STUDY ON NASAL NITRIC OXIDE MEASUREMENT IN ALLERGIC RHINITIS PATIENT WITH ASTHMA.

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STUDY ON NASAL NITRIC OXIDE MEASUREMENT IN ALLERGIC RHINITIS PATIENT WITH ASTHMA.

by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

n	Number of participants
ppb	Parts per billion
p-value	Probability value
SD	Standard Deviation
US$	United States Dollar
ml/s	Millilitres per second

LIST OF ABBREVIATIONS

AR	Allergic Rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
BHR	Bronchial Hyperresponsiveness
COAD	Chronic Obstructive Airway Disease
COPD	Chronic Obstructive Pulmonary Disease
CRS	Chronic Rhinosinusitis
eNOS	Endothelial Nitric Oxide Synthase
ELISA	Enzyme-Linked Immunosorbent Assay
FeNO	Fractional Exhaled Nitric Oxide
GINA	Global Initiative for Asthma
HDMs	House Dust Mites
HPUSM	Hospital Pakar Universiti Sains Malaysia
ICS	Inhaled Corticosteroids
IgE	Immunoglobin E
iNOS	Inducible Nitric Oxide Synthase
IPF	Idiopathic Pulmonary Fibrosis
ISAC	Immune-Solid Phase Allergen Chip
LABAs	Long-Acting β2-Agonists
NO	Nitric Oxide
nNO	Nasal Nitric Oxide
NOS	Nitric Oxide Synthases
nNOS	Neuronal Nitric Oxide Synthase
ORL-HNS	Otorhinolaryngology Head and Neck Surgery
SACRA	State of the Impact of Allergic Rhinitis on Asthma
SCIT	Subcutaneous Immunotherapy
SLIT	Sublingual Immunotherapy
SNOT-22	Sino-Nasal Outcome Test-22
SPT	Skin Prick Test
SPSS	Statistical Package for Social Science
TB	Tuberculosis
TB-nNO	Tidal Breathing Nasal Nitric Oxide

VAS	Visual Analogue Scale

LIST OF APPENDICES

KAJIAN PENGUKURAN NITRIC OKSIDA NASAL DALAM KALANGAN PESAKIT RINITIS ALERGI YANG MENGHIDAP ASMA

ABSTRAK

Kajian ini meneroka potensi oksida nitrik hidung (nNO) sebagai penanda biologi untuk membezakan pesakit resdung (AR) yang mempunyai dan tidak mempunyai asma. Kajian dijalankan di Klinik Otorinolaringologi Kepala dan Leher (ORL-HNS), Hospital Pakar Universiti Sains Malaysia, melibatkan 154 peserta: kumpulan asma (n=77) dan tanpa asma (n=77). Objektif utama adalah untuk membandingkan tahap nNO antara kedua-dua kumpulan dan menilai korelasi antara tahap nNO dengan tahap keterukan gejala yang diukur menggunakan Skala Visual Analogue (VAS). Pesakit AR dengan asma menunjukkan tahap nNO yang jauh lebih rendah (median 74.0 ppb, julat antara kuartil 49.00–151.00 ppb) berbanding pesakit AR tanpa asma (median 440.0 ppb, julat antara kuartil 238.00–687.00 ppb), dengan nilai p <0.001. Penemuan ini mencadangkan bahawa asma memberi kesan ketara terhadap tahap nNO, berkemungkinan disebabkan oleh halangan pada ostium sinus yang lebih lazim dalam pesakit yang mengalami kedua-dua keadaan. Walaupun korelasi keseluruhan antara tahap nNO dan skor VAS tidak signifikan, korelasi positif yang signifikan ditemui dalam kumpulan AR tanpa asma, menunjukkan bahawa tahap nNO yang lebih tinggi dikaitkan dengan gejala yang lebih teruk. Ini menonjolkan potensi nNO sebagai penanda untuk penilaian gejala dalam pesakit AR tanpa asma. Sebaliknya, kekurangan korelasi signifikan dalam pesakit AR dengan asma menunjukkan hubungan yang lebih kompleks antara tahap nNO dan keterukan gejala, mungkin disebabkan oleh interaksi antara keradangan saluran pernafasan atas dan bawah. Analisis demografi menunjukkan bahawa pesakit asma secara amnya lebih

berumur (purata umur 39.7 tahun) berbanding pesakit tanpa asma (purata umur 33.3 tahun), dengan nilai p yang signifikan 0.006. Jantina dan etnik tidak menunjukkan perbezaan ketara antara kumpulan. Skor VAS menunjukkan bahawa pesakit asma mengalami gejala yang lebih teruk secara keseluruhan (purata skor VAS 10.3) berbanding pesakit tanpa asma (purata skor VAS 7.9), dengan nilai p yang signifikan 0.007. Kesimpulannya, tahap nNO adalah jauh lebih rendah dalam pesakit AR yang mempunyai asma, mencadangkan kaitan antara tahap nNO yang rendah dan kehadiran asma, berkemungkinan disebabkan oleh halangan sinus. Walaupun tahap nNO tidak menunjukkan korelasi konsisten dengan keterukan gejala secara keseluruhan, pesakit AR tanpa asma menunjukkan hubungan positif di mana tahap nNO yang lebih tinggi dikaitkan dengan gejala yang lebih teruk, manakala pesakit berasma menunjukkan corak yang lebih kompleks akibat keradangan saluran pernafasan gabungan. Penemuan ini menekankan kepentingan mempertimbangkan teknik pengukuran dan faktor anatomi yang berbeza dalam mentafsir tahap nNO dalam pesakit AR. Kajian lanjut perlu memberi tumpuan kepada saiz sampel yang lebih besar, reka bentuk longitudinal, dan penerokaan mekanisme asas yang menyumbang kepada perbezaan tahap nNO yang diperhatikan. Kajian ini menyumbang kepada pemahaman tentang nNO sebagai penanda biologi dalam AR dan peranannya dalam membezakan pesakit asma dan bukan asma, sekali gus meningkatkan pengurusan dan hasil pesakit.

