observed in the seasonal AR patients (Calderon *et al.*, 2007). SCIT has also been observed to be effective and safe to use in perennial AR adults and children with HDM sensitization (Eifan *et al.*, 2013). Similar to SCIT, SLIT is safe and effective to treat seasonal AR (Canonica *et al.*, 2014) but its efficacy in treating HDM allergy in perennial AR patients especially in AR children is less convincing (Calderon *et al.*, 2013). Currently, there is no specific lower age limit to start allergen-specific immunotherapy (Canonica *et al.*, 2014). However, SLIT is a favorable and attractive option of allergen-specific immunotherapy in young children and their caregivers compared to SCIT. This is because SLIT can be administered to the young children without the usage of needles and frequent trips to the medical clinic. Furthermore, SCIT is not frequently prescribed to the young children mainly because of concern that they may have difficulty in communicating the symptoms of systemic reactions during the immunotherapy program (Canonica *et al.*, 2014). The third update of Allergen Immunotherapy: A Practice Parameter (Cox *et al.*, 2011) states that immunotherapy can be given to the young children aged less than five years old if recommended. The recommendations must be based on the severity of the disease, risk/benefit ratios, and the physician's ability to correlate the clinical presentation with suitable allergy testing. This practice parameter of allergen immunotherapy was updated after studies that evaluated the safety of SCIT in children aged less than five years old reported a similar incidence and severity of