Peanut Allergic Children Residing in Olmsted County, MN: An Examination of the Prevalence in 2007, Incidence Rates from 1999-2007 and Association between Peanut-Specific IgE Level, Tolerance and Reaction Severity

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DEDICATION

This dissertation is dedicated to my sons, Joseph and James, and all the children and families suffering from food allergies. Joseph, age 3 years, introduced our families to the incredible impact of food allergies on a child and their family after his first anaphylactic reaction to peanuts at 1 year old. Shortly later, our son James was diagnosed with sesame and milk allergies following cutaneous reactions at 1 year old. Like many families worldwide, our family is faced with the task of teaching our children how to remain safe in a world in which food allergens are ubiquitous and food safety is of paramount importance. It is my hope that this research will contribute to our understanding of the clinical relevance of peanut-specific IgE levels regarding tolerance and reaction severity, which will provide physicians with further information to counsel patients and answer important questions for families. In addition, through investigation of the burden and incidence rate of peanut allergy diagnoses in Olmsted County, MN, I hope to open avenues and provide further support for research into etiology, prevention, awareness, educational, safety and policy efforts in regards to food allergies.

ABSTRACT

INTRODUCTION

Peanut allergy is a significant concern due to increased prevalence over the last few decades, potential severity of a reaction, and the large percentage of children who do not acquire tolerance. This dissertation evaluated rates of diagnosis over time and examined whether peanut-specific IgE level has clinical relevance regarding tolerance and reaction severity.

METHODS

Data on all children with a peanut allergy diagnosis between 1999-2007 in Olmsted County, MN was collected using the Rochester Epidemiology Project. The first study estimated the prevalence in 2007 and incidence rate of peanut allergy diagnoses from 1999-2007. This study further examined whether the number of peanut allergy diagnoses from 1999-2007 varied by reaction severity. The second study estimated the percentage of children that developed tolerance to peanuts and assessed the association between peanut-specific IgE level and tolerance. The third study evaluated the association between peanut-specific IgE level and reaction severity.

RESULTS

The 2007 prevalence of peanut allergy was 0.59%. There were statistically significant lower rates of peanut allergy diagnoses among females (82.0%) as compared to males and among children aged 3-17 years (99.9%) as compared to those aged 0-2 years. There was a significant 1.7-fold increase in peanut allergy diagnoses from 3.84 cases per 10,000 children in 1999-2001 to 6.53 per 10,000 children in 2005-2007. There

was not a significant difference in the number of children having had mild as compared to moderate/severe reactions over time. In this sample, 16.4% developed tolerance to peanuts. Children with peanut-specific IgE class levels 4-6 as compared to those with levels 1-3 had a significant 91.0% reduced likelihood to develop tolerance after adjustment for number of atopic conditions and a 2.15 non-significant greater odds for a moderate/severe reaction after adjustment for age at diagnosis.

CONCLUSION

The incidence rate of peanut allergy increased irrespective of severity of first reaction and most children did not acquire tolerance. The majority of those diagnosed with peanut allergy were males and 0-2 years old. Peanut-specific IgE level was not associated with initial reaction severity, but was found to be a useful prognostic tool for tolerance.

1.1 LIST OF TABLES

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2.0 INTRODUCTION

Peanut allergy is a significant clinical and public health concern due to the increase in prevalence to approximately 1% of children over the last two decades in conjunction with the small percentage of children who acquire tolerance to peanuts and the significant morbidity associated with the condition [1]. An accurate estimation of the prevalence in 2007 and incidence rate of peanut allergy diagnoses from 1999-2007 is necessary to understand the burden of disease and enables better preparation for patient care. Further, an investigation as to whether the reported increase in affected children is real or a result of an increase in diagnosis of mild cases in recent years addresses an important unanswered question and provides support for further research into etiology and prevention. Examination as to whether IgE level is associated with developing tolerance and reaction severity is necessary due to a conflicting literature base. In conjunction with other reported associations, the results of these studies will aid in providing the evidence necessary for clinicians to provide prognostic information in regards to one's likelihood to develop tolerance and expected reaction severity upon exposure to peanut.

This dissertation document begins with an introduction and discussion of the pathophysiology of type 1- hypersensitivity reactions, of which include reactions to peanuts [1]. A comprehensive discussion of peanut allergy follows including a definition of peanuts, epidemiology, risk factors and clinical management of peanut allergies. Next, details specific to the research are introduced including the data resource and setting. Thereafter, the three studies are introduced followed by in depth reporting of each investigation and an overall summary. The first study estimates the prevalence in 2007

and incidence rates from 1999-2007 of peanut allergy diagnoses in children aged less than 18 years residing in Olmsted County, MN. In addition, study one assesses whether the reported increase in peanut allergy diagnoses over time varies by reaction severity. The second analysis estimates the percentage of children that developed tolerance to peanuts in this sample and assesses the association between peanut-specific serum IgE level and peanut tolerance. The third paper examines the association between peanut-specific IgE level and reaction severity.

3.0 BACKGROUND

3.1 Type I Hypersensitivity Reaction

3.1.1 Definition

The term hypersensitivity denotes a condition in which an immune response results in an exaggerated or inappropriate reaction that is harmful to the host [1]. Hypersensitivity diseases are classified on the basis of the principle immunologic mechanism that is responsible for tissue injury and disease [1]. Food allergy is classified as one of four types of hypersensitivity conditions called type I or immediate hypersensitivity [2]. Type 1 hypersensitivity is a rapid, IgE antibody, mast cell mediated vascular and smooth muscle reaction provoked by re-exposure to a specific type of antigen referred to an allergen [1]. An initial contact is a necessary preliminary event that induces sensitization to that allergen [2]. Exposure may be by ingestion, inhalation, or direct contact.

Immediate hypersensitivity reactions are a type of allergy, or atopy, and individuals with a propensity to develop such reactions are said to be "atopic" [1].

Allergies are the most frequent disorder of the immune system and are estimated to affect about 20% of the population [1]. The prevalence of food allergy has increased significantly over the last several decades [3-5].

3.1.2 Pathophysiology

T cells belong to a group of white blood cells in the immune system known as lymphocytes. T helper cells (T_h cells) are a sub-group of lymphocytes that play a major role during immune responses by recognizing foreign antigens and activating other components of the cell mediated immune response to eliminate antigens [6]. Once activated, T_h cells divide rapidly and secrete small proteins called cytokines, which are used extensively in cellular communication and are often secreted by immune cells that have encountered a pathogen thereby activating and recruiting further immune cells to increase the system's response to the allergen [1]. When a person encounters a pathogen, dendritic cells integrate information collected by their receptors, travel to a nearby lymph node, express certain combinations of co-stimulatory molecules and subsequently deliver a signal to T helper cells that informs them of which cytokines to secrete.

The critical step for the development of IgE-mediated immune responses is the differentiation of T lymphocytes into one of two distinct phenotypes, T_h1 or T_h2, which differ based on their cytokine profile. The T_h1 cytokines, IFN-gamma (y), IL-2, and TNFB, help defend against viral and bacterial attack in the blood and tissues [5]. The T_h2 phenotype is characterized by secretion of cytokines, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, which assist in making antibodies to defend against foreign proteins [5]. By secreting their respective set of cytokines, T helper cells create an immune response that is deemed appropriate for a given situation [7].

A variety of factors determine whether T_h1 or T_h2 cells develop. The cytokine profile and balance induced by the antigen are critical. For example, IL-12 is a potent stimulus for production of INF-gamma and Th1 cellular differentiation [1, 5]. INF-gamma is produced early during viral infections and is a strong inducer of the IL-12 thus, favoring a T_h1 response. Conversely, early production of IL-4 will favor the generation of T_h2 cells. Other factors that influence the T_h1/T_h2 profile include the dose of the antigen, the cytokines secreted from cells, the genetic background of the host, and their environment [6].

Once a T cell response to an antigen has deviated towards either the Th1 or Th2 response, positive feedback sustains and enhances the response. Cytokines from the T_h1 and T_h2 cells not only provide a positive feedback loop for their respective processes but also inhibit the activities of the other. Specifically, IL-4, secreted in the T_h2 response, provides a positive feedback for further T_h2 responses while suppressing T_h1 differentiation [1]. Similarly, IL-12 in the T_h1 response stimulates production of T_h1 cells and inhibits T_h2 differentiation [1]. IL-4 induces differentiation of naïve T_h0 cells into T_h2 cells whereas IL-12 induces differentiation of naïve T_h0 cells into T_h1 cells [1]. This causes an immune response to tend towards either a T_h1 or T_h2 type.

For reasons that are not entirely clear, when atopic individuals encounter otherwise innocuous antigens such as pollen, certain foods, or animal dander, the dominant T cell response is the T_h2 profile which results in the production of cytokines, in particular IL-4, and subsequent production of IgE. IgE is an antibody that causes a cascade of events including mast cell and basophil degranulation and subsequent release of chemical mediators, which results in a type I hypersensitivity reaction [8]. Atopic

people favor a T_h2 phenotype and as such, the T_h2 response is often associated with allergic diseases. Non-atopic individuals respond to the same antigen by launching a T_h1 response which does not lead to an allergic reaction [1]. It is believed that a skewed T_h2 response manifests early in life as a result of genetic and environmental influences [5].

A type I hypersensitivity reaction against an allergen, encountered for the first time in an atopic individual, develops as a consequence of activation of T_h2 cells in response to protein antigens (allergens) [1]. Allergen specific T_h2 cells produce various cytokines, including interleukin-4 (IL-4) and interleukin-13 (IL-13). The action of these cytokines in conjunction with co-stimulatory signals from the T_h2 cells stimulate B cells specific for the foreign antigen to class switch to producing IgE antibodies. Immunoglobin E (IgE) is a class of antibody that plays an important role in allergy and especially in type 1-hypersensitivity reactions.

The antigen specific IgE antibodies secreted from the B cells circulate in the blood and bind to high affinity receptors, FcεRI, expressed on the surface of immune cells called mast cells and basophils [1, 2]. A mast cell is a resident cell of several types of tissues and contains many granules rich in mediators such as histamine [8]. Basophils, another granule containing white blood cell, are recruited from the blood by signals given off by mast cells responding to an allergen [8]. Thus, in an atopic individual, mast cells and basophils are coated with IgE antibodies specific for the antigen(s) to which the individual is allergic. The process of coating the cells with IgE is called sensitization because coating with IgE specific for an antigen makes the mast cells sensitive to activation by subsequent encounter with that antigen [1]. The IgE coated mast cells and

basophils are considered sensitized to the allergen [1]. Sensitization often occurs in the first two years of life [9].

Allergens are small proteins with a repeating structure to which many IgE antibodies can bind close together [7]. Upon a subsequent exposure to the same allergen, the allergen cross-links the IgE molecules on the mast cell and basophils surfaces dragging the FcεRI receptors together. This clustering of FcεRI receptors signals activation of the sensitized cell[7]. Activated mast cells and basophils undergo a process called degranulation, during which biochemical signals form the signal transducing chains on the FcεRI cause rapid release of mediators [7].

The release of mediators is responsible for a variety of physiologic responses including the acute vascular and smooth muscle reactions and inflammation associated with hypersensitivity reactions. The mediators include vasoactive amines, proteases, lipid mediators, and cytokines [9]. The major amine, histamine, causes the dilation of small blood vessels, increases vascular permeability, and stimulates the transient contraction of smooth muscles [9]. Proteases include enzymes such as mast cell chymase, tryptase and serine esterases, which cause tissue damage [9]. Lipid mediators and the release of additional cytokines perpetuate the T_h2 response and contribute to both the immediate and late phase responses. The lipid mediators, prostaglandin, leukotrienes and platelet activating factor, causes smooth muscle contraction, increased vascular permeability and mucus secretion[9]. Cytokines such as tumor necrosis factor (TNF) induce local inflammation. The mediators and cytokines, including IL-4 and IL-13, contribute to the influx and activation of leukocytes, which contribute to a late phase response[1, 9].

Late Phase Reaction

Hypersensitivity reactions are divided into two phases. The first is an acute response that occurs immediately after exposure to an allergen. The immediate reaction is due to the release of chemical mediators that cause a rapid increase in vascular permeability and the contraction of smooth muscle [9]. After the chemical mediators of the acute response subside, a late phase response can occur. Cytokines produced by mast cells stimulate recruitment of leukocytes, which cause the late phase reaction[9]. The principle leukocytes involved in the late reaction are eosinophils and neutrophils. The late response may involve a second phase smooth muscle contraction, sustained edema, and asthma symptoms[9]. The reaction is usually seen 2-24 hours after the original reaction. The severity of late phase symptoms is highly variable, being either more or less severe than the initial symptoms[10].