STUDY ON NASAL NITRIC OXIDE MEASUREMENT IN ALLERGIC RHINITIS PATIENT WITH ASTHMA.

ABSTRACT

This study explored the potential of nasal nitric oxide (nNO) as a biomarker to distinguish allergic rhinitis (AR) patients with and without asthma. The study was conducted at the Otorhinolaryngology Head and Neck (ORL-HNS) Clinic, Specialist Hospital Universiti Sains Malaysia, which included 154 participants: asthmatic (n=77) and non-asthmatic groups (n=77). The primary objectives were to compare nNO levels between these groups and to examine the correlation between nNO levels and symptom severity, measured by the Visual Analogue Scale (VAS). Asthmatic AR patients had significantly lower nNO levels (median 74.0 ppb) with interquartile range of 49.00-151.00 ppb, compared to non-asthmatic AR patients (median 440.0 ppb) with interquartile range of 238.00-687.00 ppb, with a p-value of <0.001. This finding suggests that asthma significantly impacted nNO levels, potentially due to factors such as sinus ostium obstruction, which is more common in patients with both conditions. While the overall correlation between nNO levels and VAS scores was not significant, a significant positive correlation was detected in the non-asthmatic AR subgroup, indicating that higher nNO levels were associated with greater symptom severity. This highlighted the potential of nNO as a biomarker for symptom assessment in non-asthmatic AR patients. In this study, nNO is a potential biomarker for distinguishing between AR patients with and without asthma. It serves as a biomarker for airway inflammation and symptom assessment, particularly in non-asthmatic AR patients, where higher nNO levels are associated with greater symptom severity. In contrast, the lack of significant correlation in asthmatic AR patients suggested a more complex

relationship between nNO levels and symptom severity, possibly due to the interplay of lower and upper airway inflammation. Demographic analysis showed that asthmatic patients were generally older (mean age 39.7 years) compared to non-asthmatic patients (mean age 33.3 years), with a significant p-value of 0.006. Gender and ethnicity did not show significant differences between the groups. VAS scores indicated that asthmatic patients experienced higher overall symptom severity (mean VAS score 10.3) compared to non-asthmatic patients (mean VAS score 7.9), with a significant p-value of 0.007. From this study, it can be concluded that nNO levels are significantly lower in AR patients with asthma, suggesting a potential association between lower nNO and asthma presence, possibly due to sinus blockage. While nNO levels didn't consistently correlate with symptom severity overall, non-asthmatic AR patients showed a positive relationship such that higher nNO levels matched worse symptoms, whereas asthmatic patients showed a more complex pattern due to combined airway inflammation. The findings underscored the importance of considering different measurement techniques and anatomical factors when interpreting nNO levels in AR patients. Future research should focus on larger sample sizes, longitudinal studies, and exploring the underlying mechanisms contributing to the observed differences in nNO levels. This study contributed to the understanding of nNO as a biomarker in AR and its potential role in differentiating between asthmatic and non-asthmatic patients, ultimately improving patient management and outcomes.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Allergic rhinitis (AR) is a chronic disease caused inflammation of the nasal mucosal membranes, characterized by sneezing, rhinorrhea, nasal itching, and nasal congestion without a fever (Hansel, 1929). The prevalence of AR is the highest among allergy diseases. It is estimated that 500 million people globally had AR, and its occurrence had considerably increased in the last ten years, in accordance with epidemiological data (Schuler IV & Montejo, 2019). Several studies in Asian countries showed the prevalence rates of AR in general population was 39.4%, which was an extremely high compared to other developed countries (Okubo et al., 2011).

Asthma is a common inflammatory disorder of the lower airways, characterized by fluctuating and recurrent symptoms such as wheezing, shortness of breath, chest tightness, and coughing, which often worsen at night or with exposure to triggers like allergens, cold air, or exercise, reversible airway obstruction, and bronchial hyperresponsiveness (BHR). This disease affects 8.4% of children and 8.1% of adults in the United States (Song et al., 2014). According to several studies, over 300 million people globally have asthma, with prevalence rates varying between countries (Bousquet et al., 2009). The National Health and Morbidity Survey of 2011 reported asthma prevalence in Malaysia as 6.3%. On top of that, a cross-sectional national surveillance study in Japan called SACRA (State of the Impact of Allergic Rhinitis on Asthma Control) found that 67.3% of asthmatics had rhinitis, and that individuals with rhinitis experienced significantly worse asthma control (Ohta et al., 2011).

The prevalence of asthma is increasing among AR patients, and there has been much discussion on the close relationship between asthma and AR. According to, asthma and AR are interconnected through the unified airway theory, which considers the upper and lower airways as a single functioning unit. A study by Samitas et al. (2018) further supported this by identifying shared immune-pathological pathways between the two conditions. Furthermore, Licari et al. (2017) also emphasized that AR and asthma exert a bidirectional influence on each other, meaning that changes in one can affect the other regardless of the relationship between the nose and the lung in allergic airways disease. Notably, treating AR has been shown to alleviate asthma symptoms, as highlighted by GINA (2016), reinforcing the importance of integrated management in allergic airway diseases.

