A CASE CONTROL STUDY TO DETERMINE THE ASSOCIATION OF BLOOD GROUPS AND ALLERGIC RHINITIS

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2022

A CASE CONTROL STUDY TO DETERMINE THE ASSOCIATION OF BLOOD GROUPS AND ALLERGIC RHINITIS

by

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Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

August 2022

ACKNOWLEDGEMENT

This master project is made feasible thanks to the assistance and guidance of several parties. First and foremost, I would want to express my heartfelt appreciation to Universiti Sains Malaysia (USM) for providing the opportunity and financial aid to complete this project.

I would like to convey my sincere gratitude to my research supervisor, Dr. Siti Mardhiana binti Mohamad, for her dedication to mentoring and supporting my master's study and research. Her guidance and advice carried me through all the stages of this master research. This project would not have been completed without her encouragement and insightful comments.

Next, I would like to express my appreciation and thanks to all my co-supervisors: Dr. Noorsuzana binti Mohd Shariff, Dr. Muhamad Yusri bin Musa, Professor Dr. Baharudin bin Abdullah, and Dr. Nurul Khaiza binti Yahya for their constant supervision and their support in completing this endeavour. The completion of this study could not have been possible without them imparting their knowledge and expertise.

Finally, I want to thank my parents and friends for their love, prayers, and ongoing support throughout my research endeavour. This endeavour would not have been feasible without their encouragement and assistance during my studies.

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LIST OF ABBREVIATIONS

AD	Atopic dermatitis
AMDI	Advanced Medical and Dental Institute
AR	Allergic rhinitis
ARIA	Allergic Rhinitis and Its Impact on Asthma
EDTA	Ethylenediaminetetraacetic acid
ENT	Ear, Nose, Throat
FA	Food allergy
FUT2	Fucosyltransferase-2
HDM	House dust mites
HUSM	Hospital Universiti Sains Malaysia
IgE	Immunoglobulin E
Rh	Rhesus
RMA	Red meat allergy
SPT	Skin prick test
Th	T helper
vWF	Von Willebrand factor

LIST OF APPENDICES

KAJIAN KES KAWALAN UNTUK MENENTUKAN HUBUNGKAIT KUMPULAN DARAH DAN PENYAKIT RESDUNG

ABSTRAK

Penyakit resdung adalah keradangan pada epithelium hidung yang dirangsang oleh tindak balas imun yang dimediasi oleh imunoglobulin E setelah terdedah kepada alergen dan mempunyai gejala seperti hidung berair, hidung gatal, dan bersin. Penyakit resdung dianggarkan mempengaruhi sekitar 400 juta orang di seluruh dunia. Kumpulan darah ABO dan rhesus terbukti meningkatkan kerentanan dalam penyakit seperti kanser gastrik, kanser pankreas, dan penyakit arteri koronari. Hipotesis kajian ini adalah kumpulan darah ABO dan rhesus mempunyai hubungkait dengan penyakit resdung dan kumpulan darah ABO adalah faktor risiko kepada penyakit resdung. Objektif kajian ini adalah untuk mengesahkan hubungankait kumpulan darah ABO / rhesus dan penyakit resdung. Tujuan kajian ini juga adalah untuk mengetahui hubungan faktor demografi (jantina dan bangsa) pesakit penyakit resdung dan kumpulan darah ABO. Oleh kerana tidak ada kajian mengenai kelaziman dan hubungan kait kumpulan darah ABO dan *aeroallergen* di Malaysia, kajian ini akan menjadi yang pertama untuk mengkaji hal ini. Sebanyak 326 sampel yang terdiri daripada 163 sukarelawan yang sihat dan 163 pesakit resdung dipilih berdasarkan kriteria seperti subjek yang berumur 18 ke atas dan berumur 60 ke bawah, sukarelawan yang tidak mempunyai alergi atau penyakit lain-lain dan pesakit resdung yang didiagnos oleh pakar ENT. Ujian cucuk kulit *(skin prick test)* menggunakan kawalan positif dan negatif, *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blomia tropicalis,* lipas Jerman, dan bulu kucing dilakukan pada pesakit. Sampel darah dikumpulkan daripada pesakit dan kawalan. Kaedah tiub digunakan untuk

membezakan kumpulan darah. Kemudian analisis statistik dilakukan dengan menggunakan SPSS versi 27.0. Berdasarkan kajian ini, kumpulan darah O adalah kumpulan darah yang paling ramai dalam kedua-dua kumpulan, diikuti dengan kumpulan darah B dan A, dan kumpulan darah AB adalah kumpulan darah yang paling kurang. Kekerapan rhesus positif lebih tinggi daripada rhesus negatif dalam kedua-dua kumpulan. Kajian ini menyimpulkan bahawa tidak terdapat hubungan antara kumpulan darah ABO / RhD dan penyakit resdung. Di samping itu, tidak ada hubungan antara kumpulan darah ABO dan penyakit resdung. Selain daripada itu, kajian ini juga tidak dapat menunjukkan hubungan antara kumpulan darah ABO dan jantina pesakit resdung. Kajian ini menunjukkan bahawa *aeroallergen* yang paling sering menyebabkan reaksi positif pada pesakit adalah *house dust mite*. Kajian ini tidak menunjukkan hubungan yang signifikan antara kumpulan darah ABO dan pelbagai jenis *aeroallergens* yang diuji. Kajian ini akan membantu dalam praktik klinikal dan seterusnya menyokong peranan kumpulan darah ABO / rhesus sebagai faktor risiko penyakit resdung. Jikalau dibuktikan kumpulan darah tertentu mempunyai hubungkait dengan penyakit resdung, kita boleh mencegah perkembangan penyakit resdung dengan membuat saringan awal. Walaupun demikian, penyelidikan selanjutnya yang mengunakan ukuran sampel yang lebih besar dan berbilang kaum disarankan untuk mengesahkan bukti hubungankait ini.