Local and Systemic Reactions

The symptoms and severity of type-1 hypersensitivity reactions differ depending on the individual, the allergen, dose and route of entry[9]. Type I hypersensitivity reactions may be local or systemic reactions. Many reactions are localized to the site of entry of allergen into the body and are referred to as local type 1 reactions. Examples of local reactions include hay fever in which the interaction of allergen with mast cell bound complementary IgE antibodies occurs in the nasal submucosa and in the conjunctival tissues causing sneezing, mucus secretion and itchy, teary eyes. Another example is asthma in which the interaction of allergen with IgE antibodies occurs in the submucosa of the airways resulting in increased mucus secretion, coughing and constriction of airways that leads to difficulty breathing and wheezing. A third example is food and drug allergies in which the interaction of allergen with mast cell bound complementary IgE

antibodies occurs in the submucosa of the intestines resulting in fluid accumulation, peristalsis (with cramps), vomiting and diarrhea. Food allergens may also cross the intestinal epithelium and diffuse through the blood to others sites in the body, where they may react with mast cell bound IgE to elicit type I reactions. The most common symptoms are hives. Some food allergens can cause skin symptoms without gastrointestinal symptoms.

Type 1 hypersensitivity reactions may also be generalized, involving multiple sites in the body [9]. Anaphylaxis is the most severe type 1 hypersensitivity reaction because it is a life threatening systemic allergic reaction in which symptoms occur in multiple organ systems and may also cause cardiovascular symptoms [9]. Such systemic type 1 reactions are triggered by the interaction of allergen with IgE antibodies on mast cells and basophils in many tissues and in the blood, with subsequent degranulation of those cells**.** The resulting generalized inflammation leads to capillary dilation with increased vascular permeability and smooth muscle contraction all over the body [8]. The increased vascular permeability often causes swelling of the lips, tongue and larynx, thus making swallowing and breathing difficult. The smooth muscle contraction in the lungs results in constriction of the airways that further impedes breathing, leading to an increase in the ratio of carbon dioxide to oxygen in the blood. This can result in loss of consciousness from an inadequate supply of oxygen to the brain. The widespread dilation of capillaries and larger blood vessels cause a fall in blood pressure. A drastic fall in blood pressure, shock, is often fatal.

Systemic type 1 reactions occur in response to allergens that are either injected directly into the blood or diffuse from the site of entry to other sites [9]. The most common

causes of anaphylaxis are food, medications and insect stings [11]. The foods most commonly associated with anaphylaxis are peanuts, tree nuts and shellfish [9, 11]. Although the mechanisms of each response are fairly well understood, why some people develop anaphylaxis while others suffer from less severe reactions such as hives is still under investigation.

3.2 Peanut Allergy

3.2.1 About Peanuts

3.2.1.1 What is a peanut?

Peanuts (botanical name Arachis Hypogaea) are members of the legume family, which also includes lentils, soybeans, peas, black eyed peas, chick peas, lima beans, kidney beans, green beans, and garbanzo beans[11]. Peanuts are vegetables and tree nuts are fruits. Unlike tree nuts, which grow on a tree, peanuts grow in the ground. Although peanuts belong to the legume family, less than 15% of peanut allergic patients react to other members of the legume family. However, despite the fact that tree nuts belong to a different botanical family, 25-35% of people who are allergic to peanuts are also allergic to tree nuts[4].

Peanuts are a nutritious food source, containing many important nutrients, such as vitamin E, niacin, folacin, calcium, phosphorous, magnesium, zinc, iron, riboflavin, thiamine and potassium [12]. Peanuts are a great source of protein and edible oil. Furthermore, peanuts are inexpensive to produce and purchase, which is important for families and in the context of world hunger.

3.2.1.2 Why are peanuts so allergenic?

3.2.1.2a Proteins

Allergic reactions result from the body's immune response to proteins. The allergenic peanut proteins are the seed storage proteins vicilin (Ara h1), congutin (Ara h2), and glycinin (Ara h3) [11]. These proteins are considered major allergens because 95% of peanut allergic patients react to Ara h1 and Ara h2 and 50% of peanut allergic patients react to Ara h3 [11]. Four additional minor proteins (Ara h5, Ara h6, Ara h7, Ara h8) have been identified, but are considered secondary as peanut allergic individuals react to them less than 50% of the time [11, 12].

3.2.1.2b Quantity

Peanut is a ubiquitous food and allergic people are faced with numerous hurdles to avoid ingestion [13]. Threshold studies have been conducted to identify the minimum dose at which allergic people may react however, the results vary due to differences in patient selection, the form of peanut used for testing, and study procedures. Nonetheless, all studies agree that exposure to minute quantities can induce severe reactions in people who are sensitive to peanuts.

It is estimated that one peanut contains approximately 200 mg of protein [14]. In many allergic people, symptoms develop after substantially less than 1 peanut is ingested and highly allergic people may react to trace amounts[15]. A study published by Hourihane and colleagues indicated that the lowest does of peanut protein that caused observable symptoms was 2 mg or 1/100th of a peanut[14]. Another study by Wensing and colleagues indicated that threshold doses for allergy symptoms ranged from 100 micrograms to 1 gram of peanut protein. Fifty percent of subjects in this study reacted to 3 mg of peanut protection or approximately 1/65 of a peanut [5, 16]. In sum, microgram amounts of protein can induce allergic symptoms which emphasize the importance of allergy management

strategies including education on proper label reading and the potential for cross contamination in food manufacturing and preparation facilities.

3.2.2 Epidemiology

3.2.2.1 Food Allergy

The prevalence of food allergy is highest in infants and toddlers [17]. An estimated 2.5% of infants suffer from cow's milk allergy and up to 8% of children less than 3 years of age are allergic to cow's milk, egg, soy, peanut, wheat, fish, shellfish, and tree nuts [17]. The prevalence of food allergy decreases with age, affecting an estimated 4% of the general population [17, 18 5, 19, 20]. The perceived prevalence of food allergies is substantially higher than that confirmed by diagnostic testing [5]. Population based studies indicate that 40%-60% of parents believe their child has a food allergy yet only 4%-8% had symptoms that were reproduced in an oral food challenge [5].

Eight types of food, milk, eggs, peanuts, tree nuts, fish, shellfish, soy and wheat, account for over 90% of allergic reactions[17]. The most common food allergens for children are milk, eggs, soy, wheat, peanut, tree nuts and shellfish [17]. More than 90% of acute systemic reactions to food in children are from eggs, milk, soy, wheat and peanuts with the majority of remaining reactions a result of tree nuts and shellfish [17, 20]. The common adult food allergens are tree nuts, peanut, fish and shellfish, which cause 85% of allergic reactions [5].

Food allergy among children is believed to be on the rise [5]. The National Health Interview Study indicates that an estimated 3 million children under the age of 18 years (3.9%) suffered from any kind of food or digestive allergy in the last 12 months [3]. The

study indicates that the prevalence of reported food allergy increased 18% among children under the age of 18 years from 1997 to 2007 [3].

3.2.2.2 Peanut Allergy

Peanut allergy is an IgE-mediated disease that affects approximately 1% of children under the age of 18 years and 0.6% of the general population [1, 19, 21-25]. The prevalence of peanut allergy has reportedly doubled over the last two decades among children [22]. Peanut allergy is the third most common food allergy in American children following milk and egg. Allergy to peanuts is the most common cause of fatal and near fatal anaphylaxis [11].

A voluntary registry for peanut allergy and a medical chart review at Duke University indicate that the median age of first exposure is approximately 12 months and the median age of first reaction was 14 months [26]. Ingestion was the most common mode of exposure (91%) followed by presumed skin contact (8%) and presumed airborne exposure (1%) [26]. First reactions occurred primarily at home (72%) with reactions occurring outside the home at day care (22%), grandparent's homes (8%) and school (3%). [26, 27]

3.2.2.3 Morbidity and Mortality

Allergy to peanuts is the most common cause of fatal and near fatal anaphylaxis [28]. As accidental ingestion of even minute quantities of peanut by an allergic individual can immediately provoke life-threatening symptoms, patients must maintain strict avoidance. However, owing to the frequent use of peanut based products in common foods in conjunction with the potential for cross-contamination in bakeries, restaurants, and processed food lines, complete avoidance is difficult and accidental ingestions are

common [29, 30]. Fatal and near fatal food anaphylactic reactions tend to occur away from home after an unintentional ingestion of a food allergen by individuals with a known food allergy to that food [31]. It is estimated that 25% of peanut allergic people have had an accidental ingestion and reaction in the past year and reactions are projected to occur once in every three years [11]. A study of schoolchildren in Montreal, Quebec, Canada reported an annual incidence rate of accidental ingestion of 14.3% [32].

Allergy to peanuts is the most common cause of fatal and near fatal related anaphylaxis [23]. In the United States, fatal food anaphylaxis occurs in 150 people each year and 90% of deaths are a result of peanut or tree nut allergies [11, 33]. In a retrospective review, peanut was the most common cause of anaphylaxis accounting for 18% of food reactions followed most closely by tree nuts (17%) [34]. The Food Allergy and Anaphylaxis Network reported that 54% of fatal food allergic reactions reported in the United States from 2001-2006 were from peanuts [28]. Risk factors for death from anaphylaxis are adolescence or young age, underlying asthma and delay in or lack of administration of epinephrine [4, 15, 20, 21].

Unfortunately, only 20% of children diagnosed with peanut allergy become tolerant and thus, the majority of people face a lifetime of avoidance [19]. Children with peanut allergy are faced with many challenges and social restrictions due to the potentially life threatening nature of their disease. Their quality of life is greatly impaired, even compared with children with other chronic diseases such as diabetes [13].

3.2.2.4 Natural History

Sensitivity to many foods, especially milk, eggs and soy tend to resolve with age, whereas peanut allergy remits less frequently [13]. Peanut allergy established under the age

of two years resolves for approximately 20% of young children by school age [21, 23, 27, 35]. In comparison, 85% of children outgrow milk and egg allergies by age five [11]. Children diagnosed as having food allergy after three years of age are less likely to lose sensitivity [21, 35]. Of those who outgrow peanut allergy, 9% may relapse and become allergic again [11].

Why food allergy persists in some people and not others is unclear [21]. Research investigating the association between many factors including the size of the skin test wheal, level of peanut-specific IgE antibody, severity of reactions, number of additional atopic diseases including other food allergies, age at diagnosis and tolerance status have been conducted indicating conflicting results. [21, 35,36] It is believed by some that strict avoidance increases one's chance of outgrowing peanut allergy however, there are limited data to support this notion [17, 18, 21, 35]. In a study by Hourihane and colleagues the resolvers tended to report successful avoidance of peanuts for longer than those people in which the allergy persisted. However, the authors note that the children not experiencing reactions may have had a less severe allergy to peanuts or their allergy may have resolved over time [23].

3.2.2.5 Risk Factors for Peanut Allergy

There has been an increase in allergic disease among children, with a doubling in the number of children with asthma, environmental allergies, eczema, and food allergies including peanut allergy [21]. There are numerous theories attempting to answer the question as to why allergic conditions are more prevalent in children today as compared to a generation ago. It is likely that both genetic and environmental influences affect development of peanut allergy. One environmental theory that receives much support is

the Hygiene Hypothesis [28, 30]. Other theories under investigation include increased consumption of peanuts by mothers and young children, early feeding when the immune system is immature, sensitization in utero or through breast milk, allergenicity of roasted forms, and use of topical ointments containing peanut.

3.2.2.5a Genetics

Allergic disease in general has been shown to have a genetic predisposition, although the development of peanut allergy has not been linked to a specific genetic profile [19]. Genetic risk factors associated with food allergy include a personal or family history of atopy including allergic rhinitis, asthma, atopic dermatitis and food allergy [20, 21]. Infants with moderate to severe atopic dermatitis have the highest occurrence of food allergy [21]. Children who develop a type-1 hypersensitivity reaction to one food are at a greater risk of developing reactions to other foods [21]. Peanut allergies are more common in male children and female adults [30, 37].

The risk of allergy tends to run in families however, affected family members may have different allergies in response to different allergens [21]. It is the risk of allergy that is inherited and not the specific allergy per se. Thus, parents with allergic diseases will have children at higher risk of developing allergic disease, including food allergy. For instance, the rate of observed food allergy in children born to families with parental asthma is approximately 4-fold higher [14]. Food allergy is more common in close relatives, such as siblings, parents and other relatives, of those who have allergic disease. Research indicates that peanut allergy is more common in siblings of those with peanut allergy than in the general population (7% vs.1.3%) [14]. A study conducted by Sicherer and colleagues indicates a strong genetic component to peanut allergy in that the

concordance rate of peanut allergy in siblings was 7% whereas 64% of identical twins were both allergic to peanuts [31]. Peanut allergy is inherited more strongly through maternal lines [14].

The immunologic basis for allergies is a defect in immune regulation in which allergen specific helper T cells are strongly polarized toward the Th2 cytokine profile, resulting in the production of allergen specific IgE antibodies [7]. The genes a person inherits can make him or her less susceptible to allergies, and exposure to environmental variables such as infections may influence whether susceptible individuals become atopic [7].