The gas nitric oxide (NO) is a free radical gas derived from L-arginine by three enzymes known as nitric oxide synthases (NOS): inducible (iNOS), endothelial (eNOS), and neuronal (nNOS). The NO is released in the human respiratory tract. Inside the human body, the nose and paranasal sinuses serve as a physiological reservoir for endogenous NO synthesis, which is essential for both defense mechanisms and the regulation of inflammatory diseases (Lundberg & Weitzberg, 1999). NO production by paranasal sinus ciliary epithelial cells is predicted to happen constantly without the presence of acute triggers. In order to optimize mucociliary clearance and control blood flow across the airways, NO is needed for boosting mucociliary movement. Additionally, it functions as a bronchodilator nerve's neurotransmitter in the lower airways, preventing bronchoconstriction through cholinergic neural mechanisms (Hewitt & Lloyd, 2021).

The amount of NO present in exhaled air from the lungs and nasal airways could be tested using a non-invasive equipment. Nasal NO (nNO) and fractional exhaled NO (FeNO), both quantify NO from the nasal and lungs correspondingly, are two separate sampling procedures that could be used to noninvasively quantify the majority of NO that is detected in exhaled air from the lungs and nasal airway (Luo et al., 2021). FeNO has been found to be a valuable biomarker for detecting asthma and identifying AR (Heffler et al., 2020). Research has demonstrated that FeNO levels are much higher in AR and asthma when compared to the healthy control group (Kalpaklioglu & Kalkan, 2012). On the other hand, nNO is used to measure the NO level in the upper airway primarily nose and paranasal sinuses. Studies show that nNO in AR patients are significantly higher than the healthy group (Nesic et al., 2016). However, the application of nNO measurement is very limited.

Despite its proven effectiveness in evaluating AR, nNO is still not widely used to assess asthma. Research by Nesic in 2016 demonstrates that patients with AR exhibit significantly higher nNO levels (696.5±136.0 ppb) compared to controls (460.5±133.3 ppb). Currently, nNO levels in AR remain at an experimental stage and lacked an acknowledged reference range. nNO may serve as a clinical biomarker for upper airway inflammation. Therefore, the purpose of this study is to measure AR patients in asthma and non-asthma through nNO measurement.

1.2 Problem Statement and Study Rationale

Several published studies have demonstrated nNO measurements in AR and CRS; however, investigations of nNO measurements in asthmatic and non-asthmatic AR patients in Malaysia are very limited.

The measurement of nNO not only aiding in diagnosing AR but also helps in monitoring treatment responses and understanding the pathophysiological mechanisms underlying the relationship between AR and asthma. Elevated nNO levels in AR patients may indicate a higher risk of developing asthma, highlighting the importance of monitoring these patients closely (Luo et al., 2021).

In summary, the association between AR and asthma can be observed through the measurement of nNO, which serves as a valuable biomarker for airway inflammation. Although nNO is a promising biomarker for detecting airway inflammation in individuals with AR and asthma, its clinical application remains limited due to the absence of standardized reference ranges and clear diagnostic guidelines. This gap hinders consistent use in patient assessment and integrated care. To overcome these challenges, further research is needed to establish validated nNO thresholds, strengthen clinical evidence supporting its utility, and develop comprehensive management protocols that incorporate nNO measurements. By doing so, healthcare providers can improve diagnostic accuracy and deliver more effective, personalized treatment for patients suffering from both AR and asthma. This relationship underscores the need for integrated management strategies for individuals suffering from both conditions.

1.3 Justification and Benefits

In the respiratory system, nitric oxide produced by epithelial cells lining the airways exhibits anti-inflammatory and antibacterial activity. Measuring nNO entails determining the concentration of this gas produced in the nasal passages. nNO levels reflect inflammation of the nasal passages, which is frequently present in AR or asthma.

nNO measurement is an innovative and effective evaluation method that does not require invasive diagnostic procedures. Unlike blood tests, bronchoscopy, or biopsy, nNO measurement involves few painless steps and can be completed quickly. Patients blow air into the nasal prob through the nasal passages to measure NO concentration, providing immediate results. This non-invasive technique is suitable for children and patients with severe symptoms who may be distressed by invasive diagnostic methods. Consequently, nNO measurement increases patient compliance and facilitates regular monitoring of chronic diseases such as AR and asthma, thereby improving diagnostic experience and reducing patient distress associated with diagnostic processes, ultimately enhancing quality of life and outcomes (Dahlan et al., 2024).

nNO has proven useful for assessing the degree of inflammation and the clinical response to therapy since it provided a prognostic value. This is important in designing tailoring intervention strategies to ensure patients receive individualized treatment plans, which improve disease management and clinical (Marcuccio et al., 2023).

Monitoring nNO levels enables clinicians to detect early changes in airway inflammation and initiate action before the situation deteriorated. The potential progression from mild allergic rhinitis to asthma is avoided and the burden of the two diseases in patients and healthcare provision is reduced. Regular follow-up also helps in assessing the sustained benefit of the treatments given and changes that would improve patient care (Ren et al., 2019).

The use nNO to monitor for early identification, treatment could be started on time, reducing the intensity of symptoms and improving patient outcomes.

Additionally, by identifying individuals at risk of getting asthma and putting preventative measures in place, this proactive approach could help lower the occurrence of the illness.

1.4 Research Questions

1. What is the mean value of nasal Nitric Oxide patients in AR with asthma and without asthma?
2. Does nasal nitric oxide useful to assess asthma in AR patient?

1.5 Study Objectives and Hypothesis

1.5.1 Main Objective

To compare the mean value in AR patients with asthma and without asthma using the nNO levels.

1.5.2 Specific Objectives

1. To compare the nNO levels between AR patients with and without asthma.
2. To determine the correlation and association between nNO and VAS score of AR patients with asthma.
3. To determine the correlation and association between nNO and VAS score of AR patients without asthma.
4. To compare the correlation between nNO and VAS score of AR patients with and without asthma.