A CASE CONTROL STUDY TO DETERMINE THE ASSOCIATION OF BLOOD GROUPS AND ALLERGIC RHINITIS

ABSTRACT

Allergic rhinitis (AR) happens when the epithelial lining of the nose is inflamed, stimulated by the immunoglobulin E-mediated immune response after exposure to allergens. Symptoms of AR include nasal obstruction, watery nose, and sneezing. It is estimated to affect 400 million people worldwide. The ABO/rhesus blood groups are proved to increase susceptibility in diseases like gastric cancer, pancreatic cancer, and coronary artery disease. Hypothesis of this study is that the ABO/rhesus blood groups are related with allergic rhinitis and blood group O may increase susceptibility in allergic rhinitis. Therefore, the primary objective is to discover the association between ABO/rhesus blood groups and allergic rhinitis. Also, this study aimed to find out the association of demographic factors (gender and races) of patients and ABO blood groups. Since no study on the prevalence and association between ABO blood groups and aeroallergens conducted locally, this study will be the first to study this. A total of 326 samples comprised of 163 healthy volunteers and 163 patients were selected based on the criteria. Inclusive criteria for volunteers include adults aged between 18-60, without AR or any chronic illnesses. While inclusive criteria for patients is adults aged 18-60, diagnosed with ARIA according to ARIA guidelines by ENT specialist. Skin prick test using positive and negative controls, *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blomia tropicalis*, German cockroach, and cat dander was performed on patients. Blood samples were collected from both patients and controls. The tube method was employed for blood group typing. Then SPSS version 27.0 was used for statistical

analysis. Based on this study, the prevalence of distribution of ABO blood groups in both allergic rhinitis and non-allergic rhinitis group was O, followed by B then A and lastly AB. Blood group O was the commonest blood group in both groups, which is 66 out of 163. The frequency of rhesus positive is higher than the rhesus negative in both groups. 98.16% of patients and 97.55% of healthy subjects with rhesus positive. This study concludes that no associations were found between ABO/RhD blood groups and allergic rhinitis. Furthermore, there was also no association between ABO blood groups and races of allergic rhinitis patients. This study also did not show any association between ABO blood groups and the gender of allergic rhinitis patients. This present study found out that the aeroallergens that most frequently in causing a positive reaction in patients was house dust mites. This study did not show an association between ABO blood groups and different types of aeroallergens tested. This study will be helpful in clinical practice and further support the role of ABO/rhesus blood groups as risk factors for allergic rhinitis. If blood group O is proved to have association with AR, we can do early screening and treat the disease as soon as possible. Nonetheless, future research with large sample size and multiracial is recommended to validate the merging evidence on this relationship.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Allergic rhinitis (AR) is a symptomatic condition when the epithelial lining of the nose is inflamed, that is stimulated by the immunoglobulin E (IgE) mediated-immune response after exposure to the allergens that characterized by one or more symptoms including blocked nose, runny nose and itchy nose (Bousquet *et al.*, 2008).

People of all ages and ethnic groups are affected by AR worldwide. Its prevalence is increasing especially among the younger generation in Asian countries (Asher *et al.*, 2006). Prevalence of AR contributed to a range between 10-40% of the population (Brożek *et al.*, 2010), which estimated about 400 million people worldwide (Bousquet *et al.*, 2008). The frequency of AR is 27% among the paediatric community with a higher prevalence in the 12-14 year-old-group in Malaysia (Quah *et al.*, 2005). Approximately 10-32% of the adult population is to have AR including Malaysia, although regional data is minimal (Pawankar *et al.*, 2012). According to a study in 2011, it was reported that the prevalence of allergic rhinitis is 7.1% (Katelaris *et al.*, 2011).

AR can be caused by many factors and these include genetic, family history, allergens exposure, and pollutants (Brożek *et al.*, 2010). Family history is shown to be a crucial risk factor for AR. To date, the ABO blood groups have yet to be considered as risk factors for AR in the present knowledge. Meanwhile the ABO blood groups are proved to increase susceptibility in several diseases such as gastric cancer (Aird *et al.*, 1953; Edgren *et al.*, 2010; Liumbruno and Franchini, 2014), pancreatic cancer (Abegaz, 2021; Yamamoto *et al.*, 2012), coronary artery disease (Zu *et al.*, 2017), and infectious diseases (Garratty, 2000).

According to the scoping review written by Dahalan et al. in the year 2020, there are only four studies has been conducted to study on the association of ABO blood groups and allergic rhinitis (Carpeggiani, 2011; Dahalan *et al.*, 2020; Falsarella *et al.*, 2011; Hamad, 2016; Topno *et al.*, 2019). Currently, study on the association of ABO/rhesus blood groups and allergic rhinitis has not been conducted in Malaysia. Hence, this proposed research is vital to explore the association of ABO/rhesus blood groups and allergic rhinitis and further identify ABO/rhesus blood groups as a risk factor for allergic rhinitis.

1.2 Objectives

1.2.1 General

To study the association of blood groups and allergic rhinitis in case and control group.

1.2.2 Specific

1. To determine the prevalence of ABO blood groups in allergic rhinitis and non-allergic rhinitis.
2. To determine the association between the blood groups (ABO and Rhesus) and allergic rhinitis.
3. To determine the association between the socio-demographic factors (races and gender) and allergic rhinitis in case and control groups.
4. To determine the prevalence of ABO blood groups in different types of aeroallergens (*B. tropicalis*, *D. farina*, *D. pteronyssinus*, German cockroach, cat dander).

5. To determine the association between ABO blood groups and aeroallergens (*B. tropicalis*, *D. farina*, *D. pteronyssinus*, German cockroach, cat dander) in case group.

1.3 Hypothesis

1.3.1 Null Hypothesis

1. There is no difference in prevalence between case (AR) and control (healthy) groups.
2. There is no association between ABO blood groups and allergic rhinitis.
3. There is no association between ABO blood groups and socio-demographic factors (races and gender) of allergic rhinitis patients.
4. There are no differences in the prevalence of ABO blood groups between aeroallergens (*B. tropicalis*, *D. farina*, *D. pteronyssinus*, German cockroach, cat dander).
5. There is no relationship between ABO blood groups and types of aeroallergens (*B. tropicalis*, *D. farina*, *D. pteronyssinus*, German cockroach, cat dander).