3.2.2.5b Environment: The Hygiene Hypothesis

In 1989, David Strachan studied the epidemiology of hay fever in a national sample of British children born in March 1958 and followed until age 23. The results of this longitudinal study indicated that hay fever at 11 and 23 years was inversely related to the number of children in the household at age 11 [38]. Strachan concluded that the risk of allergic disease was reduced by infections in infancy transmitted by older siblings. As a result of this study, Strachan proposed the "hygiene hypothesis" which suggests that protection against developing allergies was conferred by older siblings through greater exposure to viruses at an early age, leading to a down-regulation of the allergic response [38]. The hygiene hypothesis postulates that a lack of early childhood exposure to infectious agents, symbiotic microorganisms (i.e. gut flora), and parasites increases susceptibility to allergic diseases by modulating immune system development [38].

Strachan further speculated that declining family size, improvements in household amenities, prevention of viral infections and higher standards of personal cleanliness,

which are characteristics of the "Western" lifestyle, may be associated with the increase of atopic diseases seen over the past few decades [39, 40]. The theory is used to explain the increase in allergic diseases that has been seen since industrialization, and the higher incidence of allergic diseases in more developed countries. Since 1989, the hygiene hypothesis has been extensively investigated by researchers and has become an important theoretical framework for the study of allergic disorders.

Laboratory studies in animals and humans suggest that the immune system in early infancy is primed to recognize and fight infections [39]. In the absence of infections, the immune system targets innocuous items in the child's diet and environment resulting in abnormal reactions to harmless things such as food, pets, pollen and other environmental allergens. It is believed that exposure in the perinatal period and in the first few years of life are critical to determining whether the child develops atopic disease.

Biological Plausibility

The biological plausibility of the hygiene hypothesis relates to the maturation process of the immune system. On a cellular basis, the development of T lymphocyte responsiveness to allergens is a prerequisite for allergic reactivity. The T-helper 2 cells (T_h2 cells) determine the level of involvement of the mast cells and basophils in the host response. Through secretion of the cytokine IL-4, T_h2 cells regulate the synthesis of IgE antibodies [9]. However, if significant other cytokines, for example, interferon-y (IFN-y), which counteract the effects of IL-4 are present, the reaction is assumed to be pushed toward a T_h1 phenotype, which causes the secretion of IgG antibodies and the removal of

the allergen [9]. The T_h1 and T_h2 phenotypes are characteristic of people without and with atopy respectively.

The immune maturation approach conceptualizes atopic disorders as a failure of the immune development that should normally select for T_h1 cells during allergen driven immune response in early life. Allergic diseases are caused by inappropriate immunological responses to harmless antigens driven by a T_h2 mediated immune response. Many bacteria and viruses elicit a T_H1 mediated immune response, which down-regulates T_h2 responses. The hygiene hypothesis proposes that insufficient stimulation of the T_h1 arm of the immune system leads to an overactive T_h2 arm, which in turn leads to allergic disease [7].

It is believed that neonatal and childhood exposure to infections creates a balanced T_h1/T_h2 response [7]. Exposure to infections in early childhood may provide important signals to the child's maturing immune system. At birth, most individuals have T cells with a bias towards T_h2 type cytokine production, possibly as a result of cytokines such as IL-4 and IL-10 secreted by the placenta [7]. A T_h1 response includes the production of interferon-y, which inhibits the proliferation of T_h2 cells [1, 9]. Therefore, infections that stimulate a T_h1 response during this critical period of maturation may play an important part by inhibiting the predominantly Th2 response that is present in newborn infants. The absence of such inhibitory signals during infancy may allow the expansion and maturation of T_h2 memory cells, resulting in the persistence of a more atopic phenotype [7]. This concept of "immune deviation" is consistent with the increased incidence of allergies and the corresponding decreased incidence of infections in developed countries. The hygiene hypothesis suggests that exposure to organisms that

promote a T_h1 response early in life deviates the immune response away from the T_h2 response [8].

3.2.2.5c Maternal and Infant Diet

Other factors under consideration include the influence of maternal and infant diet, in particular, maternal ingestion during pregnancy and lactation, timing of solid food introduction, timing of introduction of highly allergenic foods (including peanuts) and breastfeeding duration. Previously, the American Academy of Pediatrics advised mothers of children at risk of developing allergy, as defined as having a first degree relative (parent or sibling with food allergy) to consider eliminating highly allergenic foods (peanuts, tree nuts, eggs, cow milk and fish) from their diet during pregnancy and lactation [41-43]. The AAP also recommended parents to delay solid food introduction until 4-6 months of age and for those children at risk for atopic disease, delaying eggs until 2 years, peanuts, tree nuts and fish until 3 years of age [41-43].

In January 2008, the AAP revised its recommendations and concluded, due to recent studies indicating mixed results, that maternal dietary restriction during pregnancy and lactation is unlikely to contribute significantly to the development of food allergy in infants [42]. Furthermore, the AAP revised their recommendation to state that there is not any convincing evidence that delaying the introduction of complementary foods beyond 4-6 months of age, including delaying introduction of highly allergenic foods, is protective against the development of atopic disease, including food allergy [41, 43]. Current feeding guidelines continue to recommend delaying introduction of solid foods until 4-6 months of age [41, 43].

Interestingly, some researchers postulate that the lack of ingestion of highly allergenic foods early in life may increase the possibility of becoming sensitized to these foods. There is the possibility that the introduction of small amounts of peanuts early in life may prevent sensitization [44]. Results from the Koala Birth Cohort Study suggest that delaying the introduction of cow's milk or other food products may not be favorable in preventing the development of atopy [43]. However, the authors recognize that there may be several alternative explanations for their findings and caution against interpretation of causality [41]. Inadequate study design, paucity of data and mixed results limit the ability to draw firm conclusions about etiology of food allergy and atopy prevention in regards to dietary interventions [42].

3.2.2.5 d Processing and Consumption

Regional dietary habits and processing practices of peanuts are hypothesized to play a role in the differing prevalence of food allergies in various countries around the world [25]. The introduction of new trends such as vegetarian and health food diets that incorporate a high consumption of nuts have been suspected to contribute to the rise in allergy in some cultures [12]. As the vegetarian diet becomes more popular and demand grows for quick, nutritional foods, exposure to peanuts has increased in pregnant/nursing mothers and in infants [5].

In the United States, more than five billion pounds of peanuts are produced each year and the average American eats eleven pounds of peanuts annually. The majority of peanut consumption in the U.S. is in the form of peanut butter (55%) and the remainder is consumed as table nuts and in baked goods and candies [11]. Peanuts and peanut products, particularly peanut butter, are inexpensive and convenient sources of food that are

commonly eaten as snacks and as quick meals [11]. Therefore, the majority of American children are exposed to peanuts (i.e. peanut butter) within the first two years of life when the immune system is immature. In countries where peanut butter is rarely eaten, such as Denmark and Norway, peanut allergy is much less common [45]. Furthermore, peanut allergy is the fourth most common food allergy among children in Israel behind sesame allergy whereas in the U.S., peanuts are the third most common food allergen [11]. This difference may reflect the fact that in Israel, sesame products and sesame paste are more commonly eaten than peanuts products and peanut butter.

China is the world's leader in peanut production and consumption is comparable to that in the U.S., however peanut allergy was previously reported to be less common in China [25]. Recent data indicates that this may not be true as there are believed to be more children with peanut allergy in China than previously thought, though the data are still inconclusive [19]. One explanation for the potentially different rates of allergy between China and the U.S. is the Chinese method of preparation in which peanuts are usually boiled or fried. Studies indicate that high processing temperatures used in manufacturing peanut butter and dry roasted peanuts, the forms of peanuts commonly eaten in the U.S., results in an increase in allergenicity of the peanut proteins. The lower cooking temperatures used in boiling or frying peanuts do not cause this increase in allergenicity resulting in less allergenic forms of food for consumption. The prevalence of peanut allergy is similar among the children of Chinese immigrants to the United States and the children of native born Americans [25].

3.2.2.5e Epicutaneous Exposure

Another hypothesis is that epicutaneous exposure to peanut proteins early in life contributes to the development of peanut allergy. The Avon study in the United Kingdom suggests a correlation between topical application of oils containing peanut protein and peanut allergy. In a part prospective, part retrospective study of preschool children, Lack found an association between the use of skin preparations containing peanut oil and peanut allergy (OR 6.8 95% CI 1.4-32.9) [46]. The authors concluded that sensitization to peanut protein may occur in children through the application of peanut oil to inflamed skin. This study raises concerns that non-oral routes may be an important risk factor, especially for atopy prone infants with a damaged skin barrier that may allow increased absorption and sensitization to topical allergen exposure.

3.2.2.6 Prevention

Several food allergy prevention strategies have been investigated, as reported in detail in the risk factors section. As stated, the effectiveness of these strategies for safeguarding against the development of food allergies has not been established and is currently being debated in the literature [21]. As this time, there are not any known, proven preventative strategies.

3.2.3. Clinical aspects of peanut allergy

3.2.3.1 Symptoms of a type-1 hypersensitivity reaction to peanut

Individuals with allergic reactions to peanuts may experience a variety of symptoms that range from mild to severe. Most often the peanut must be eaten before symptoms occur, however, in severe cases, symptoms may be triggered by skin or air contact with peanut protein [19]. The symptoms of a type-1 hypersensitivity food reaction are typically related to the skin, gastrointestinal tract and respiratory tract [19]. Skin

symptoms include acute urticaria, angioedema, or a pruritic erythematous skin rash. Gastrointestinal symptoms range from nausea, vomiting, and diarrhea to severe abdominal pain. Respiratory symptoms include wheezing, stridor, cough, dyspnea, throat tightness and nasal congestion. Anaphylaxis is the most severe type-1 hypersensitivity reaction to food because it is a life threatening systemic allergic reaction in which symptoms occur in multiple organ systems and may also include cardiovascular symptoms such as hypotension and dysrhythmia [5, 19, 20].

Symptoms of a type-1 hypersensitivity reaction can develop within seconds and up to two hours after ingestion of even a few milligrams of peanut protein (one peanut has approximately 200 mg of protein) [19]. Studies indicate that clinical signs of a reaction develop rapidly with 85% developing within 10 minutes after ingestion whereas 93% began within 30 minutes and 95% within 1 hour [26]. Reactions to peanuts are often times severe and the severity of reactions may vary with exposure [15]. The Food Allergy and Anaphylaxis Network's voluntary registry study indicates that 50% of children surveyed had allergic manifestation in 1 organ system, 30% had symptoms in 2 systems and 10-15% in 3 systems, and 1% in four systems. A study of 101 children found that 50% had two or more organ system involvement and 20% required epinephrine treatment for their first reaction [29]. A 5-year retrospective review indicates that anaphylaxis is predominantly a childhood disease, estimated to occur in 1 out of 170 children [34].

3.2.3.2 Diagnosis of peanut allergy

Step 1

The primary tools available to identify peanuts as the cause of a type-1 hypersensitivity reaction include patient medical history and allergy testing for peanut-

specific IgE antibodies [21]. Aspects of the patient's dietary history including route of exposure of suspected allergen, quantity of food consumed, timing of onset following ingestion, description of symptoms, and reproducibility of symptoms with ingestion of the same food, are all important to proper diagnosis [4, 21]. Details of the patient's history that indicate a type-1 hypersensitivity reaction would include, but not limited to, a rapid onset of symptoms following ingestion (typically within minutes, but may be delayed for one to two hours), consumption of small quantities of peanut elicited a reaction and symptoms consistent with an IgE-mediated peanut allergy.

Step 2

If history indicates type-1 hypersensitivity reaction, specific IgE testing is the next step [21, 47]. Methods of testing for food specific IgE include skin prick tests (SPTs) and serum tests for peanut-specific IgE. The tests are highly sensitive (>95%) and are thus, able to exclude the diagnosis of food allergy well. However, they are only modestly specific (50%) and therefore well suited for use when suspicion of a particular food is high [21]. The tests are not effective for the purpose of screening. Because of the poor positive predictive value of food specific diagnostic tests, a positive test result does not always equate with clinical food allergy. Therefore, the diagnostic test results must be paired with other diagnostic evaluations, including patient history, to make a firm diagnosis [21].

Typically, a blood test, called a RAST, a radioallergosorbent test, which determines peanut-specific IgE antibodies in serum is conducted first to confirm the allergy [21, 47]. The test involves drawing blood from the patient's arm and sending it to a laboratory to be analyzed for the presence of antibodies to specific allergens. The

amount of allergen specific IgE contained in the patient's blood is calculated to determine how much antibody that person has to that specific allergen.

The RAST test, which was invented in 1974, was replaced, in 1989, with a superior test named the ImmunoCAP. The Immunocap by the company PHADIA is the food specific IgE antibody blood test, which is used to diagnose peanut allergy in Olmsted County currently [47]. The assays use a total serum IgE heterologous reference curve based on a World Health Organization IgE standard and quantitative results are reported in classes (class 1 through 6) or units of concentration that range from 0.35 to 100 kilo-units per liter (kU/ml) [21]. The ImmunoCAP is the only specific IgE assay to receive FDA approval to quantitatively report to a detection limit of 0.1 kU/l. The levels of allergen correlate with how active your symptoms are, and if these levels decline, it may indicate that a person is losing the allergy. The ImmunoCAP class ratings and associated IgE levels are outlined in Table 1.