1.5.3 Research Hypothesis

nNO levels are higher in AR patients without asthma compared to those with asthma. This hypothesis aligns with the study's main objective to evaluate and compare nNO levels between AR patients with and without asthma. Additionally,

it addresses the specific aim of investigating the correlation between nNO levels and symptom severity, measured by the Visual Analogue Scale (VAS), in both groups. The alternative hypothesis put forward that significant differences exist in nNO levels between the two groups and that there is a meaningful correlation between nNO levels and symptom severity in at least one group, highlighting the potential of nNO as a biomarker for differentiating disease status and assessing symptom burden in AR patients with or without asthma.

CHAPTER 2

LITERATURE REVIEW

2.1 Allergic Rhinitis

The clinical definition of AR is an immunoglobin E (IgE)-mediated inflammation of the nasal membranes following allergen contact, and characterized by symptoms such as sneezing, rhinorrhea, nasal obstruction and itching (Bousquet et al., 2009). AR significantly affects quality of life by disrupting sleep, daily activities, work performance, and increasing healthcare utilization. Importantly, AR frequently coexists with asthma, a chronic inflammatory disease of the lower airways. Research indicates a strong epidemiological and pathophysiological link between AR and asthma, often described under the "unified airway" concept, where inflammation in the upper and lower airways was interconnected Licari et al., 2017; Samitas et al. (2018).

Patients with both AR and asthma tend to experience more severe symptoms, reduced asthma control, and greater disease burden compared to those with AR without asthma. Even though AR was not a life-threatening illness with significant morbidity and mortality, patients frequently sought medical attention for it from doctors (Brożek et al., 2017). This had a severe detrimental effect on the economy, such that in the US, AR-related expenses were projected to be as high as US$20.9 billion (Pawankar, 2014). In societies with developing economies, such as those experiencing productivity losses brought on by AR, the financial impact is greater and leads to yearly per-patient expenses ranging from US$184 to US$1,189. Thus, it was important to recognize that AR could have a significant financial burden (Kushnir & Kaliner, 2015).

2.1.1 Epidemiology and Pathophysiology

According to Brożek et al. (2017), allergic rhinitis (AR) was a common persistent illness occurring in a large percentage of people worldwide, having rates of

prevalence that varied between 10% to 40%. Within the past 10 years, studies on epidemiology demonstrated that AR was more prevalent in city and industrialized environments than in the countryside, most likely as a result of increased contact with allergens and pollutants (deShazo, 2020).

There was a complicated interaction between hereditary and biological factors in the pathophysiology of AR. NO production within the respiratory tract contributed to the allergic reaction linked to AR. Nitric oxide synthase (NOS), an enzyme that increased in reaction to allergen exposure, produces NO (Bjermer et al., 2019). Radical molecules of nitrogen were produced as the outcome, which stimulated the chain of inflammatory reactions. Histamine and other cytokines circulated from basophils as well as mast cells during the early-phase reaction in allergic rhinitis (AR), resulting in symptoms such sneezing, itching, and rhinorrhea (deShazo, 2020). Eosinophils, T cells, and other infectious cell types were attracted towards the nasal mucosa as a component of the late-phase reaction, which caused remodelling of tissues and ongoing inflammation (Bjermer et al., 2019).

The function of NO in AR as an indicator and transmitter of disease was emphasized by current studies. Patients with AR were reported to have a higher concentration of exhaled NO, which was correlated with the severity of the disease and the number of symptoms (Bjermer et al., 2019). As a result, researchers were looking at NO as a possible indicator to track the progression of this condition and its reaction to therapy. Furthermore, therapies aimed at the NO process, including NOS inhibitors, were researched as possible AR treatments (deShazo, 2020). The Allergic Rhinitis and Its Impact on Asthma (ARIA) recommendations highlighted the significance of treating AR with an extensive plan that incorporated immunotherapy, pharmacology, and allergy management (deShazo, 2020). The basic treatment continued to be

pharmacological interventions such as intranasal corticosteroids and antihistamines, but new therapies which concentrated on certain processes, such as the NO process, were being researched (Bjermer et al., 2019).

2.1.2 Diagnosis of AR

The primary methods used to identify AR symptoms were the patient's medical history, allergies, and a physician's endoscopic nasal examination (Bousquet et al., 2009). If a patient exhibited two or more of the evaluated symptoms, including watery anterior rhinorrhea, paroxysmal sneezing, nasal blockage, nasal pruritus, and conjunctivitis lasting longer than an hour on most days, they might be diagnosed with AR (Bousquet et al., 2009). While non-nasal symptoms like chronic cough, headaches, eye, ear, and throat pain, as well as impairment in mental function (cognitive), were common in AR patients, the evaluation of these symptoms was not part of the diagnostic process (Spector et al., 2003). The severity of AR could be further supported by evaluating these non-nasal symptoms (Wallace et al., 2008).

The presence of nasal secretions, erythematous or pale nasal mucosa, nasal septum deviation, inferior turbinate hypertrophy, narrow internal nasal valve, and nasal polyps were the characteristics that were checked during a nasal endoscopic examination (Ziade et al., 2016). The purpose of examining these indicators was to rule out other sinus infections (Maru & Gupta, 2016). The diagnosis of AR could be further supported by nasal symptoms such as the transverse crease of the nose (Ramot et al., 2010) and the dark circle beneath the eyes, which was also referred to as allergy shiners (Chen et al., 2009).