1.3.2 Alternative Hypothesis

6. There is a difference in prevalence between case (AR) and control (healthy) groups.

1. There is an association between ABO blood groups and allergic rhinitis.
2. There is association between ABO blood groups and socio-demographic factors (races and gender) of allergic rhinitis patients.
3. There are differences in the prevalence of ABO blood groups between aeroallergens (*B. tropicalis*, *D. farina*, *D. pteronyssinus*, German cockroach, cat dander).

4. There is a relationship between ABO blood groups and types of aeroallergens (*B. tropicalis*, *D. farina*, *D. pteronyssinus*, German cockroach, cat dander).

1.4 Justification

The ABO/rhesus blood groups have been related with increased susceptibility to numerous diseases such as gastric cancer (Aird *et al.*, 1953; Edgren *et al.*, 2010; Liumbruno and Franchini, 2014), pancreatic cancer (Abegaz, 2021; Yamamoto *et al.*, 2012), coronary artery disease (Zu *et al.*, 2017), and infectious diseases (Garratty, 2000). As reported by the scoping review written by (Dahalan *et al.*, 2020) Dahalan et al., there are only four studies has been conducted to study the association of ABO blood groups and allergic rhinitis, and the findings remained controversial (Carpeggiani, 2011; Dahalan *et al.*, 2020; Falsarella *et al.*, 2011; Hamad, 2016; Topno *et al.*, 2019).

Furthermore, there are limited data which studies the prevalence of allergic rhinitis according to blood groups in Malaysia. The relationship of ABO/rhesus blood groups and allergic rhinitis has not been studied locally. Not to mention the relationship between ABO blood groups and demographic factors (gender, races) in patients with allergic rhinitis has not been investigated in Malaysia too. This data would be helpful in clinical settings as early screening can be conducted to screen, publish diagnosis, and treat accordingly for patients that have high risk of getting allergic rhinitis.

There are a scarce number of studies published on the prevalence of aeroallergens, and most were conducted on the prevalence of house dust mites. No study was conducted to determine the relationship of ABO blood groups and different types of aeroallergens. This present study will be the first study conducted on the

relationship between ABO blood groups and aeroallergens in Malaysia. Aeroallergens such as house dust mites have been proved to cause the most positive reaction in skin prick test (Liam *et al.*, 2002; Lim *et al.*, 2015; Sinniah and Thakachy, 2014). Cockroach allergen has been proved to cause positive reaction in patients with allergic rhinitis, second to house dust mites (Sam *et al.*, 1998). Animal dander such as cat and dog dander are also the common aeroallergen in Asia-Pacific (Oncham *et al.*, 2018). Thus, a skin prick test panel including these aeroallergens was chosen to be included in our panel.

Hence, the proposed study will provide evidence-based data that will help in identifying ABO/rhesus blood groups as a risk factor in developing allergic rhinitis. By recognizing ABO blood groups as a risk factor, it can be used as a preventive measurement in treating allergic rhinitis. We can identify patients earlier by screening patients with high-risk blood group. Also, it will give benefit in discovering the relationship between ABO blood groups and aeroallergens. Consequently, it will help to better facilitate the consultation process and revise the management for allergic rhinitis in the clinical setting.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Allergic Disorders

Allergic disorders are a group of immune-mediated disorders caused by hypersensitivity to normally innocuous substances found in the environment (allergens) (McConnell, 2014). These diseases are hay fever, food allergy, atopic dermatitis, bronchial asthma, and allergic rhinitis.

Allergic disorders are prevalent worldwide, affecting males and females of various ages, socioeconomic backgrounds, and ethnic groups. Allergic diseases are increasing their prevalence with increasing urbanization. The prevalence of allergy ranges from 30-40% worldwide (Y. Kumar and Bhatia, 2013). "39% of children and 30% of adults have been diagnosed with one or more allergic diseases such as asthma, eczema, and hay fever in the United Kingdom" as stated in a study (Gupta *et al.*, 2004). Eczema, asthma, and allergic rhinitis afflict more 'South Asian' and 'Black' people than 'White' people. (Gupta *et al.*, 2004).

The "hygiene hypothesis" was proposed by Professor David Strachan in 1989 (Strachan *et al.*, 1996). Professor Strachan observed that children who grew up in family with larger number of family members, may have been exposed to more microbes, because of unsanitary interaction with older siblings. From this observation, he hypothesised that early and high microbial exposure may protect infants and children from developing immunological hypersensitivity later in life (Stiemsma *et al.*, 2015). However, few pieces of literature held opposing opinions on this hypothesis recently. In 2003, Rook proposed the "Old Friends" mechanism and it suggests that chronic inflammatory illnesses including allergies, autoimmunity, and inflammatory bowel disease are better controlled in low-income countries than in high-income and

urbanized countries. (G. A. W. Rook, 2010; Graham A. W. Rook *et al.*, 2013). Transition in lifestyle and environment, along with expeditious urbanization, altered diet with less fibre, and excessive usage of antibiotics have caused a detrimental effect on the diversity of the human microbiome, thus, causing an increased risk of allergic diseases (Bloomfield *et al.*, 2016).

Risk factors of allergy can be classified into two groups: host and environmental factors (Grammatikos, 2008). Host factors include heredity, gender, ethnic and age. Previous studies showed that genetic disposition plays a significance influence in allergic diseases. A study proved that children have a higher risk to develop asthma if parents have asthma (Burney *et al.*, 1997). Furthermore, environmental factors play a pivotal factor in developing allergic disorders. Allergens, pets, parasites, air pollution, infection, diet, and tobacco smoking are all contributing to the risk factors of allergic disorders (Nicolaou *et al.*, 2005).