Table 1. IgE Class Ratings for ImmunoCap

Class	IgE Level (kU/L)	Comment
0	<0.35	Negative Result
1	0.35-0.69	Low level of allergen specific IgE
2	0.70-3.49	Moderate level of allergen specific IgE
3	3.5-17.49	High level of allergen specific IgE
4	17.50-49.99	Very high level of allergen specific IgE
5	50.0-100.0	Very high level of allergen specific IgE
6	>100.0	Extremely high level of allergen specific IgE

A few of the older laboratory results from Mayo patients and many of the less recent tests performed for Olmsted County patients were performed by an outside laboratory that uses the older RAST technology. The reported IgE values from RAST tests are based on a different scale than the Immunocap. The results from RAST testing

are reported as peanut-specific IgE levels in a range from 0 to 40,000 mass units or greater. The levels have associated class ratings and similar to the ImmunoCap, increasing values represent greater levels of peanut-specific IgE in serum. Studies comparing serum analyzed by multiple laboratories and technologies indicate that the class ratings are comparable between the two technologies when a level of 750 is used as the cut-off for a positive result for the RAST test [48]. The class ratings and associated IgE levels for the RAST test are outlined in Table 2.

Table 2. IgE Class Ratings for RAST

Class	IgE Level	Comment
0	<750	Negative Result
1	751-1600	Positive with increasing amounts of peanut
2	1601-3600	Specific IgE antibody
3	3601-8000	
4	8001-18000	
5	18001-40000	
6	>40000	

Step 3

If blood test results are negative (<0.35), yet the patient has a positive history of a type-1 hypersensitivity reaction from peanut, an allergy skin test is conducted to confirm diagnosis [21, 47]. A serum blood test is preferable as the first diagnostic tool, however, for several reasons:

- The patient does not have to discontinue medications that may interfere with test results or cause medical complications
- In the event that a patient suffers from severe skin conditions such as widespread eczema or psoriasis

• A patient has such a high sensitivity level to the suspected allergen that any exposure may result in potentially serious side effects

Furthermore, the serum test may be a better way to follow children for the presence of food allergy since skin tests can remain positive even when clinical activity lessens as a child "outgrows" the food allergy [20].

A skin prick test (SPT) is done to test the occurrence of an immediate (within 20 minutes) wheal and flare reaction after application of the allergen to skin [21]. Positive and negative controls are used to verify normal skin reactivity. During a SPT, the skin is pricked or scratched to introduce a tiny drop of a suspected allergen into the surface of the skin. The peanut-specific skin prick test is made by Hollister Stier and is in a 5 ml vile (concentration, 1:20 (wt/vol)) [47]. If a patient is allergic to the allergen, their mast cells will already be sensitized to it and the person will have a histamine-mediated reaction indicated by the appearance of a small hive where the skin was pricked. Results of SPTs are considered positive if a hive appears with a mean wheal diameter of 3 mm or greater than the negative control [20, 47]. Skin tests have excellent sensitivity (95%) and negative predictive value (95%) but modest specificity (30%-60%) and positive predictive value (50%) [15, 20]. A wheal size of 8 mm has been determined to have a 95% positive predictive value [10].

Conclusions

The larger the skin test diameter and the higher the Immunocap level, the more active the allergy is and the more likely a reaction will occur on exposure. It is unclear at this time, however, if peanut-specific IgE level correlates with severity of a reaction. A reaction to any given exposure depends on many factors including the amount of

exposure, the route of exposure (i.e. ingestion, skin), and health status at the time of exposure (i.e. whether you have active allergy and/or asthma symptoms) [45].

3.2.3.3 Management of peanut allergy

Currently there are not any medical interventions to prevent or cure peanut allergy. Once the diagnosis of food allergy is established, the only way to minimize one's risk for an allergic reaction is strict avoidance of the food. Management of peanut allergy is based on strict avoidance of peanuts, prompt recognition of allergic reactions, and rapid initiation of treatment [11]. Because of the likelihood of an inadvertent exposure to peanut, education of the patient and guardian, including information as to the importance of label reading and potential for cross contamination, is essential to management [11, 49]. In addition to education regarding strict avoidance of peanuts, informing patients of the early signs of anaphylaxis and proper usage of self-injectable epinephrine are essential [49].

3.2.3.4 Investigational Treatments

Immunotherapy for food allergies is under investigation and considered experimental at this time due to the high incidence of side effects and the lack of large, controlled studies documenting the safety of such treatments. In 1992, a randomized placebo controlled study of peanut immunotherapy in patients with a history of peanut allergy and anaphylaxis was conducted by Drs. Nelson and Leung at the National Jewish Hospital in Denver, Colorado [11]. The patients that received peanut immunotherapy had a significant decrease in symptoms in double blind placebo controlled food challenges and a decrease in skin reactivity to peanut despite experiencing a high rate of systemic reactions (13.3%) yet no incidents of anaphylaxis during the process [11]. This was the first

study to demonstrate that immunotherapy may be an effective treatment for peanut allergy when done in a well-controlled manner.

Two recent studies of immunotherapy treatment show promising results. A study conducted in the United States in 2008 involved 20 children aged 1 to 16 years who had moderate peanut allergy [44]. The children received multiple doses of peanut flour the first day, starting with 0.1 mg of peanut protein, which was increased to 50 mg at the end of the day. The children took peanut protein every day at home, starting at 50 mg/day and increasing by 25 mg every two weeks. The increasing doses stopped 300 mg/day at which time the children remained on this maintenance dose for up to 42 months. Of 20 children, 18 were eventually able to eat the equivalent of 13 peanuts with few or no allergy symptoms [44]. Mean levels of IgE increased initially and then gradually declined over time.

A small-scale study conducted in the United Kingdom, the Addenbrooke's Hospital study**,** gave four children aged 9 to 13 years, who were highly allergic to peanuts, small doses of peanut flour every day and then increased that dose over six months [50]. Specifically, the children were given a five-milligram serving of peanut flour at the beginning of the trial. This was slowly built up over six months until the volunteers trained their bodies to tolerate at least 800 milligrams, equivalent to approximately five whole peanuts [50].

The main drawback to immunotherapy with peanut extracts is the likelihood for severe reactions. A new type of immunotherapy, peptide therapy, modifies the allergen protein to contain only the portion that is recognized by the patient's immune system (epitope) and to not have the portion that will bind to IgE on mast cells (peptide) [11]. This

will allow the immune system to generate the same type of immunity to peanut as traditional immunotherapy without any chance for allergic reactions to the injections. Peptide therapy has been successfully studied in humans with cat allergen and ragweed pollen but not yet studied with peanuts.

Additional experimental approaches are under investigation. Researchers are testing an anti-IgE vaccine that decreases the amount of circulating IgE in a patient. A study was conducted in 2003 by Dr. Leung and colleagues on several patients with severe peanut allergy [51]. After receiving injections of an anti-IgE vaccine for three months, the patients were able to tolerate eating up to nine peanuts without a reaction. In addition, a DNA vaccine that contains the DNA coding for peanut allergen Ara h2 was tested successfully in animal models and may be applied to humans in the future [11]. Injection of the DNA induces a suppressive immune response that turns off the response to Ara h2 and prevents an allergic reaction to peanut. Both vaccines are under investigation.

Researchers are using the principles of the Hygiene Hypothesis to turn off an allergic immune system with bacterial proteins. Scientists found that a combination of certain heat killed bacteria with modified peanut protein prevented anaphylaxis in peanut allergic mice, though this has not been tried in humans [11]. Another investigational approach based on the Hygiene Hypothesis is the use of probiotics, which are considered good bacteria, and are found in such things as yogurt and sold as supplements [11]. Probiotics have not yet been studied for peanut allergy but are currently being examined with other allergic diseases such as asthma and hay fever.

4.0 INTRODUCTION TO RESEARCH

4.1 Overview of Dissertation Aims

The diagnosis of peanut allergy has a significant impact on the lives of the patients and families. Allergic individuals and their families have constant anxiety related to preventing an inadvertent exposure in a world in which peanut is a ubiquitous product. The reported increase in the prevalence and incidence rates of peanut allergy diagnoses over the last two decades in conjunction with the low rate of acquiring tolerance, significant morbidity and the many health care dollars spent on patient care makes the issue of childhood peanut allergy a significant clinical and public health concern. In order to move the field forward, accurate prevalence and incidence rate estimates are needed, which will enable precise estimation of the burden of disease and answer the question as to whether the reported increase in rates is real. Secondly, it is important to understand factors associated with gaining tolerance and reaction severity in order to manage and advise patients appropriately. Specifically, furthering the utility of the current diagnostic test, peanut specific IgE level, by expanding our understanding to include the association between IgE level, reaction severity and tolerance will enhance medical management and answer important questions for patients.

4.2 Data Resource and Setting

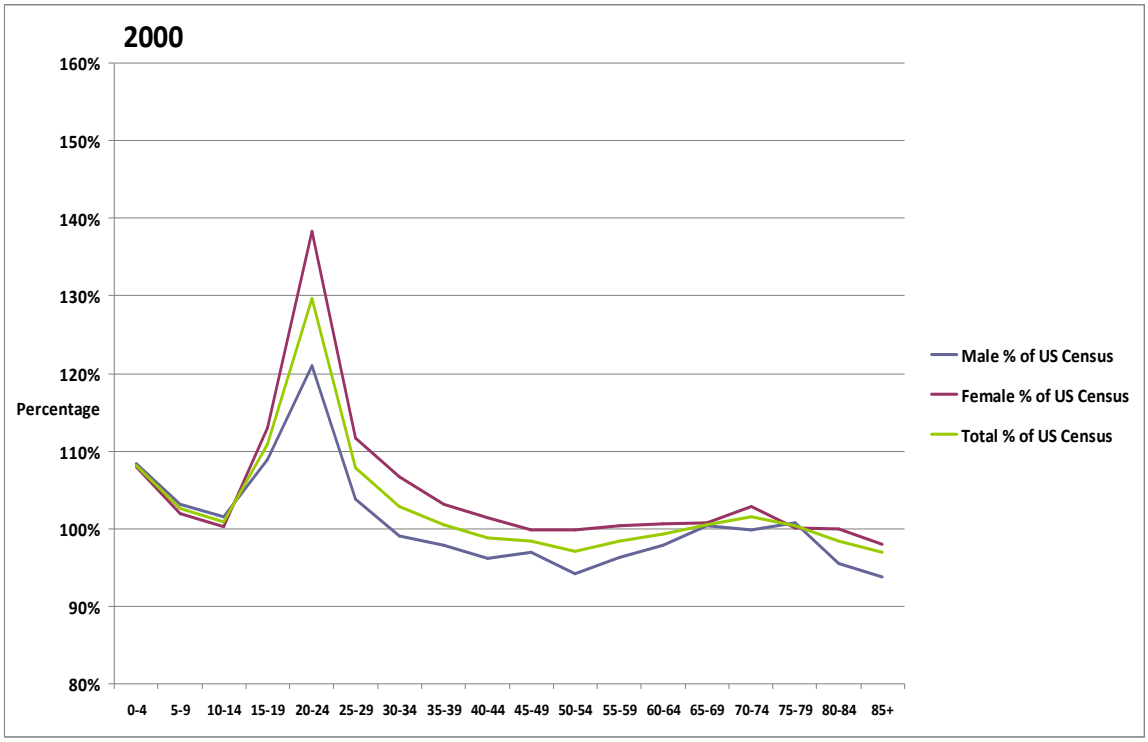
Olmsted County, MN is one of the few places in the world where one can conduct detailed epidemiologic studies in a defined geographic location. The Rochester Epidemiology Project (REP) is a population based medical records linkage system in which medical data from the main providers of care in Olmsted County have been retained in a central bank. The REP records represent the patient population well. Ninety-eight percent of Olmsted County residents seen from 1998-2007 granted

permission for at least one of their medical records to be used for research and ninety-one percent granted permission for all of their records to be used for research. [52,53]

The largest provider of care is the Mayo Clinic, which has maintained a common medical record with its two large affiliated hospitals, St. Mary's and Rochester Methodist since 1907. In 1966, Mayo's indexing system was extended to the other providers of medical care in Olmsted County, including the second largest provider, Olmsted Medical Center and its affiliated hospital. A third provider, Rochester Family Medical Center, a local family medicine practice also participates in the REP. Two small Rochester medical facilities, a Target store outpatient clinic and a small nurse practioner's office, are the only providers of medical care in Olmsted County that do not participate in the REP.

The REP is an excellent representation of the Olmsted County population. The vast majority of residents of Olmsted County receive care at one of the three facilities included in the REP. As compared to 2000 U.S. Census population estimates for Olmsted County, the REP did a better job of estimating the population aged less than 18 years than did the U.S. Census (see Figure 1) [52].