A range of diagnostic methods is currently utilized to detect the allergens contributing to sensitization in patients with AR. The enzyme-linked immunosorbent

assay, or ELISA, was one test that determined a person's susceptibility to aeroallergens.(Vidal et al., 2005). Nevertheless, the specific aeroallergens that produced sensitization in individuals with AR could not be identified by this technique. To validate the underlying allergic sensitization that caused AR, the clinical and laboratory gold standard allergy tests were skin prick test (SPT) (Nevis et al., 2016) and allergen-specific IgE immunoassay utilizing blood samples (Posa et al., 2017), respectively. The dietary and aeroallergens that AR patients were sensitized to, could be identified using both testing. Research indicated that there were strong associations between *in vitro* specific IgE immunoassay and *in vivo* SPT (Cho et al., 2014; Wongpiyabovorn et al., 2018). Thus, to identify the allergens causing the problem, a diagnostic test such as SPT or specific IgE immunoassay could be employed. Both assessments, though, offered benefits and drawbacks of their own. Allergists frequently utilized SPT as their main method of allergy detection because of its great sensitivity, speed, and affordability (Cox et al., 2008; Wongpiyabovorn et al., 2018). This test was easy to do because no equipment was needed. As a machine-required test, specific IgE immunoassay often emerged as the other method for identifying the allergens causing the problem. Compared to SPT, it was more costly and produced benefits more slowly. Nevertheless, drugs and skin conditions had no effect on the test's results. Notably, compared to an *in vivo* SPT, there was no chance of a severe allergic reaction for allergy sufferers when using an *in vit*ro immunoassay (Wongpiyabovorn et al., 2018).

An intradermal skin test was a further diagnostic procedure that could identify the allergens to which AR patients were sensitized. According to research, following an intradermal skin test, AR patients with negative SPT findings for HDMs seemed to be positive for HDMs (Erel et al., 2017). Thus, if *in vitr*o diagnostic assays were not accessible, intradermal skin testing might be used as a substitute *in vivo* diagnostic test

to identify the allergens causing sensitization in AR patients. The immune-solid phase allergen chip (ISAC) (Griffiths et al., 2017; Van Hage et al., 2017) allowed for the simultaneous detection of many allergen components of particular IgE antibodies. The allergen-specific IgE antibody profile of individuals with AR might be obtained using this multiplex *in vitro* diagnostic technique. Study indicated that ISAC was capable of being used as an aid to diagnosis in polysensitized AR patients to determine whether allergen elements showed cross-reactivity with HDMs (Yadzir et al., 2014).

The seriousness of AR was divided by two classifications based on the ARIA suggestion: mild and moderate-severe (Bousquet et al., 2009). Four criteria were evaluated: sensations which made one uncomfortable, deterioration of routine tasks, degradation in work or academic achievement, and inconsistent bedtime. The ARIA guidelines further classified the symptoms of AR into intermittent and persistent groups depending on the length of time of signs for people with AR. Intermittent symptoms were those that occurred for less than four days per week or less than four weeks in a series, while persistent symptoms were those that occurred for more than four days per week and more than four weeks in a row. Figure 2.1 showed the classification of symptoms together with a summary of AR seriousness.

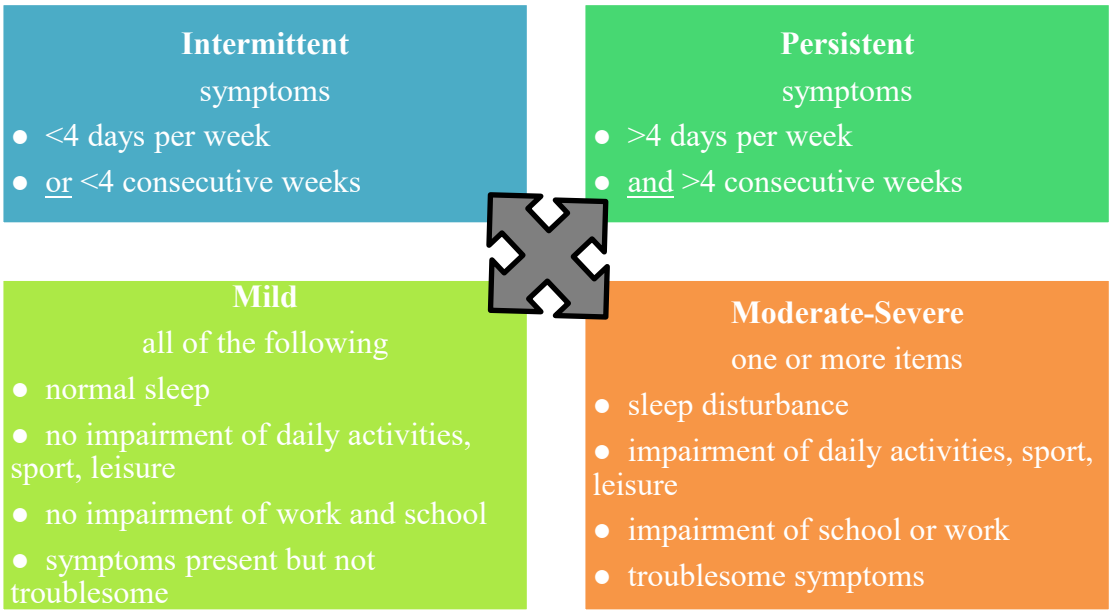

Figure 2.1 Summary of Allergic Rhinitis severity and classification of symptoms based on ARIA guidelines (Bousquet et al., 2009).

2.1.3 Treatment of AR

Pharmacotherapy (May & Dolen, 2017), immunotherapy (Rajakulasingam et al., 2018), and avoidance of allergens could all be used to treat AR. Since traditional allergy avoidance did not yield beneficial outcomes in the management of AR (Solelhac & Charpin, 2014), medication was thought to be the cornerstone in controlling the majority of AR patients (Ridolo et al., 2014). It was demonstrated that avoiding allergens by using mite-proof sleeping coverings lowered exposure to HDMs, but there was no discernible improvement in the clinical symptoms of AR patients (Terreehorst et al., 2003). This finding suggested that the allergens breathed by AR patients were not reflected in the measured HDMs on the mattress surface ("Bed Covers and Dust Mites," 2003).