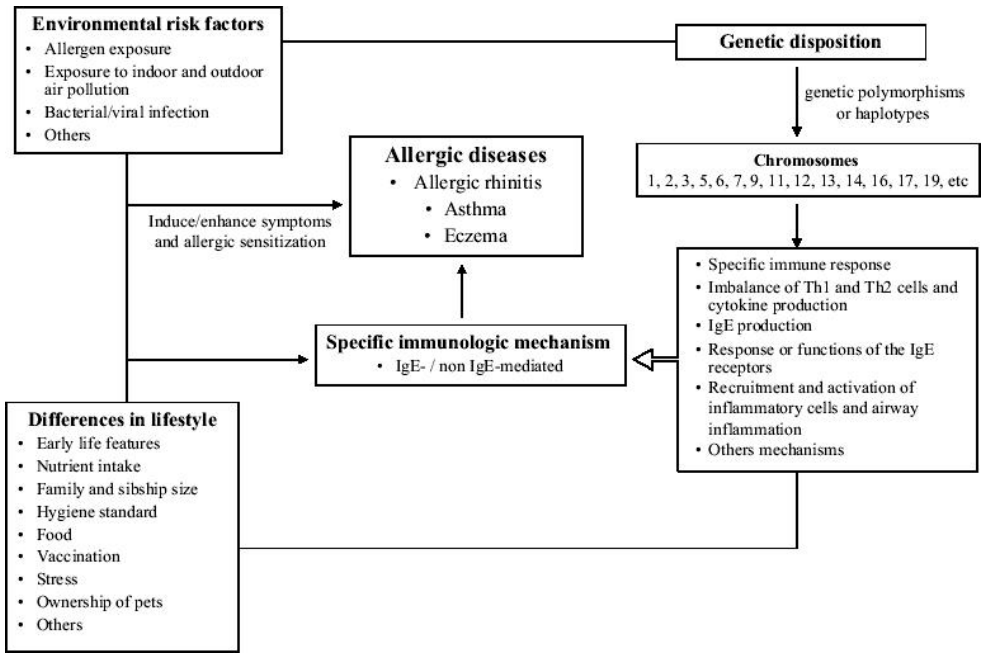

Figure 2.1 Risk factors of allergic diseases. Figure adapted from (D.-Y. Wang, 2005).

Allergic diseases are mainly IgE-mediated immediate reactions, which otherwise known as the type I hypersensitivity reaction. During the sensitization process, dendritic cells will capture the foreign antigens and convey the antigen information to T helper (Th) cells (Liu and Yang, 2015). The Th2-type cytokines are interleukins 4, 5, and 13, which promote the production of mast cells, immunoglobulin E (IgE), basophils, and eosinophils (Berger, 2000). By attaching to the IgE-specific receptors on the surface of mast cells, IgE will sensitise them. When sensitised mast cells are re-exposed to the same allergen, the activated mast cells and basophils undergo a process known as degranulation, which results in the release of mediators such as histamine, prostaglandins, and leukotrienes. This will initiate allergic symptoms such as itchiness, rash, shortness of breath, swelling and sneezing.

Allergic reactions can result from food, come in contact with airborne particles, or come into contact with skin. In cases when in contact with airborne particles, symptoms normally occur in areas like the eyes, nose, and lungs. For example, allergic rhinitis causes nasal inflammation, sneezing, nasal itching, and redness of the eyes. Allergic reactions that result from food are abdominal pain, vomiting, and diarrhea. When the allergen comes in contact when skin, it will cause allergic reactions such as rashes, swelling, or inflammation of the skin. The symptoms might be systemic (classical anaphylaxis) or confined to specific body systems, depending on the individual, allergen, and manner of exposure.

Since there is no gold standard diagnostic test for allergic disorders, symptoms like wheezing, shortness of breath, irritation of the skin, and rashes are commonly used to diagnose allergic diseases. Skin prick test (SPT) and patch test can both be done to determine if a specific substance creates an allergic reaction to the skin. Laboratory tests to measure the total serum IgE in the blood such as enzyme immunoassay and

radioallergosorbent test can be used to exclude other aetiology such as parasite infection.

The principal management of allergies is allergen avoidance. Patients should recognize what substances that might trigger the allergy and avoid the allergen. Most commonly prescribed medications for patients with allergic disorders include antihistamines, glucocorticoids, epinephrine, mast cell stabilizers, and antileukotriene agents in order to improve the symptoms (Frieri, 2018). Immunotherapy is recommended in management for allergy to insect bites and asthma. Immunotherapy includes exposing patients to increasing doses of allergen with the purpose of altering the immune system's reaction. Types of immunotherapies include subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT), and medications. Immunotherapy has been proved to show improvement in overall quality of life, comorbid illnesses, and medication requirements (Cox, 2016).

2.2 Allergic Rhinitis

2.2.1 Definition

Allergic rhinitis (AR) is an inflammation of the epithelial lining of the nose that is stimulated by the immunoglobulin E (IgE) mediated immune response after exposure to the allergens with “the three cardinal symptoms of sneezing, nasal obstruction, and rhinorrhea” (Bousquet *et al.*, 2008). The symptoms of AR are reversible and occur as a result of exposure to allergens and triggering factors. AR is a serious airway disease that can result in severe morbidity and reduces a patient’s functional ability and his quality of life.

2.2.2 Epidemiology

Over the last several decades, the global prevalence of allergy disorders such as allergic rhinitis and asthma has increased. Prevalence of AR contributed to a range between 10-40% of the population (Brożek *et al.*, 2010), which estimated about 400 million people worldwide (Bousquet *et al.*, 2008). According to the "International Study of Asthma and Allergies in Childhood (ISAAC) Phase III" study, the frequency of AR is increasing globally, especially among the younger population in Asian countries (Asher *et al.*, 2006). A previous study conducted on the Asian-Pacific population reported that the average prevalence of AR among children and adults was 8.7% (Cazzoletti *et al.*, 2015). In Malaysia, the frequency of AR is 27% among the paediatric community with a higher prevalence in the 12-14 year-old-group (Quah *et al.*, 2005). In the previous study on Asia-Pacific, it is estimated about 10-32% of the adult population have AR including Malaysia although regional data is minimal (Pawankar *et al.*, 2012). According to a study in 2011, it was reported that the prevalence of allergic rhinitis is 7.1% (Katelaris *et al.*, 2011).

2.2.3 Risk Factors

Allergic rhinitis can be caused by many factors, normally caused by interactions between genes and the environment. Risk factors of AR include genetic predisposition and familial history, early-life exposure, ethnicity, allergens exposure, pollutants, and social class (Brożek *et al.*, 2010).