Figure 1: REP Estimation of U.S. Census 2000



This REP's dossier type records contain both inpatient and outpatient data, and the diagnosis and surgical procedures recorded in these records are entered into a computerized index. Patient medical records were all paper files prior to 1994, mixed paper and electronic records from 1994-2002 and electronic after 2003. The significance of the REP is its ability to provide accurate, descriptive information for many diseases and the ability to conduct population based analytical studies of disease etiology and outcome. The REP was used as the data source for all three analyses for this dissertation.

The major city located in Olmsted, County is Rochester, MN and greater than 70% of the county population resides within the city limits. In 2000, the population was 124,277 with 90% white and 29.5% of the population less than 18 years (7.2% under 5 years, 7.3% 5-9 years, 7.9% 10-14 years, 7.1% 15-19 years). In comparison to the 2000 US Census data, Olmsted County was shown to be less ethnically diverse than the entire

US population (90.3% vs. 75.1% White), more highly educated (91.1% vs. 80.4% high school graduates), and wealthier ($51,316 vs. $41,994 median household income) (personal communication, Dr. Jennifer St. Sauver) [52,84].

4.3 Case Definitions and Identification

The data for this study was collected by identifying all cases of peanut allergy documented in the REP from 1999-2007. All patient diagnoses have been coded with the Hospital Adaption of the International Classification of Diseases (H-ICDA), Second Edition, codes or the International Classification of Diseases (ICD), Ninth Revision, codes. These codes are indexed for computerized retrieval through the REP. Using the retrieval system, a search was conducted for all patients with diagnostic code related to peanut allergy from 1999-2007. In an effort to capture all diagnoses of peanut allergy, a comprehensive search of the medical records under all potential ICD and associated H-ICDA codes for peanut allergy was performed including the following: 693.1 allergy food, allergy food with dermatitis, allergy peanuts, v15.05 allergy food personal history, v15.01 allergy peanuts personal history, 995.61 anaphylaxis shock peanuts, 995.0 anaphylaxis, 995.3 allergic reaction.

The Mayo Clinic Medical Records Retrieval experts generated a list of all patients with a potential diagnosis of peanut allergy in the medical records from 1999-2007 for medical chart review. The unique patient identification number assigned to each patient when they first seek care at the respective medical facility organized this list. Once data collection was complete, it was insured that the list of cases did not include duplicate patients. Each patient had a unique REP identification number, which was used to check and eliminate duplicate entries.

Each potential subject's file was reviewed to determine whether they were a case. A case is a patient who has a diagnosis of peanut allergy in their record. A physician makes the diagnosis of peanut allergy if the patient has a positive history of an IgE-mediated type 1-hypersensitivity reaction to peanuts in conjunction with positive allergy testing for peanut-specific IgE antibodies [54]. All of the patient's files, lab and testing results were reviewed to confirm that the patient met the criteria for a case. Specifically, to be considered allergic to peanuts, each patient must have met the following criteria [54].

1. Positive history of an IgE-mediated type 1 hypersensitivity reaction AND
2. A positive blood test result (>=0.35 kU/l) OR
3. If blood testing is negative, positive skin prick test (>-3.0 mm) OR
4. If both blood and skin prick testing are negative, a positive double blind placebo controlled food challenge

This protocol for diagnosis was followed for all patients presenting with the full range of potential peanut allergy symptoms.

It was noted as to whether each case is an incidence and/or prevalent case. To be considered an incident case, the initial diagnosis of peanut allergy must have been made at one of the REP medical facilities from 1999-2007. The records from each medical visit were reviewed to ensure that the recorded case is the incident diagnosis. Furthermore, the patient must have been a resident of Olmsted County at the time of diagnosis. A prevalent case is defined as an Olmsted County resident that is a patient of a REP facility from 1999-2007 with peanut allergy. The allergy may have been diagnosed prior to 1999 and may or may not have been initially diagnosed at a REP facility.

4.4 Data collection

Maria Rinaldi reviewed the medical records of each potential case. This process involved reviewing patient histories at the Olmsted Medical Center, Mayo Clinic and the Rochester Family Medicine clinic. Pertinent data were entered into a pre coded, electronic data form. The data was checked for accuracy by analyzing means, medians and spread of data for continuous variables and distributions for categorical variables to locate outliers and confirm accuracy of data collected. Furthermore, a computer program was run, which was developed by REP programmers, to identify any potential duplicate entries. Each patient has a unique REP identification number. The program scanned all entries to identify duplicate entries. Duplicate patients were reviewed by Maria Rinaldi to combine the information into one entry and remove duplicate listings. A SAS data set was created for analysis.

Variables

Cases were reviewed to confirm that they met the diagnosis for the outcome of interest, peanut allergy. Furthermore, it was determined whether the case is an incidence case, initial diagnosis made at a REP facility from 1999-2007, or a prevalent case, a patient of a REP facility from 1999-2007 with peanut allergy that may have been initially diagnosed before 1999 and may or may not have been initially diagnosed at a REP facility.

Details regarding peanut allergy diagnosis were collected for all confirmed cases. The information included the date and age of the initial diagnosis of peanut allergy and a history of other atopic diseases (asthma, eczema, hay fever). Furthermore, symptoms of the child's first reaction were noted. These symptoms are divided into four types; cutaneous, respiratory, gastrointestinal, and cardiovascular [47]. The child's reactions were

graded as either mild or moderate/severe. A mild reaction is defined as one in which the child experienced cutaneous symptoms only. A moderate/severe reaction is defined as an anaphylactic reaction, a systemic type 1 hypersensitivity reaction in which a child may experience a range of potential symptoms from moderate to severe. The criteria for anaphylaxis are outlined below [54]. There are three criteria each reflecting a different clinical presentation and range of potential symptoms, which constitute anaphylaxis. Anaphylaxis is considered highly likely when any one of the following criteria is fulfilled:

Criterion 1- Acute onset of an illness involving the skin, mucosal tissue or both (i.e. generalized hives, pruritus or flushing, swollen lips, tongue, uvula) and respiratory compromise or reduced blood pressure

Criterion 2- Two or more of the following that occur after exposure to a likely allergen

- Involvement of the skin-mucosal tissue (generalized hives, itch, flush, swollen lips, tongue, uvula)
- Respiratory compromise
- Reduced blood pressure
- Persistent gastrointestinal symptoms

Criterion 3- Reduced blood pressure after exposure to a known allergen for that patient

- In infants and children, reduced blood pressure is defined as low systolic blood pressure or greater than thirty percent decrease in systolic blood pressure

Lastly, it was noted as to whether the child became tolerant to peanuts. Tolerance was defined by meeting one of two criteria. First, a child was classified as acquiring tolerance if they were diagnosed with peanut allergy previously and became tolerant as

documented by passing an in office food challenge. An in office food challenge is considered a success when the patient experiences no signs of type-1 hypersensitivity reaction to peanut as determined by the patient's physician who is present during the challenge [47]. In office food challenges are performed on children who have a negative serum IgE level (<0.35) and a negative skin prick result (less than 3 mm* 3mm wheal and flare) [47]. A child was also be considered to have acquired tolerance if one of the following is true:

- The child's IgE testing level becomes negative (<0.35 kU/l), while their SPT test is still positive, they did not have a skin text or their skin test is negative.

  OR

- SPT changes to a negative status, less than 3 mm* 3mm wheal and flare, while IgE results were always negative

4.5 Human Subjects

Pertinent medical information was abstracted from the medical charts of children residing in Olmsted County between 1999 and 2007. The research did not involve experimentation on human subjects and was limited to a retrospective medical record review. The research was based on children aged less than 18 years, as children are at highest risk for peanut allergy and for which the research question is pertinent.

Patient Consent

This study provided little risk to patients because it consisted only of retrospective medical record review. Since January 1, 1997, Minnesota State Law requires a general authorization to review medical records for research from each patient who attended a health care facility after this time. All health care providers who participate in the REP

have implemented systems to track and document patient research authorizations to comply with this law. Records were not reviewed from patients who did not provide this authorization. Ninety-five percent of patients receiving care at one of the three REP facilities consented to review of their medical records for research purposes [52].

Confidentiality

Several steps were taken to insure privacy of patient medical information. Information was abstracted from medical records and stored in an electronic database. Maria Rinaldi underwent all necessary training modules in regards to the safe and proper handling of medical records. Maria was the only person that had access to medical records and the electronic database. The data were analyzed anonymously and policies and safeguards enforced by the Department of Health Sciences Research at the Mayo Clinic and the Health Insurance Portability Act were used to protect the confidentiality of patient records. Data on individual patients was not shared. All results will be published in the aggregate. Comparisons between health care institutions will not be made.

Benefits

While this study may not directly benefit the involved children, the results are expected to enhance our medical knowledge in regards peanut allergy.

Gender/Minority Mix

All residents of Olmsted County aged less than 18 years of age whose parents consented to the review of their medical records for research purposes were eligible for this study. The study population is expected to reflect the underlying demographic composition of Olmsted County. According to the 2000 U.S. census , it is expected that

approximately 50% of the children will be female and 10% will belong to a racial or ethnic minority group.

5.0 Study 1: An examination of the prevalence of peanut allergy in 2007 and the incidence rate of peanut allergy diagnoses among children aged less than 18 years residing in Olmsted County, MN from 1999-2007

5.1 Specific Aims

Peanut allergy is a major health concern in the United States and worldwide with an estimated prevalence of 1% among children [19, 21-25]. Peanuts are one of the most common food allergies and account for most cases of fatal and near fatal anaphylactic reactions to food. Recent studies indicate a two-fold increase in the prevalence of peanut allergies in the last two decades [22]. Peanut allergy presents early in life and only a minority of cases resolve (20%) [21, 23, 27, 35]. In highly sensitive people, trace quantities of this ubiquitous food can induce severe allergic reactions. Currently, the only available treatment is strict avoidance, recognition of early symptoms of a reaction and usage of emergency medication.

There exists a debate in the literature as to the prevalence of peanut allergy and whether the incidence is truly increasing among children due to limitations in the literature including reliance on self-report data and inaccurate diagnostic criteria for a positive allergy. An incomplete understanding of and agreement upon estimates of prevalence and whether the increase in affected children is real or a result of an increase in diagnosis of mild cases in recent years prevents proper management from a clinical and public health standpoint.

The objective of this study was to fill this gap in the literature by providing an accurate estimation of the prevalence in 2007 and incidence rates of peanut allergy from 1999-2007 among children aged less than 18 years residing in Olmsted County, MN. The central hypothesis was that the current prevalence estimate of 1% is approximately accurate and that the increase in children diagnosed with peanut allergy over the last decade is accurate. This hypothesis was formulated based on results from well-designed studies, despite several discrepancies and shortcoming in the literature overall. To test this hypothesis, the follow specific aims were addressed:

1. To estimate the prevalence of peanut allergy among Olmsted County residents aged less than 18 years in 2007

2. To estimate the incidence rate of peanut allergy diagnoses among Olmsted County residents aged less than 18 years from 1999-2007

3. To examine whether the increase in peanut allergy rates varies by initial reaction severity

This information is significant to our medical and public health communities. The results will provide important information to clinicians in terms of counseling patients. As the numbers of peanut allergic children rise, public health plays an increasingly important role in awareness, safety and educational efforts. Further, public health policy plays a pivotal role in regards to labeling laws, and bans of peanuts in high-risk settings. Lastly, confirmation of an increase in rates provides support for further research into etiology, prevention and therapeutic techniques, all of which are critical to address this significant health issue among children.

5.2 Background and Significance

5.2.1 Introduction

There is a general consensus that allergic disorders are increasing in prevalence especially in the Western world. Atopy and its phenotypic expression, asthma, allergic rhinitis, atopic dermatitis (eczema) and food allergies are an increasing burden for sufferers and their healthcare providers. Allergic diseases are now the most common chronic disorders in childhood in the developed world[12]. Of particular interest to this study, is the suggestion by many that the incidence rate of peanut allergy has also increased in recent years.

5.2.2 Etiologic Basis

There are numerous theories attempting to answer the question as to why allergic conditions, including peanut allergies, are more prevalent in children today as compared to generations ago. It is likely that both genetic and environmental influences affect the development of peanut allergy. Theories under investigation include the hygiene hypothesis, maternal and infant diet, including increased consumption of peanuts by mothers and young children, early feeding when the immune system is immature, sensitization in utero or through breast milk, food processing methods, in particular the allergenicity of roasted forms, and non-oral exposure including the use of topical ointments containing peanut.

Despite evidence suggesting an increase in incident diagnoses, some question whether the reported increase in peanut allergy diagnoses over time is a reflection of more cases of mild reactions being captured now as a result of several potential factors including heightened awareness, improved surveillance, changes in diagnostic criteria,

health insurance, or food availability and not truly a reflection of an increase in the rate of disease.