The standard therapy for individuals with AR consisted of intranasal corticosteroids (Trangsrud et al., 2002) and antihistamines (Hernandez-Trujillo, 2009). As an outcome of the enhanced incidence of osteoporosis (Aasbjerg et al., 2013) and

diabetes, addressing AR alongside systemic steroids was not suggested, even if it was an underlying condition (Blanca et al., 2015; Campo et al., 2013). It was shown that intranasal corticosteroids reduced nasal mucosa inflammation through their anti-inflammatory actions, which in turn relieved the signs of AR (Bousquet et al., 2009). AR was treated with first- and second-generation antihistamine drugs (Hoyte & Katial, 2011). It might not have been advisable to take first-generation antihistamines since they penetrated the brain and caused drowsiness, fatigue, and sedative that could impair cognition and driving ability (Church & Church, 2011). In the present day, the most often used second-generation antihistamines for the treatment of AR were loratadine, desloratadine, rupatadine, bilastine, levocetirizine, and fexofenadine (Recto et al., 2017). It was shown that these drugs improved the standards lifestyle for the majority of AR patients while rapidly alleviating the disease's visual and nasal effects (Demoly et al., 2014).

Allergen-specific immunotherapy was a targeted treatment for IgE-mediated allergic diseases, such AR, allergic asthma, atopic dermatitis and insect venom hypersensitivity. It works by gradually desensitizing the immune system to specific allergens, thereby reducing symptoms and potentially modifying the course of the disease (Oppenheimer & Marshall, 2018). Sublingual immunotherapy (SLIT) and subcutaneous immunotherapy (SCIT) were used in the treatment of AR patients (Durham & Penagos, 2016). As an alternative treatment option for managing their disease, patients with AR may receive allergen-specific and displaying unmanageable signs might have decided to consider allergen-specific immunotherapy (Bousquet et al., 2009). Because seasonal AR patients showed a considerable reduction in their symptom ratings, SCIT proved to be an extremely successful treatment for seasonal AR (Calderon et al., 2007). Children with HDMs and adults with persistent AR also showed a good

response to SCIT, which was safe to use (Eifan & Durham, 2016). Like SCIT, SLIT was safe and successful in treating seasonal AR; however, it was less compelling in treating HDMs in patients with persistent AR, particularly in youngsters (Canonica et al., 2014). When compared to SCIT, SLIT was a more appealing and beneficial choice for allergen-specific immunotherapy for children and their caregivers (Calderon et al., 2013) . This was so that the kids could receive SLIT without requiring unnecessary and frequent appointments to the clinic (Canonica et al., 2014). Moreover, the youngsters were not often prescribed SCIT, mostly due to the worry that they would not be able to express the symptoms of systemic responses throughout the immunotherapy programme (Canonica et al., 2014).

2.1.4 Impact on Quality of Life

The quality of life was greatly diminished by allergic rhinitis (AR), which affected individuals mentally as well as physically. Acute pain and exhaustion might have resulted from the enduring signs of AR, which included rhinorrhea, sneezing, nasal congestion, and itching (Bernstein et al., 2024).These signs frequently caused disruptions to regular activities, such as social contacts, work, and education, which lowered performance and productivity. Due to nasal congestion and postnasal drip, which could disrupt good sleep and cause fatigue during the day and dementia, sleep disorders were a typical side effect of AR (Ozdoganoglu et al., 2012). The following might have made individuals become even more irritable and frustrated, which lowered their quality of life in general. Furthermore, it might have been extremely taxing to constantly control symptoms and stay away from triggers, which raised tension and worry. The negative perceptions attached to nose blowing, constant sneezing, and the outward manifestations of nasal irritation, which could cause social distancing and humiliation, contributed to the emotional cost of AR (Bernstein et al., 2024).

Proper treatment of AR was demonstrated by research to considerably enhance such findings. Medication, such as antihistamines and intranasal corticosteroids, improved the quality of sleep and reduced signs, which could improve daily performance and general health (Ozdoganoglu et al., 2012). By decreasing exposure to allergens, immunotherapy provided an ongoing treatment that could result in long-lasting pain reduction and an enhanced quality of life (Ozdoganoglu et al., 2012). Beyond that, patient awareness and techniques for self-management enabled patients to take charge of their health and lessen the burden of their symptoms on day-to-day living. By assisting patients in assessing their signs, prescribed medications, and external factors, medical devices and smartphone applications promoted self-management and improve prevention of illness.

Additionally, research indicated a substantial correlation connecting allergic disorders including eczema and asthma and AR, and this might exacerbate the effects on quality of life. Improving well-being required an integrated strategy to patient treatment which took into account these concurrent conditions (Bernstein et al., 2024). Through an understanding of the complex effects of AR on quality of life, physicians could create all-encompassing therapy programs which improved interpersonal and mental besides physical wellness.

2.1.5 Recent Advances and Future Directions

Novel possibilities for study and therapy were made possible by recent improvements in our knowledge and approach to treating allergic rhinitis (AR), which drastically enhanced care for patients. The invention of biologic treatments that concentrated on methods associated with allergy symptoms was among several significant innovations. Some drugs, which included interleukins (e.g., dupilumab) and monoclonal antibodies targeting IgE (e.g., omalizumab) demonstrated progress in

symptom reduction and quality of life improvement for individuals having chronic AR who were resistant to standard therapy. Furthermore, the accuracy of managing AR improved with the discovery of prospective biomarkers for detection and reaction to therapy. Biomarkers that could aid in personalizing therapy include periostin, nasal nitric oxide levels, and eosinophil cationic protein. These indicators provided information regarding the mechanisms that caused inflammation.

Understanding the biological and behavioural elements which triggered the onset and course of the illness was one of the next paths for AR research. Acquiring knowledge about these variables could help discover new areas for treatment and create more potent remedies. Furthermore, investigations into the involvement of the microbiome in AR were still underway. Findings from these investigations indicated that changes to the gut and nasal microbiota may affect immunological response and allergen sensitivity.