Family history has been proven to be a major risk factor for AR expression in several studies. In 1971, Edfors-Lubs discovered that AR is inheritable in twins (D.-Y. Wang, 2005). Previous studies showed that children whose parents have AR, have increased risk in contracting AR (Alsowaidi *et al.*, 2010; Li *et al.*, 2014). Another study

proved that children would have a higher risk in contracting asthma if parents have asthma (Burney *et al.*, 1997).

The term "atopic march" was used to characterise the evolution of atopic illnesses from infantile atopic dermatitis (AD) to childhood allergic rhinitis and asthma. According to Spergel et al, among patients with AD, approximately half will develop asthma, and two-thirds will have allergic rhinitis in adulthood (Spergel, 2003). It was suggested that the defect in the epithelial barrier of patients with atopic dermatitis could not fend off infectious agents and allergens from entering the body, therefore it initiates the occurrence of systemic allergy and increases the susceptibility of an individuals to AD, AR, and asthma (Bantz SK, Zhu Z, 2014).

Exposure to perennial or seasonal allergens prevalent in our indoor and outdoor settings is a common cause of allergic rhinitis. Allergens can be classified as outdoor and indoor allergens. Common outdoor allergens are pollens and moulds, while indoor allergens include house dust mites (HDM), animal dander, insects, and moulds. HDM are the most common indoor allergens for allergic rhinitis. The most common allergens causing AR worldwide are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* as stated by a study (Bousquet *et al.*, 2008). HDM are commonly found in carpets, mattresses, and fabrics.

The ethnic group is another risk factor for AR. One study showed that the Chinese have the highest risk for AR, followed by Malays and Asian Indians have the least risk for AR (D.-Y. Wang, 2005). Another research in Singapore revealed considerable ethnic diversity in the genetic polymorphism among Chinese, Malays, and Indians, but no link between the genetic polymorphism and atopy in any of the three ethnic groups (Tan *et al.*, 1999). There is currently little evidence that genetic

variation among ethnic groups is a significant factor in the development of allergy disorders.

2.2.4 Pathogenesis

Allergic rhinitis happens when an immunoglobulin E (IgE) mediated response is triggered by an extrinsic protein or an allergen (Skoner, 2001). This type of response is known as an immediate hypersensitivity reaction. Immunoglobulin E (IgE) mediates the release of histamine and other mediators from mast cells and basophils, resulting in the immediate hypersensitivity reaction (Buelow, 2015). During sensitization, allergens that are encountered for the first time, such as pollens, animal dander, or dust mites, are presented by antigen-presenting cell, which triggers a response in Th2 lymphocyte, which interact with B cells and will then produce IgE antibodies (Norman, 1995). The allergen will bind to the IgE molecules on the mast cells' or basophils' surfaces. Cross-linking of the IgE and Fc receptors occurs when one or more IgE-receptor complexes meet the same allergenic molecule and activate the sensitised cell. Activated mast cells and basophils then degranulate, releasing histamine as well as other inflammatory chemical mediators such as cytokines, interleukins, leukotrienes, and prostaglandins. Vasodilation, mucus production, and smooth muscle contraction are only a few of the systemic effects.

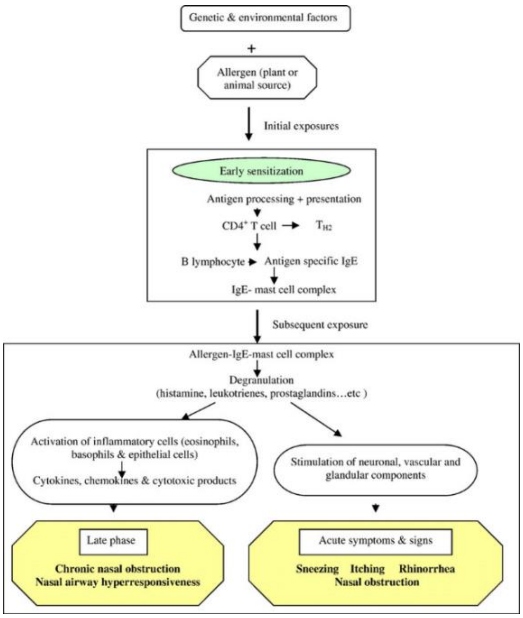

Figure 2.2 Pathogenesis of allergic rhinitis. Figure adapted from (Alsuleiman and Walker, 2007).

2.2.5 Diagnosis

To diagnose allergic rhinitis, a combination of a history of allergy symptoms and diagnostic procedure is utilised. There are several types of skin tests can be used to diagnose allergy: skin prick test (SPT), intradermal test (IDT), and Laboratory studies such as skin prick test (SPT), total serum IgE and total blood eosinophil counts are usually used to confirm the diagnosis of AR.

When two or more symptoms of sneezing, nasal blockage, watery rhinorrhea, and nasal pruritus last for more than one hour on most days, AR is suspected. The severity of AR can be classified according to the "Allergic Rhinitis and Its Impact on Asthma (ARIA)" guidelines (Pawankar *et al.*, 2012). AR is categorized depending on the duration or continuousness of symptoms and severity of the symptoms. If symptoms last for fewer than four days per week or less than four weeks per year, AR is classified as intermittent. Meanwhile, if symptoms last more than four days per week or more than four weeks per year, AR is categorized as persistent. Mild AR is

defined as a condition in which symptoms do not interfere with sleep, everyday activities, or job or school performance. When symptoms of AR interfere with sleep, everyday activities, and job or school performance, it is called severe AR. (Bousquet *et al.*, 2008).