The Hygiene Hypothesis, a theory that receives much support, postulates that a lack of early childhood exposure to infectious agents increases susceptibility to allergic diseases by modulating immune system development [28, 38,30]. The United States, among other westernized countries, is an industrialized society concerned with cleanliness and germ prevention. These assumptions regarding germ prevention and the concurrent rise in the incidence rate of diagnoses in the U.S. provides support for the hygeine hypothesis. Although intriguing, no cause has been proven at this time.

In addition to an increase in rates over time, it is established that rates of atopic diseases differ according to gender and age [42, 62]. Gender differences in atopic disease, assessed as total serum IgE levels, have been reported to be consistent across the lifespan, with levels in females being lower than those in males [62]. The physiological pathway leading to such gender differences is reported to be a result of immune dimorphism, the term given to differences in immune responses and regulation between the sexes [62].

Lastly, children aged 0-2 years are more susceptible to food allergies as a result of their immature immune system, which is more likely than an older child's to deem certain food proteins as foreign and launch an allergic reaction [42]. Further, the introduction of new foods typically begins in this age range and if a reaction were to occur, it would be expected to surface at this time. [42, 62]

5.2.3 Review of the literature

Emmet and colleagues conducted a survey of 2000 households in Great Britain in 1995 to determine the prevalence of self-reported peanut allergy [37]. The survey results

indicate an overall prevalence of peanut allergy of 0.48%. The prevalence varied among age groups with the youngest age groups suffering the highest prevalence of peanut allergy (0.61% of children aged 0 to 14 years, 0.53% of adults aged 15 to 44 years and 0.3% of respondents older than 44 years) based on self-report [37]. The author concludes that having found similar estimates in children and adults argues against a marked rise in prevalence. However, this study did not compare the prevalence of allergy within an age group over time [37].

A cross sectional study of 4,339 children in kindergarten through grade three was conducted to estimate the prevalence of peanut allergy in Montreal in 2002 [24]. Parents of selected children were asked to complete a questionnaire regarding their child's peanut ingestion. Final diagnosis of peanut allergy was based on self-reported history and confirmed with diagnostic testing and oral peanut challenges when appropriate. The diagnosis of peanut allergy was made if children who had an uncertain clinical history had either a positive SPT wheal response, a diameter at least 3 mm larger than the negative control and a peanut-specific IgE level of 15 Ku/L or greater or a positive SPT response and a positive food challenge. A child was also considered peanut allergic if they had a convincing clinical history of a type-1 hypersensitivity reaction to peanuts, as defined as a minimum of 2 mild symptoms or 1 moderate or 1 severe symptom within 60 minutes of ingestion or contact, and had a positive SPT response or peanut-specific IgE. The results indicate that the prevalence of peanut allergy is 1.34% in this population [24].

A follow up to the earlier study led by Kagan was conducted to determine whether the prevalence of peanut allergy increased from 2002 to 2007 among children in Montreal. The methodology and diagnostic criteria utilized in this study were the same

as the earlier study [24]. The results indicate that the prevalence of peanut allergy increased from 1.34% in 2002 to 1.62% in 2007 with a non-significant difference of 0.28% (95% CI -0.15% to 0.70%) [55].

The Surveying Canadians to Assess the Prevalence of Common Food Allergies and Attitudes towards Food Labeling and Risk study, launched in 2008, was designed to estimate the prevalence of food allergies in Canada using a random telephone survey of 3,613 households and 9,667 individuals. Food allergy was defined using three terms: perceived, based on self-report, probably, based on convincing history or self-report of physician diagnosis, or confirmed, based on history and evidence of confirmatory test. The prevalence of probable peanut allergy was 1.68% and confirmed allergy was 1.03% among children. The estimates are believed to be low as a result of their inability to obtain confirmatory tests. The results of this study exhibit the disparities between perceived and confirmed food allergy, which likely contributes to the wide range in published estimates [53].

The U.S. National Health and Nutritional Examination Survey, NHANES, a population based survey, is conducted by the National Center for Health Statistics to determine the health and nutritional status of the US population [56]. The NHANES study conducted from 1988-94 included allergy skin testing for peanut in an effort to estimate the prevalence of sensitization to peanut in the U.S. The study administered skin prick allergy testing in 10,863 people between the ages of 6-19 years. The results indicated that 8.6% of Americans in this age group had a positive skin prick test, as determined by a mean wheal diameter 3 mm greater than the control, which indicated sensitivity to peanuts [56].

The National Health and Nutrition Examination Survey 2005-2006 measured food-specific serum IgE to peanut, cow's milk, egg white, and shrimp [85]. Estimations of clinical food allergy risk were calculated based on previous studies correlating clinical outcomes to food-specific IgE concentrations. The estimated prevalence of clinical peanut allergy was 1.3% in the entire population and 1.8% among 1-5 year old children.

Studies were conducted on three cohorts of children born on the Isle of Wight, UK to determine whether the prevalence of peanut allergy has changed between 1994 and 2004. Tariq and colleagues conducted the initial population birth cohort study of 1,218 children born on the Isle of Wight between January 1989 and February 1990 [57]. The results indicate that by 4 years of age, 1.1% of children were sensitized to peanut, defined as a positive skin prick test response with a mean wheal diameter of at least 3 mm greater than the control [57]. Approximately half the children (0.5%) suffered from symptomatic peanut allergy, defined as a positive skin prick test response and parental report of a reaction [57].

The second study on the Isle of Wight was conducted with 1,273 children aged 3 to 4 years who were born from 1994-1996 [22]. This study provided the opportunity to study a change in prevalence in the intervening 6 years and to perform open peanut challenging to confirm diagnosis, when appropriate. The study reports a 2-fold, non-significant increase in the prevalence of symptomatic peanut allergy as reported by parents in a questionnaire (0.5% to 1.0% $p=0.17$) and a significant 3-fold increase in peanut sensitization, as defined as a mean SPT wheal diameter of 3 mm or greater than the control (1.1% to 3.3% $p<.001$) during this period [22]. Children with a positive SPT response, yet lacked a convincing history of a reaction to peanut, were invited to undergo

an open challenge. Overall, 1.5% of children were considered to have symptomatic allergy to peanut as defined by a positive oral challenge following a positive skin prick test or a positive skin prick test accompanied by self-report of a convincing clinical reaction to peanut [22].

The third cohort of 891 children were born between 2001 and 2003 and assessed at age 3 years. The study results indicate that peanut sensitization increased significantly from 1.3% in 1989 to 3.3% in 1996 but then reduced to 2.0% in 2002, though this change was not significant. Similarly, clinical peanut allergy increased significantly from 0.5% in 1989 to 1.4% in 1996 yet, reduced non-significantly to 1.2% in 2002. Thus, the prevalence changed from 1989 to 2004 with an initial significant increase through 1996 and then a stabilization and slight decrease, though not significant, through 2004. The results may have been influenced by differences in participant ages, participation rates, and selection between their studies [58, 59].

Sicherer and colleagues investigated the prevalence of peanut and tree nut allergy among the general population in the United States in 1997 by using a nationwide, cross sectional, random digit dial telephone survey of 4,373 households representing 12,032 individuals [29]. Peanut, tree nut or both allergies were self-reported in 1.1% of the general population or 3 million Americans. The prevalence of reported peanut allergy was 0.6% with 0.7% of adults aged 18 years and older and 0.4% in children younger than 18 years[29].

In 2002, Sicherer and colleagues sought to determine the prevalence of self-reported peanut and tree nut allergy among the general population in the United States to compare the results with the estimates they obtained 5 years earlier in 1997. The

researchers conducted a second nation-wide, cross-sectional, random telephone survey of 4,855 households representing 13,493 individuals. Peanut, tree nut allergy or both was self-reported in 1.4% of the individuals, which is not significantly different than the estimate obtained in 1997 (1.1%) [30]. The prevalence of reported peanut allergy (not confirmed by diagnostic testing) in children increased significantly over the 5 year period from 0.4% in 1997 to 0.8% in 2002 (p=.05) [30]. The overall prevalence of peanut allergy (0.6%) did not differ from the 1997 survey [30].

Sicherer and colleagues most recently conducted another nationwide, cross sectional survey of 5,300 households and 13,534 subjects in 2008. The survey results indicate that peanut allergy, tree nut allergy or both was self-reported by 1.4% of subjects compared with 1.4% in 2002 and 1.1% in 1997. The increase in prevalence of peanut allergy in children in from 0.4% in 1997 to 1.4% in 2008 represents a statistically significant increase ($p<0.0001$) [60].

A retrospective study was conducted over 13 years from 1995 to 2007 in the Australian Capital Territory (ACT) of 778 patients aged 4 months to 66 years with confirmed peanut allergy. The study indicates that the incidence of peanut allergy has risen over the 13-year time frame studied. The study reports that the incidence of peanut allergy by the age of 6 years had increased 2.5-fold in children born in 2004 (1.15%) as compared to those born in 1995 (0.47%) [61].

A large national database was utilized to estimate the incidence, prevalence, and trends of general practitioner recorded diagnosis of peanut allergy in the English population [86]. The age-sex standardized incidence rate of peanut allergy in 2005 was 0.08 per 1000 person-years (95% CI, 0.07- 0.08), and the prevalence rate was 0.51 per

1000 patients (95% CI, 0.49-0.54). During the study period, the incidence rate of peanut allergy remained fairly stable, whereas the prevalence rate doubled from 0.24 per 1000 patients in 2001 (95% CI 0.22-0.26) to 0.51 per 1000 in 2005 (95% CI 0.49-0.54). The increase was highest in the age group 10 to 14 years and higher in males compared to females. In those under 18 years of age, the crude lifetime prevalence rate was higher in males than females. The prevalence rates reported in this study are lower than other reported estimates, which may be explained in part by under recording of peanut allergy in general practice [86].

As can be seen from the reported studies, the prevalence of peanut allergy varies from 0.5% to 8.6% depending on the criteria used to diagnosis peanut allergy [22,56, 85,86]. The studies that rely on a history of a reported reaction in conjunction with positive diagnostic testing as compared to solely self-report of a reaction or a positive diagnostic test only reveal prevalence rates that range from 0.5%-1.62% [22, 24, 30, 37, 56,61]. Furthermore, while some studies indicate a significant increase in the prevalence and incidence of peanut allergy diagnoses over time, others do not support this notion. Reported increases over time range from 0.28% to a 2.5-fold [30, 60, 61,86].

5.2.4 Limitations in literature

Studies from the United States, United Kingdom and Canada estimate the prevalence of peanut allergy in children to be approximately 0.5%-1.62% for clinical reactivity and 1.1%-8.6% for sensitization to peanut [22, 24, 30, 37, 56,61] Several of the reported studies used questionnaire surveys. There is a risk of non-response bias associated with the use of questionnaire surveys to generate prevalence estimates because those with peanut allergy are more likely to agree to participate causing an

overestimation of the prevalence. For instance, only 43% of the target population agreed to participate in the study by Grundy and colleagues which may cause an overestimate of the prevalence in the population because those with a history of allergic diseases in the family or child may be more likely to participate [22]. Furthermore, perceived food allergy has been shown to be higher than food allergy confirmed by medical diagnosis [11]. In an effort to avoid the potential for bias and misclassification error, incident diagnosis of peanut allergy must be based upon medical guidelines, a physician's diagnosis of a type-1 hypersensitivity reaction in conjunction with diagnostic testing [69].

Lastly, the definition of peanut allergy is not consistent between studies. Self-report of an adverse food reaction or evidence of sensitization does not equate with a clinical allergy. It is known that estimates of self-reported peanut allergy are much higher than those diagnosed by a physician [11]. Further, evidence of sensitization alone does not indicate that a child will experience a clinical reaction upon exposure to that food. Nonetheless, studies rely on all three criteria, self-report of a reaction, sensitization and diagnosis of clinical allergy, to define cases. Thus, the specific criteria used to identify cases of peanut allergy will have a significant impact on prevalence and incidence estimates [35].

5.2.5 Need in the literature

Current research is needed to further examine the prevalence and incident rate of peanut allergy diagnoses based on a physician's diagnosis of clinical allergy. It is important that the definition of peanut allergy is in accordance with physician's best practice, which is a history of a type-1 hypersensitivity reaction to peanut, as diagnosed by a physician, in conjunction with positive diagnostic testing [21, 47]. Population-based

studies have estimated the prevalence of peanut allergy through the use of surveys, but few corroborated personal report of history with diagnostic testing [55, 61.] Further, only one reported study estimated the incident rate of new diagnoses over time [69].

5.3 Research Design and Methods

5.3.1 Overview

The prevalence of peanut allergy in 2007 among children was estimated from a population based descriptive study of Olmsted County, Minnesota residents using the Rochester Epidemiology Project (REP). All children less than 18 years old who were residents of Olmsted County from January 1, 1999 through December 31, 2007 and received medical care at one of the REP facilities were eligible for inclusion in the study. In addition to prevalence, the incidence rates of peanut allergy diagnoses were estimated for the years 1999-2007. These objectives were accomplished through a medical chart review of all patients with a diagnosis of peanut allergy as defined in section Case Definition and Identification.