All things considered, the identification, treatment, and management of allergic rhinitis had a lot of future potential, thanks to the recent developments in biologic medicines and our growing understanding of genetics. These advancements highlighted how crucial it was to consider AR study and treatment via a comprehensive perspective, including knowledge from immune systems, inheritance, and microbiology to improve the health of patients.

2.2 Asthma

Worldwide, many people suffered with asthma, an ongoing inflammatory condition affecting the respiratory system (Bousquet et al., 2009). Wheezing, dyspnoea, chest pain, and coughing were its hallmarks; these attacks usually occurred at night or in the early morning (GINA, 2024). Variable airflow restriction, which could be treated

or resolved spontaneously, was linked to these symptoms. In recent years, contributing factors of asthma were finally discovered, and treatment options greatly advanced.

Despite this, over half of patients persisted with poor asthma management, which raised the death rate and required consistent medical appointments. Despite these advancements, a sizable portion of patients still received insufficient care for their asthma, which increased their risk of dying and necessitates repeated hospital admissions (Abbafati et al., 2020; Tomisa et al., 2024). According to O'Byrne & Pavord (2020), the Global Initiative for Asthma (GINA), asthma was a diverse condition that was often characterized by persistent irritation of the airways. Due to the many different aspects of asthma, which included immunology, ecological, as well as biological factors, this variation resulted (Kapri et al., 2023). Gaining a knowledge of these elements was essential to creating therapeutic and preventive plans that would lower the prevalence of asthma worldwide and enhance the quality of life for sufferers (Kapri et al., 2023).

2.2.1 Epidemiology and Pathophysiology

Asthma rates differed greatly between regions and populations, with adult prevalence estimates ranging from 1% to 21% (Tomisa et al., 2024). Socioeconomic status, environmental variables, and genetics all influenced this variance (Genuneit et al., 2017). Over the past decade, research indicated that asthma prevalence was rising in low- and middle-income countries while being steady or declining in certain wealthy ones (Tomisa et al., 2024). This pointed to a complicated interplay between environmental and genetic factors in the development of asthma.

A combination of environmental and genetic variables contributed to the underlying processes of asthma, which resulted in persistent inflammation of the airways (Hill & Wood, 2009). The main component related to these inflammatory

reactions was nitric oxide (NO). Nitric oxide synthase (NOS) comprised the enzyme which produced it, and its levels rose with irritation (Decker, 2020). Increased fractional exhaled NO (FeNO) readings among asthmatic individuals were proof of an increase in airway irritation (Decker, 2020). FeNO was utilized to monitor asthma and direct therapy after extensive study (Decker, 2020). Inducible NOS (iNOS), which were present in a variety of cells, including those in the airways, were the primary source of NO synthesis (Decker, 2020). Increased mucus production, bronchoconstriction, and airway abnormalities were all made worse by excess NO (Decker, 2020).

Recent studies investigated NO's role in asthma development and its potential as a treatment target. NO not only served as an inflammation marker but also influenced airway tone and immune responses (Decker, 2020). Eosinophilic inflammation, which was prevalent in allergic asthma, was associated with elevated FeNO levels (Decker, 2020). In an effort to lower inflammation and enhance patient outcomes, this gave rise to novel NO-focused therapies such NOS inhibitors and NO scavengers (Decker, 2020). The GINA recommendations emphasized the value of individualized asthma management, tailoring treatment regimens with biomarkers such as FeNO (Strange, 2022). Although bronchodilators and inhaled corticosteroids were essential for treating asthma, novel treatments that targeted certain inflammatory pathways had a lot of potential (Strange, 2022).

2.2.2 Diagnosis of Asthma

Chronic irritation of the airways and the airflow problems were symptoms of asthma that could be verified by pulmonary function tests such as spirometry. GINA stated that assessing asthma included finding common signs, including coughing, chest tightness, wheezing, and shortness of breath, as well as unmistakable signs of shifting airflow restrictions (GINA, 2020).

Additionally, GINA recommendations emphasized the application of biomarkers such as blood eosinophil counts and FeNO in the management of asthma, particularly to distinguish it among other breathing disorders (Decker, n.d.). According to the recommendations, a comprehensive patient history and physical examination should be the first stage in the diagnostic process (GINA, 2022). Lung function tests should then be performed, and a specialist referral might be necessary for additional evaluation.

2.2.3 Treatment of Asthma

Through encouraging for systematic methods, the GINA served as instrumental in revising asthma treatment recommendations (GINA, 2024). Among a large number of patients, inhaled corticosteroids (ICS) in combination with long-acting β2-agonists (LABAs) remained the cornerstone of asthma treatment (Calhoun & Chupp, 2022). But those with severe asthma frequently required extra treatments.

Natural medicines aimed at certain mechanisms implicated in asthma were currently accessible. Omalizumab, mepolizumab, reslizumab, benralizumab, and dupilumab represented some of medications that were proven effective in reducing asthma attacks and improving lung function in long-term patients (Calhoun & Chupp, 2022; Kyriakopoulos et al., n.d.). Thus, emphasizing on many aspects of the type 2 inflammatory reaction, such medications offered better targeted medication.

Furthermore, many patients found the high cost of biologic therapy to be a major obstacle that restricted their access to these crucial medications. To guarantee that all patients might take advantage of the latest developments in asthma treatment, efforts had to be made to lower expenses and increase accessibility (Kyriakopoulos et al., n.d.).