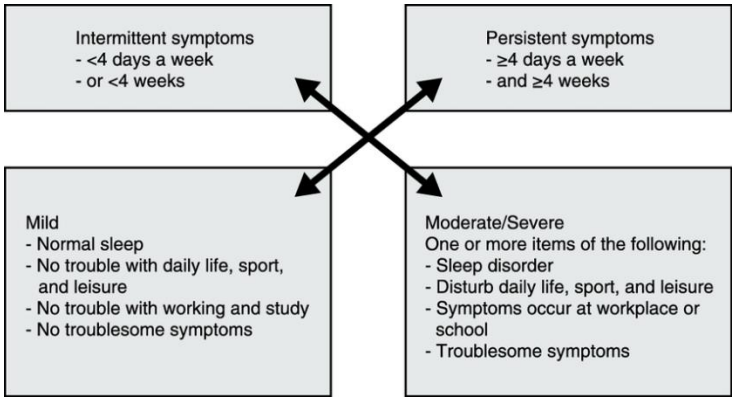

Figure 2.3 Classification of allergic rhinitis according to "ARIA" guidelines. Figure adapted from (Bousquet *et al.*, 2008).

Skin prick test (SPT) is an important diagnostic method used to diagnose IgE-mediated allergic disorders such as asthma, atopic dermatitis, food allergy, and allergic rhinitis (Heinzerling *et al.*, 2013). It is a test where an extract of suspected allergen, positive and negative controls are introduced percutaneously. The interpretation of results after 15-20 minutes and a wheal reaction that larger than 3mm will be produced if positive. The advantages of SPT are that it is minimally invasive, not expensive, and the results are almost immediate.

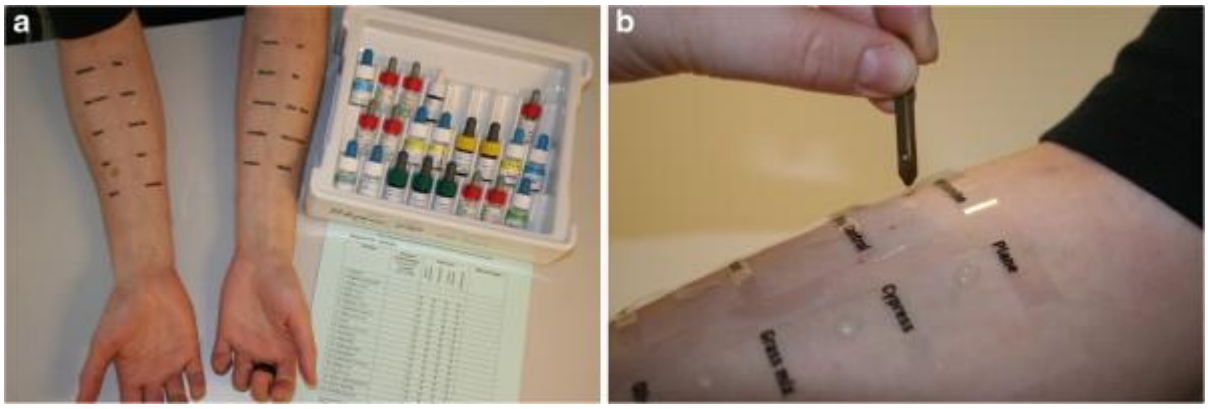

Figure 2.4 SPT procedures. (a) Preparation of SPT on forearm of patient. (b) Lancet is used to prick the skin through a drop of allergen. Figure adapted from (Heinzerling *et al.*, 2013).

The measurement of total serum IgE, serum specific IgE, and eosinophil counts is not specific for AR, but it is helpful when combined with other factors such as results of SPT and history of allergy symptoms. Patient with AR will show an increased IgE value when compared to non-allergic patients. Total serum IgE is a conventional measure to diagnose allergic diseases, however due to its low specificity, it is commonly used for screening (Chang *et al.*, 2015). Serum-specific IgE is an objective measure to diagnose allergic diseases with a high sensitivity, making it a valuable piece of evidence for allergy diagnosis (Chang *et al.*, 2015).

Both SPT and serum specific IgE measurement are used in the diagnosis of AR. The serum-specific IgE measurement has a high sensitivity and positive predictive value, but it has a poor specificity to individual allergens hence it may lead to a false-positive diagnosis of allergic disease (Raj Kumar *et al.*, 2015). SPT can provide information on hypersensitivity to individual allergens. In addition to that, SPT is less expensive and can offer the patient and the clinician with rapid and educational information (Tschopp *et al.*, 1998). Nonetheless, it is encouraged to carry out both SPT and serum specific IgE measurement to confirm the diagnosis of AR.

A nasal provocation (allergen challenge) testing is another research tool that helps with the diagnosis of AR. The patient is asked to inhale the allergen and then monitor for possible symptoms or production of secretion (Gendo and Larson, 2004).

2.2.6 Treatment

The management of AR can be classified into three categories: allergen avoidance, pharmacotherapy, and immunotherapy (Jean, 2021).

Allergen avoidance is a means for the prevention and treatment of AR. Allergens or irritants that trigger the symptoms of AR should be identified. Then patients should avoid such allergens. The most common indoor allergens that have been proved to trigger symptoms are HDM, pets, cockroaches, and molds. Nonspecific triggers like rapid changes in temperature, smoke and strong perfumes should also be avoided.

To date, most of the treatment for AR is supportive treatment. Supportive treatment is carried out to relieve the symptoms by providing oral antihistamines, decongestants, or both as needed. Antihistamines include cetirizine, loratadine, and levocetirizine are commonly prescribed to patients with AR. Decongestant such as pseudoephedrine is used to relieve nasal congestion in the respiratory tract by stimulating vasoconstriction. Patients with chronic AR are normally prescribed with intranasal steroid spray. Recently, immunotherapy has been discovered to be helpful in the treatment of AR. Immunotherapy involves administrating the allergen(s) subcutaneously or sublingually, that cause allergy symptoms by the physician, of increasing dose to induce clinical and immunologic tolerance (Petalas and Durham, 2013). Immunotherapies include subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT), and medications. Immunotherapy has been proved to provide

long-term symptom relief for years even after the treatment is discontinued (Cox, 2015).

2.3 ABO/Rhesus Blood Groups

In 1900, Karl Landsteiner, an Austrian scientist, was awarded the Nobel Prize for discovering the first blood group system, ABO. He discovered that the red blood cells (RBCs) of some individuals were agglutinated by others' serum. He observed the patterns of agglutination and established that blood could be divided into groups. Landsteiner and Wiener discovered the rhesus (Rh) system in 1940 (Lockyer, 1982).