This population-based study made use of the Rochester Epidemiology Project (REP), a population based medical records linkage system in which medical diagnosis data from the three main providers of care in Olmsted County have been retained in a central bank. Each REP health care provider uses a dossier type record containing both inpatient and outpatient data. The diagnosis and surgical procedures recorded in these records are entered into a computerized index. All patient diagnoses have been coded with the Hospital Adaptation of the International Classification of Diseases, Second Edition, codes or the International Classification of Diseases, Ninth Revision, codes. These codes are indexed for computerized retrieval. Using this retrieval system, a search

was conducted for all patients who had a new diagnostic code related to peanut allergy from 1999 through 2007, were residents of Rochester, Minnesota and who gave permission for their medical records to be used for research.

A list of patients with a potential diagnosis of peanut allergy in the medical records from all three facilities from 1999-2007 was generated and the medical charts were retrieved and reviewed to confirm cases of peanut allergy. Specifically, all 547 patients with codes representing the following diagnoses were reviewed: allergy food, allergy food with dermatitis, allergy peanuts, allergy food personal history, allergy peanuts personal history, anaphylaxis shock peanuts, anaphylaxis, and allergic reaction. The list was organized by the unique REP patient identification number assigned to each patient when they first sought care at the respective medical facility. Each patient's unique number was used to check and eliminate duplicate entries. Patients who met the criteria for a diagnosis of peanut allergy, as defined below, were included in the study.

Specifically, to be considered allergic to peanuts, each patient must have met the following criteria [54].

1. Positive history of an IgE-mediated type 1 hypersensitivity reaction AND
2. A positive blood test result (>=0.35 kU/l) OR
3. If blood testing is negative, positive skin prick test (>-3.0 mm) OR
4. If both blood and skin prick testing are negative, a positive double blind placebo controlled food challenge

The Mayo Foundation, Olmsted Medical Center and University of Minnesota institutional review boards approved the study.

5.3.2 Study Population

Subjects who did not provide permission for their medical records to be used for research, whose residency information was not available, who were not residents of Olmsted County at the time of diagnosis and/or did not meet the diagnostic criteria for peanut allergy were excluded from the data set. The complete medical records of each potential case were reviewed to confirm peanut allergy and collect information regarding demographics, health status, presenting symptoms, and outcome. Of the group of 547 potential cases, 171 (31.3%) met the criteria for an incident case of peanut allergy. Twelve duplicate cases were eliminated from the original sample of 183 cases, which resulted in 171 unique incident cases. From the sample of 171 incident cases, 1 case was eliminated to incorrect date information that could not be verified. The final analytic sample consists of 170 cases. All data were abstracted using an electronic data collection form.

Prevalence

A prevalent case was defined as an Olmsted County resident that was a patient of a REP facility from 1999-2007 with a diagnosis of peanut allergy. Prevalent cases are a mixture of existing and new cases of peanut allergy diagnosed in the medical records from 1999-2007. Thus, the allergy may have been diagnosed prior to 1999 and may or may not have been initially diagnosed at a REP facility. It is estimated that approximately 20% of people with peanut allergy diagnosed before the age of five years will become tolerant of peanuts. The medical records of all cases were followed in time to determine whether they became tolerant of peanuts and if so, the subject was removed from the prevalence estimate by subtracting that case from the numerator total.

Incidence

An incident case was defined as a new diagnosis of peanut allergy made at a REP facility from 1999-2007 in an Olmsted County resident.

Reaction Severity

Reaction severity, defined as the severity of the child's worst reaction to peanut before consulting a physician, was assessed in this study to address the debate as to whether the reported increase in children diagnosed with peanut allergy over the last few decades is accurate or a result of an increase in diagnosis of mild cases in recent years as a result of many potential factors including heightened awareness, improved surveillance, changes in diagnostic criteria, health insurance, or food availability.

Reaction severity is a dichotomous variable, mild versus moderate/severe. A mild reaction is defined as one in which the child experienced cutaneous symptoms only. A moderate/severe reaction is defined as an anaphylactic reaction, which is a systemic type 1-hypersensitivity reaction in which a child may experience a range of potential symptoms from moderate to severe. There are three criteria each reflecting a different clinical presentation and range of potential symptoms, which constitute anaphylaxis [16]. highly likely when any one of the following criteria is fulfilled[16]:

Criterion 1- Acute onset of an illness involving the skin, mucosal tissue or both (i.e. generalized hives, pruritus or flushing, swollen lips, tongue, uvula) and respiratory compromise or reduced blood pressure

Criterion 2- Two or more of the following that occur after exposure to a likely allergen

- Involvement of the skin-mucosal tissue (generalized hives, itch, flush, swollen lips, tongue, uvula)
- Respiratory compromise

- Reduced blood pressure
- Persistent gastrointestinal symptoms

Criterion 3- Reduced blood pressure after exposure to a known allergen for that patient

- In infants and children, reduced blood pressure is defined as low systolic blood pressure or greater than thirty percent decrease in systolic blood pressure

Tolerance

The most recent 2010 Guidelines for the Diagnosis and Management of Food Allergy in the United States refers to the point at which a person is believed to be able to consume peanut safely as the development of tolerance [69]. Tolerance was defined as the point at which a previously peanut allergic individual is able to consume peanuts without experiencing a type 1-hypersensitivity reaction [69]. A child was considered to have become tolerant to peanuts if they met criterion one and one of criteria 2-4 below:

1. No recent reactions to peanuts upon ingestion AND one of the following:
2. Passed an in office food challenge
3. The child's IgE testing level becomes negative (IgE<0.35 kU/l), while their skin prick test (SPT) test is still positive, they did not have a skin text or their skin test is negative
4. SPT changes to a negative status, less than 3 mm*3mm wheal and flare, while IgE results were always negative

5.3.3. Variables

The variables considered in the analysis are the following.

Age

Age represents the child's age at first diagnosis. Age was modeled as a categorical variable, 0-2 years and 3-17 years. Despite being a more powerful representation, age was not modeled as a continuous variable because the variable is not linear.

Year

Year represents the year of first diagnosis. Year was modeled as a categorical variable representing years 1999-2001, 2002-2004 and 2005-2007. Modeling year of first diagnosis as a categorical variable 1999-2007 led to large confidence intervals and unreliable estimates, thus the years were grouped to allow for an adequate sample size in each strata enabling a more sound statistical evaluation. Further, a three-level categorization allows an even distribution of years in each group, which enables a more natural interpretation of data.

Gender

Gender was modeled as a dichotomous variable, male and female.

5.3.4 Statistical Analysis

Participant characteristics of all cases were descriptively summarized by means and standard deviations for continuous variables or by frequencies for categorical variables. These characteristics included age of first diagnosis, year of first diagnosis, and gender**.**

An estimate of the prevalence of peanut allergy in 2007 was calculated by dividing the numerator, all prevalent and incident cases of peanut allergy in the medical records from 1999-2007, by the denominator, the Olmsted County population aged less than 18 years estimated from US census data. In calculating incidence rates, the entire

population of Olmsted County age 17 years or less was considered at risk. The overall denominator as well as age- and gender-specific person-years were estimated from decennial census data with linear interpolation between census years [63].

The incident diagnosis for each case contributed to the numerator for the incidence rate calculation for that respective year. Rates, per each year category (1999-2001m 2002-2004, 2005-2007), population-based and adjusted for age and gender, were standardized using the indirect method. Observed to expected ratios were calculated using the indirect method of standardization to data in 1999 for Olmsted County. A test for trend was conducted to assess whether there was a significant increase in the age- and gender-adjusted rates of peanut allergy diagnoses over time.

Poisson regression was used to estimate incidence rates and to examine the relationship of incidence rates to age, gender and calendar year of peanut allergy diagnosis. [64] Poisson regression is considered the ideal tool for this analysis as it allows the estimation of incidence rates for cohort data in which follow-up is variable, is well suited to rare outcomes, allows for assessment of covariates, including gender, age and year, and enables a test for departure from linearity. [64] The denominator for the incident rate calculation was estimated using person-years. In each year, the time at risk for those with a peanut allergy diagnosis was estimated as the time until they were diagnosed (i.e. A person who was diagnosed in March 1999 would contribute 0.25 person-years). For the rest of the population in that year with no disease, it was assumed they contributed one-person year.

The regression equation, which was used to estimate the incidence rates for peanut allergy for the years 1999-2001, 2002-2004 and 2005-2007 and to test for non-

linearity, whether the rates are statistically different from year to year and vary based on age of diagnosis and gender, is described below.

log (peanut allergy count)= B0+ B1Year + B2Age + B3Gender + log(person-years)

The variable year represents the year of incident diagnosis, and the variable Age indicates the age of incident diagnosis. Poisson regression allows estimation of rates from count data by offsetting the log of person-years.

A chi-square test was conducted to examine whether the number of diagnoses in 1999-2001, 2002-2004, 2005-2007 differed among those with mild as compared to moderate/severe reactions. Doing so addressed the question as to whether the reported increase in peanut allergy diagnosis over time is accurate or a result of factors such as heightened awareness or improved surveillance, which may lead to more cases of mild reactions being documented in the later as compared to a earlier years and not a true increase in rates of disease. All data were analyzed in SAS 8.0 software.

5.4 Results

There were 244 prevalent cases of peanut allergic children aged less than 18 years in Olmsted County, MN from 1999-2007. Twenty-eight prevalent cases acquired tolerance. Thus, the final prevalence estimate in 2007 is 216 cases/36,312 (the Olmsted County, MN population of children in 2007) or 0. 59%.

There were 170 incident cases of peanut allergy over the time period (Table 1). The majority of children were 0-2 years (77.7%), male (69.4%) and experienced mild reactions (76.5%). The number of incident cases of peanut allergy increased overall from 1999-2007 with 5.9% of new diagnoses in 1999 and 17.7% in 2007.

As seen in Figure 2 and 3, the overall, age and gender adjusted and standardized, rates increased 1.7-fold from 3.84 cases per 10,000 children in 1999-2001 to 6.53 cases per 10,000 children in 2005-2007. A test for trend was conducted to determine whether the annual age- and gender-adjusted rates of peanut allergy diagnoses increased statistically over time. The results indicated that there was a significant increasing trend in peanut allergy diagnoses over time from 1999-2007 (p=0.01).

Figure 2 presents the overall rates and the gender specific rates standardized using the indirect method to the 1999 Olmsted County population. Overall, rates of allergy in females were lower than the rates in males each year category. The rates among males and females increase over time from 1999-2001 through 2005-2007. Figure 3 presents the overall and age specific rates standardized using the indirect method to the Olmsted County 1999 population. As seen in the figure, the rates are the greatest among the 0-2 year old children in each year grouping as compared to the 3-17 year olds. The rates among children aged 0-2 years increase over time whereas the rates among those aged 3-17 years increase from 1999-2001 and then decrease in 2005-2007.

The multivariable analyses, as seen in Table 2, indicated that females had a statistically significant 82% lower rate of peanut allergy diagnosis as compared to males after adjustment for age and year of diagnosis (IRR=0.18 95%CI (0.07,0.48)). Furthermore, the rate of peanut allergy diagnosis increased over time as compared to the reference year, 1999-2001. Rates for peanut allergy diagnosis in 2002-2004 and 2005-2007 as compared to 1999-2001 are 2.13 (95%CI 0.61,7.41) and 5.15 (95%CI 1.63,16.3) times greater respectively, after adjustment for gender and age of diagnosis with only the years 2005-2007 indicating a significant association with peanut allergy rate. With

regards to age, as compared to children aged 0-2 years old, older children aged 3-17 years have a 99.9% significantly lower rate of peanut allergy diagnosis after adjustment for year of diagnosis and gender. In the multivariable analyses, age, gender, and year of diagnosis remained significantly associated with incident peanut allergy.

There exists a debate as to whether the recent increase in peanut allergy diagnosis is a result of more diagnoses of children experiencing mild reactions than they were previously, as a result of many potential factors including heightened awareness, improved surveillance, changes in diagnostic criteria, health insurance, or food availability and not truly a reflection of an increase in the rate of disease. The results from this analysis indicate that the number of diagnoses of peanut allergic children having both mild and moderate/severe reactions are increasing overall from 1999-2007. As seen in Table 3, there is not a significant difference in the number of children diagnosed having had mild as compared to moderate/severe reactions from 1999-2007 ($p=0.91$).

5.5 Discussion

The 2007 prevalence of peanut allergy in this sample was 0.59%, while the incidence increased 1.7-fold from 3.84 cases per 10,000 children in 1999-2001 to 6.53 cases per 10,000 children in 2005-2007. Additionally, males and children aged 0-2 years were significantly more likely to be diagnosed with peanut allergy compared to older children (3-17 years) and females.

Our 0.59% prevalence is within reported ranges yet, slightly lower than estimates of clinical allergy and much lower than estimates based on sensitization alone such as NHANES (8.6%) [3,4,5,15,19,28, 56]. It is known that sensitization is not a true estimate of

clinical allergy and thus, estimates of sensitization are expected to be much higher than those of clinical allergy, such as reported by this study [12].