2.2.4 Impact on Quality of Life

People with asthma had a substantial impact on their quality of life in various kinds of situations. Asthma had a complicated effect on an individual's psychological, physiological, and social lives, as a number of studies showed. Wheezing, breathing difficulties, and chest tightness were some of the symptoms of asthma that could significantly restrict everyday activities, leading to increased levels of pain and exhaustion (Turner & Landon, 2015). In addition to being often associated with higher rates of other disorders such as obesity and cardiovascular diseases, poorly managed asthma exacerbates physical health problems (Stanescu et al., 2019). Many people with asthma experienced anxiety and depression, which had a major negative influence on their mental health. The chronic nature of asthma and the fear of sudden worsening made managing the condition and overall quality of life more difficult when anxiety levels rose (Hossny et al., 2017).

When it comes to interacting with others, asthma imposed significant restrictions on people's lives. Preventing exposure to factors like pollution and allergies limited interaction with others and participation in various activities, leading to a reduction in support from social networks and increased feelings of isolation (Plaza-González et al., 2022). Asthma often affected how well someone can work and be productive at their job; both being present but not fully functioning and being absent happened frequently. According to studies, people with asthma frequently struggled to keep their jobs and might encounter prejudice when working (Fletcher et al., 2020). Effective asthma treatment, which included daily evaluation, consuming medicine as instructed, and changing one's lifestyle, was essential to improving quality of life. Higher general well-being, fewer emotional problems, and higher interaction with others were all linked to better asthma control, according to studies. Doctors and nurses

could help people with asthma more effectively if they were aware of the various ways that the chronic illness impacted their quality of life.

2.2.5 Recent Advances and Future Directions

Biomarkers attracted attention for their role in predicting treatment response. Blood eosinophil counts and FeNO levels were utilized to customize treatments for asthma, advancing towards a personalized approach to managing the condition (Calhoun & Chupp, 2022). This change was essential for more precise therapy targeting based on individual patient characteristics, which could lead to better results and fewer unnecessary side effects.

In order to include these developments, the GINA continuously updated its guidelines. The 2024 GINA report emphasized the need of customized treatment plans and integrating novel medicines into existing management systems (GINA, 2024). It also emphasized the importance of continuous studies on the environmental elements causing asthma worsening and the creation of strategies to reduce these dangers (Salo et al., 2024)

Additionally, the investigation of small-molecule drugs as substitutes or additions to biologics was a captivating field of study. These medications might provide patients with more affordable and easier-to-access choices, especially in areas where biologics were scarce (Calhoun & Chupp, 2022). Robust clinical trials and real-world studies would be necessary to establish the effectiveness and safety of incorporating these new treatments into clinical practice.

2.3 Nitric Oxide (NO)

A small molecule which circulated freely, nitric oxide (NO), played a crucial role in a variety of processes, both physical and mental. According to Mccartney et al.

(2013), there were three distinct forms of NOS, each with a distinct function: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS).

In order to maintain blood vessel stiffness as well as regulate blood pressure, NO was essential for the cardiovascular system (Mccartney et al., 2013). By relaxing blood vessel smooth muscle cells, it helped cure heart failure and hypertension and prevented artery hardening (Mccartney et al., 2013). Recent studies showed NO's therapeutic advantages in several domains, highlighting its importance for heart health (Mccartney et al., 2013).

As a neurotransmitter, NO played a crucial role in brain processes including developing memories and also synaptic evolution. There was a lot of thought being drawn toward comprehending and maybe treating neurodegenerative diseases like Parkinson's and Alzheimer's because of the disturbance of NO transmission that was linked to those ailments (Mccartney et al., 2013). NO played a dual role in cancer research, being able to either encourage or prevent tumor growth depending on its concentration and the tumour microenvironment (Mccartney et al., 2013). This double nature prompted studies on NO donors and inhibitors as possible cancer treatments (Mccartney et al., 2013).

Recent advancements in the area involved the creation of NO delivery systems, like nanoparticles and NO-releasing materials, with the goal of improving its therapeutic effectiveness and durability (Jia et al., 2018). Furthermore, current studies examined the practical uses of NO across different scenarios, such as its possible advantages in managing allergic rhinitis and other respiratory issues (Marcuccio et al., 2023). In general, nitric oxide continued to be a significant molecule in the field of biomedical research, as its various functions in both health and disease still drove scientific exploration and advancement in therapy.

2.4 Nasal Nitric Oxide (nNO)

nNO is a gaseous biomarker produced primarily in the upper airways, especially the paranasal sinuses, through enzymatic activity of nitric oxide synthases (NOS). Researchers repeatedly indicated that nNO levels were greater among those suffering allergic rhinitis than in normal subjects (Marcuccio et al., 2023). Inducible nitric oxide synthase (iNOS) in the nasal epithelium was an infection stimulated by association with allergic responses, which causing the level of NO to rise (Nesic et al., 2016).

Measurement of nNO has emerged as a promising non-invasive technique for assessing upper airway inflammation, particularly in AR. Several studies have demonstrated that nNO levels are elevated in AR patients compared to healthy individuals due to increased iNOS activity in inflamed nasal epithelium (Nesic et al., 2016; Marcuccio et al., 2023). However, nNO's role in evaluating asthma remains less clear, with some evidence suggesting that AR patients with concomitant asthma exhibit lower nNO levels, possibly due to sinus ostium obstruction or airway remodeling that limits NO release (Kalpaklioglu et al., 2021). With regard to research by Nesic et al. (2016), nNO measures were valuable in regular clinical environments since they could be used to differentiate between patients with allergic rhinitis and healthy persons (Nesic et al., 2016).

On top of that, it was previously proven that NO was linked to other markers of inflammation, such as serum IgE levels and eosinophil counts, which supported its role in the onset of allergic rhinitis (Marcuccio et al., 2023). The clinical uses of nNO went beyond diagnosing; it was suggested as a sign for tracking disease advancement and reaction to treatment. For instance, decreased nNO levels were seen after effective allergy prevention or medical treatment, suggesting its value as an indicator of treatment success (Marcuccio et al., 2023).