The ABO blood group system is recognized as the most significant blood group system in medicine, especially in transfusion medicine. Based on an individual's blood type, blood transfusion, organ, and hematopoietic stem cells donations can be carried out safely. Haemolytic disease of the fetus and newborn linked with ABO incompatibility can be evaluated by using the ABO blood group system (Sameer Wagle, 2017). Also, the ABO blood group system can be used to do a platelet refractory evaluation (Gonsorcik, 2018).

The four basic ABO phenotypes are O, A, B, and AB. The presence of antigens on RBCs and antibodies in the blood are used to classify ABO phenotypes. Blood group A has A antigen on RBCs and anti-B antibody in serum. Blood group B has B antigen on RBCs and anti-A antibody in serum. Blood group AB has A and B antigens on RBCs and no antibody in serum. Blood group O has no antigens on RBCs and anti-A, anti-B, and anti-A,B antibodies in serum.

According to past study, there are 44% of white, 49% of black and 43% of Asians having blood group O, 43% of white, 27% of black, and 27% of Asian having blood group A, 9% of white, 20% of black and 25% of Asian having blood group B,

and 4% of white, 4% of black and 5% of Asian having blood group AB (Gonsorcik, 2018). The most prevalent blood group is blood group O and blood group AB is the least prevalent in the most population. This is identical to the prevalence of ABO blood groups in Malaysia (Ahmed *et al.*, 2012). In Malaysia, blood group O is the most common among the Malays and the Chinese, whereas, for the Indians, blood group O and blood group B are of equal frequencies. The blood group AB is the least common among the Malays, the Chinese, and the Indians.

Nowadays, ABO blood grouping can be done using a variety of methods, including the slide or tile method, tube method, microplate method, and automated method (Mujahid and Dickert, 2015).

Microplate method is a modernised method that provides a faster and more sensitive result with the feasibility of automation. Microplates consist of a lot of small tubes containing a few µL of reagents, after centrifugation and incubation of the mixture of blood samples and reagents, the result of agglutination can be examined by an automatic read-out device (Mujahid and Dickert, 2015).

Automated method uses a machine to determine ABO grouping, Rh typing, antibody screening and cross matching (Khandpur, 2020). This method normally used in hospitals' blood banks and blood transfusion centre for blood grouping prior to blood transfusion.

To carry out the slide or tile method, a blood sample is collected. On a labelled slide or tile, one drop of anti-A, anti-B, and D reagent is deposited individually. One drop of blood sample is added to each drop of the reagent. By using a clean stick, the cells and reagents are mixed. Each mixture is spread evenly on the slide or tile over an area of 10-15mm diameter. The mixture is then left for 5 minutes at room temperature. The agglutination reaction is interpreted.

In the tube method, a blood sample is collected, then centrifuged, to separate the RBCs from the serum. A 2-5% cell suspension is prepared from the sample. A drop of anti-A, anti-B and anti-A,B is placed in separate test tubes. A drop of cell suspension is then added into each test tubes. Two drops of serum from the sample are placed in test tubes and one drop of reference cells of blood group A, B and O is added into the test tubes. All test tubes are centrifuged at 1500rpm for one minute. The agglutination reaction is read and interpreted according to the agglutination grading reaction table. The interpretation of ABO blood groups is interpreted according to Table 2.1.

Table 2.1 Determination of ABO blood groups.

Red Cells tested with known Antisera			Serum tested with known red cells		Interpretation of ABO Group
Anti-A	Anti-B	Anti-A, B	A1 Cells	B Cells	
3-4+	0	3-4+	0	3-4+	A
0	3-4+	3-4+	3-4+	0	B
0	0	0	3-4+	3-4+	0
3-4+	3-4+	3-4+	0	0	AB

Table 2.2 Advantages and disadvantages of slide or tile method and tube method.

	Slide or tile method	Tube method
Advantages	May be used as an emergency test or for preliminary grouping	More sensitive
		Both the red cells and serum grouping are done
		Can detect weaker antigens and antibodies
Disadvantages	Less sensitive	Subjective grading
	Drying up of the mixture can cause aggregation of cells, giving a false-positive result	
	Weaker reactions are difficult to interpret	

The Rh (Rhesus) blood group system is regarded as the second most significant blood group system following the ABO blood group system. Rh blood group typing is performed to assess Rh compatibility in blood donation, organ donation, and hematopoietic marrow donation (Victoria K Gonsorcik, 2018). It is also utilized to evaluate the haemolytic disease of the fetus and newborn (Sameer Wagle, 2017).

Rhesus blood group system comprises of more than 50 red cells antigens and the five prime Rh red cell antigens are D, C, c, E and e. This present study focuses on red cell antigen D. There are two separate genes, RhD on chromosome 1 that encode for the D antigen. Rh (D) positive is when D antigen is present on the RBCs, while Rh (D) negative is when D antigen does not present on the RBCs.

The prevalence of Rh (D) negative is approximately 15% in white individuals, 6% of black individuals, and 1% of Asians (Victoria K Gonsorcik, 2018). According to a previous study conducted in Malaysia, individuals with rhesus positive are more than individuals with rhesus negative (Ahmed *et al.*, 2012). According to Ahmed et

al, the distribution of rhesus positive is 99.5% in Malays, 98.5% in Chinese, and 91.7% in Indians (Ahmed *et al.*, 2012).

There are a few methods used for Rh typing, which include agglutination test, column agglutination, and solid-phase test systems. The tube method is recommended as both red cell and serum grouping is performed. For the tube method in Rh blood grouping, 2-5% cell suspension in saline from a blood sample is prepared. A drop of red cell suspension and a drop of anti-D serum is added together in a test tube. The test tube is then centrifuged at 1500rpm for one minute. The agglutination reaction is rated on a scale of 0 to 4+ and signifies a positive result (Figure 2.5).

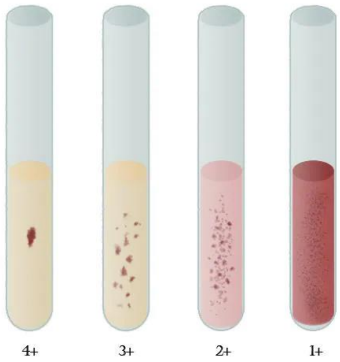

Figure 2.5 Agglutination reaction on a scale of 0 to 4+. Figure adapted from (Gonsorcik, 2018).

2.4 Association of ABO/Rhesus Blood Group with Other Diseases

The association between ABO blood groups and higher susceptibility to various disease has been studied since the early 1900s, when researchers found that antibodies and antigens may be inherited. The ABO blood groups have been linked to various diseases, including cancer, cardiovascular diseases, hematologic disorders, metabolic diseases and cognitive impairments (Abegaz, 2021).

According to World Health Organization, gastric cancer is the sixth most frequent cancer worldwide and the fourth leading cause of cancer mortality ("Cancer," 2021). Studies since the 1950s showed that individuals with blood group

A have an increased risk of getting gastric cancer compared to individuals with blood group O (Aird *et al.*, 1953; Edgren *et al.*, 2010; Liumbruno and Franchini, 2014). Chronic *Helicobacter pylori* infection has been shown to contribute to increasing the risk of gastric cancer. In relation to that, individuals with blood group A had a significantly higher chance of contracting *H. pylori* infection than individuals with non-A blood groups (Ewald and Sumner, 2016; Z. Wang *et al.*, 2012).

Pancreatic cancer is less of common cancer but it is most destructive as it has the highest mortality rate (Tomislav Dragovich, 2020). According to reliable studies, individuals with blood groups A, B, and AB had a 25% greater risk of gastric and pancreatic cancer, and a 17 percent higher risk of pancreatic cancer solely (Abegaz, 2021; Yamamoto *et al.*, 2012). Another study found that those with blood group A are more likely to get pancreatic cancer (Vioque and Walker, 1991).

Infectious diseases such as cholera, gastrointestinal epidemic caused by *Escherichia coli*, mumps, plague, tuberculosis have been linked with the ABO blood groups. A and/or B antigens, as well as the absence or presence of anti-A and/or B antibodies, offer strong or weak defence barriers against infection (Abegaz, 2021). Glycoconjugated red cell surface are used as an attachment by parasites, bacteria, and viruses, thus glycosylation polymorphisms of the ABO blood groups might result in different vulnerability among individuals with different blood groups (Yamamoto *et al.*, 2012). According to a credible study, individuals with blood group O have a higher susceptibility to cholera and plague (Garratty, 2000). Individuals who can produce anti-H antigen (blood groups A and B) will be more resistant to plague and cholera due to the presence of a "H-like" antigen on the plague bacillus and *Vibrio cholerae* (Garratty, 2000).

Numerous studies did research work on the relationship between ABO blood groups and circulatory diseases. Several studies reported that "non-O blood groups are associated with a higher risk of vascular diseases", especially coronary artery disease and venous thromboembolism (Amirzadegan *et al.*, 2006; Ohira *et al.*, 2007; Wu *et al.*, 2007). Another study also supported this and showed that blood group A has a higher susceptibility to coronary artery disease (Z. Chen *et al.*, 2016). In the context of coronary atherosclerosis, the ABO blood types have also been linked to myocardial infarction (Reilly *et al.*, 2011). The elevated level of factor VIII and the von Willebrand factor (vWF) have been linked to an increased risk factor for venous thrombosis and arterial thrombosis in patients with coronary heart disease (Kamphuisen *et al.*, 2001). Non-O blood groups have been proven to have a greater level of vWF and factor VIII, making them more vulnerable to vascular diseases (Anstee, 2010).

2.5 Association of ABO/Rhesus Blood Group and Asthma

Asthma happens when the airways are inflamed, triggered by environmental stimuli such as allergens, cold air, and smoking in individuals who are genetically predisposed. Symptoms of asthma include cough, wheezing, shortness of breath, and chest tightness. These symptoms occur due to bronchoconstriction, thickening of airway wall, and increased production of mucus.

Asthma is a chronic disease that affects patients of various ages and ethnic groups globally. Asthma is estimated to affect 5-10% of the population, or 23.4 million people (Tarlo *et al.*, 2008). According to the "National Institute of Allergy and Infectious Diseases of the United States", asthma has affected more than 18 million adults and 7 million children ("Asthma Facts," 2013). A previous study showed that

the prevalence of AR in China varied between 0.7-3.8%, in Korea varied between 3.6-5.8%, in Iran varied between 1.4-6.1%, and other South and Southeast Asian varied between 2.4-3.9% for the adult population (Song *et al.*, 2014). 4.5% of the adult population are affected with asthma based on the "National Health and Morbidity Survey 2006" in Malaysia (Ministry of Health, 2015).

Asthma can be caused by many factors, commonly caused by genetic predisposition and environmental allergens. According to a prior study, children's chances of having asthma are much increased if both parents have asthma (Burney *et al.*, 1997). Another population-based cross-sectional investigation discovered the link between asthma and the number of allergic family members (Dold *et al.*, 1992). Environmental allergens that contribute to asthma include house dust mites, animal dander, cockroach, fungi, and so on (Morris, 2016). There are a few studies that showed that house dust mites rank the highest in causing an allergic reaction in Malaysia (Liam *et al.*, 2002; Lim *et al.*, 2015; Sinniah and Thakachy, 2014). Other risk factors also include obesity, gastro-esophageal reflux disease, and rhinorrhea (Ministry of Health, 2015). Asthma can also be triggered by cold weather and the presence of irritants such as smoke, haze, strong perfumes, and exhaust fumes.

Asthma is caused by a combination of airflow restriction, bronchial hyperresponsiveness, and inflammation of the airway (Bethesda, 2007). Asthma has a complicated pathophysiology that involves various inflammatory cells and mediators, resulting in acute and chronic inflammation of the airways (Barnes and Drazen, 2009). According to one research, the establishment of a Th-2 lymphocyte-predominant immune response is influenced by genetic and environmental variables (Maddox and Schwartz, 2002). Interleukins 4, 5, and 13 are Th2-type cytokines that increase the production of mast cells, immunoglobulin E, basophils, and eosinophils (Berger,