The data suggest that there has been a statistically significant increase in peanut allergy diagnoses over time in this population. The age- and gender-adjusted rates of peanut allergy diagnoses increased 1.7-fold from 3.84 cases per 10,000 children in 1999-2001 to 6.53 cases per 10,000 children in 2005-2007, which is a consistent with reported studies [22,24,29-31,55, 56, 60,61]. There is uncertainty as to whether the reported increase in peanut allergy diagnosis is accurate or a result of an increase in diagnoses of mild cases in recent years. The data suggest that the numbers of diagnoses of peanut allergic children having both mild and moderate/severe reactions are increasing overall from 1999-2007 with no significant difference in the number of children diagnosed with mild compared to moderate/severe reactions from 1999-2007.

Further, the analyses indicate that females were significantly less likely to experience a peanut allergy diagnosis than male children, with 69.4% of cases found to be male. The literature indicates that the prevalence of peanut allergy is greater among males than females with reported estimates in the range of 63%-69% [62]. It is established that rates of atopic diseases differ according to gender [42, 62]. In population-based studies, these differences, assessed as skin test reactivity to one or more of a panel of allergens, have been reported throughout childhood and into early adulthood [62]. Specifically, rates in females were reported to be lower than males up to 15 years of age and in some studies up to 25 years of age, but are not consistently observed after age 25 [62]. Further, gender differences in atopic disease, assessed as total serum IgE levels, have been reported to be consistent across the lifespan, with levels in females being lower than those in males [62].

The physiological pathway leading to such sex differences may reported to be a result of immune dimorphism, the term given to differences in immune responses and regulation between the sexes [62].

Children aged 0-2 years were significantly more likely to be diagnosed with peanut allergy compared to older children aged 3-17 years. This conclusion is consistent with reported studies [42, 62]. Children aged 0-2 years are more susceptible to food allergies as a result of their immature immune system, which is more likely than an older child's to deem a food as foreign and launch an allergic reaction. Further, the introduction of new foods typically begins in this age range and if a reaction were to occur, it would be expected to surface at this time. [42, 62]

5.6 Strengths

The proposed study has a number of strengths. First, the questions as to the true prevalence and whether the incidence rate of diagnoses is increasing are important due to the etiologic, public health and clinical implications. Secondly, this study takes advantage of the population based medical data available through the Rochester Epidemiology Project resources [53]. This linked medical records system enables access to accurate and detailed clinical and laboratory data over many years, which are not typically available in other databases or research settings. The REP captures the bulk of residents living in Olmsted County and the majority of patients receiving care at one of the REP facilities consented to inclusion of their medical records into the REP database [52]. Thus, the patients captured in this medical record review are representative of the Olmsted County population.

Lastly, the REP medical records enable proper classification of children as peanut allergic according to the definition outlined by the Guidelines, which is a history of a type-1 hypersensitivity reaction to peanut, as diagnosed by a physician, in conjunction with positive diagnostic testing [65]. In addition, patient self-report of a reaction was determined to be a type-1 hypersensitivity reaction to peanut by a physician in this study as opposed to personal report of an adverse food reaction as a result of peanut, which is necessary to avoid the potential for misclassification bias.

5.7 Limitations

It is likely that the prevalence estimate is an underestimation of the true prevalence in this population due to the conservative case definition and method of data collection used in this study. There are several instances in which a case of peanut allergy may have been missed in the medical records because the child did not meet the diagnostic criteria for a case. For instance, a child who is labeled as peanut allergic in the records yet has no evidence of confirmatory testing was not considered a case. Also, a child who tested positive for peanut allergy yet, had never ingested peanuts was not considered a case. Further, children may have moved to Olmsted County without accessing care for their allergy.

The possibility exists that an incident event was improperly classified as a first time diagnosis leading to an overestimation of incidence. Every effort was made to review all medical records to be sure that each incident event was in fact the first time diagnosis. Further, the sample size of this study is small leading to an underpowered analysis. As such, the results must be interpreted cautiously. Lastly, Olmsted County is

a predominantly Caucasian community, which may limit extrapolation of results to more diverse communities.

5.8 Conclusions

The increase in rates of peanut allergy diagnosis among Olmsted County children experiencing both mild and moderate/severe reactions over time confirms that the increase is likely accurate and not a result of an increase in diagnoses of mild cases over time. This result provides support for the investigation into potential causes for this increase. Lastly, the higher rates among males and children aged 0-2 are consistent with biological theory, timing of age of first introduction of foods and other reported studies.

The results of this study are valuable in that an estimate of the burden of disease and confirmation of an increase in the incidence rate of diagnosis are crucial pieces of information for clinicians and public health professionals. The results provide important data to clinicians in terms of counseling patients. Further, as the numbers of peanut allergic children rise, public health will continue to play an increasingly important role in awareness, safety and educational efforts. Further, the results are important in informing public health policy, which has an important responsibility in regards to labeling laws and bans of peanuts in high-risk settings. Lastly, confirmation of an increase in rates provides support for further research into etiology, prevention and therapeutic techniques.

Future studies that address study limitations and utilize diagnostic criteria based on current clinical guidelines would be helpful to further validate the prevalence and incidence rate estimates and to confirm that the incidence rates are increasing among those children with true clinical peanut allergy regardless of severity of initial reaction.

5.9 Tables

Table 1: Characteristics of Children in Olmsted County with Incident Diagnoses of Peanut Allergy from 1999-2007

Number of cases (n)	170
Age of diagnosis in months (median and IQR)	18 (13.0-24.0)
Gender n (%)	
Male	118 (69.4)
Female	52 (30.6)
Diagnosis Year n (%)	
1999	10 (5.9)
2000	11 (6.5)
2001	19 (11.2)
2002	10 (5.9)
2003	12 (7.1)
2004	28 (16.5)
2005	22 (12.9)
2006	28 (16.5)
2007	30 (17.7)
Reaction Severity n (%)	
Mild	130 (76.5)
Moderate/Severe	40 (23.5)

Table 2: Multivariate Incidence Rate Ratios of Peanut Allergy Diagnosis

Variable	IRR	95% CI
Year		
1999-2001	Reference	
2002-2004	2.13	(0.61,7.41)
2005-2007	5.15	(1.63,16.3) *
Gender		
Male	Reference	
Female	0.18	(0.07, 0.48)*
Age		
0-2 years	Reference	
3-17 years	0.001	(0.0004,0.004)*

* Indicates a significant association ($p<0.05$)

Table 3: Number of Incident Diagnoses of Peanut Allergy in the Years 1999-2001, 2002-2004, and 2005-2007 by Severity of First Reaction

	Reaction Severity		
	Mild n=130, 76.5%	Moderate/Severe n=40, 23.5%	P value *
Year of Diagnosis			0.91
1999-2001	31(23.9)	9 (22.5)	
2002-2004	39 (30.0)	11 (27.5)	
2005-2007	60 (46.2)	20 (50.0)	

* P value from a chi-square test

5.10 Figures

Figure 1: Sample Size Waterfall

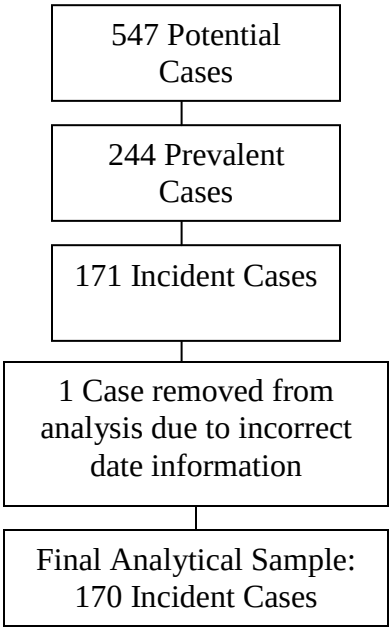


Figure 2: Gender-specific rates by year range (age adjusted) standardized to the 1999 Olmsted County population

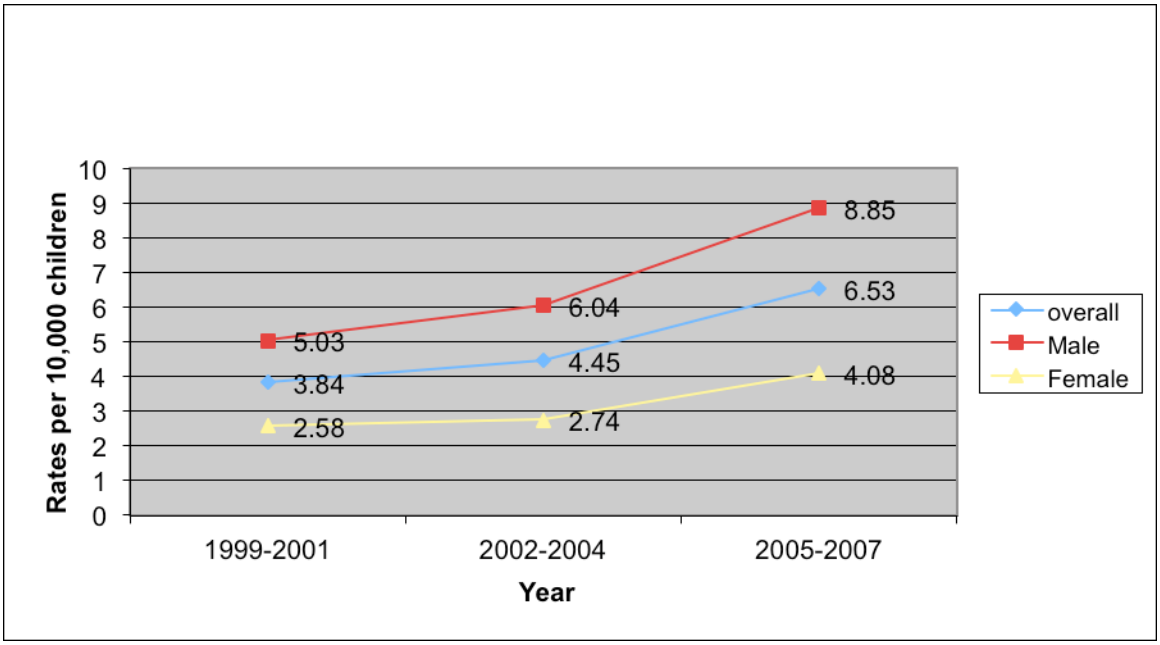
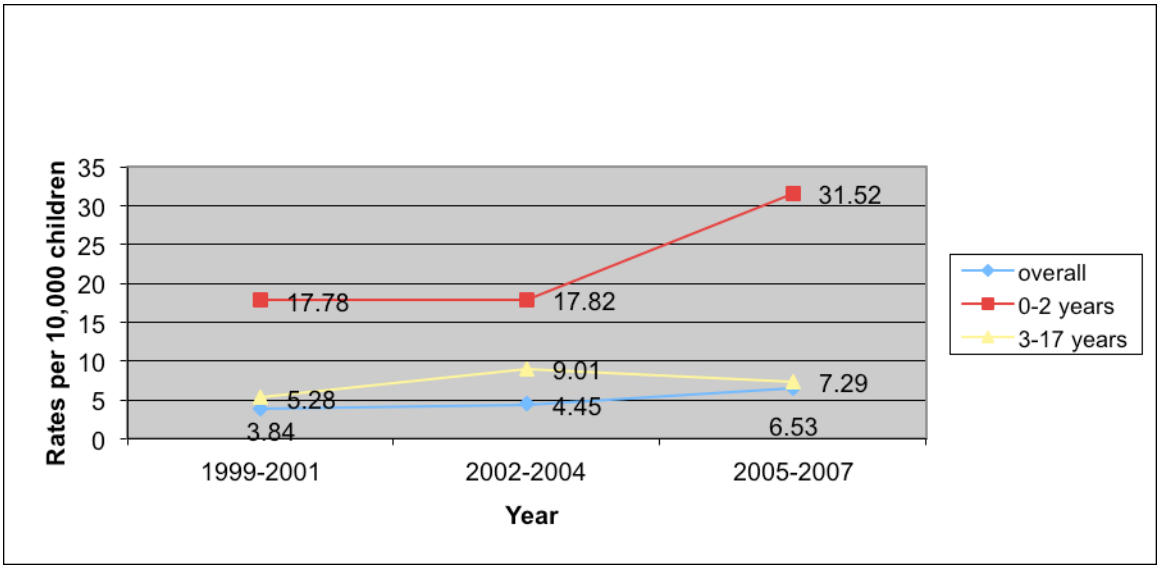


Figure 3: Age specific rates by year range (gender adjusted) standardized to the 1999 Olmsted County population



6.0 Study 2: An estimation of the number of children who develop tolerance to peanut and an examination of the association between peanut-specific IgE level and tolerance among peanut allergic children aged less than 18 years residing in Olmsted County, MN between the years of 1999-2007

6.1 Specific Aims

Peanut allergy is a significant health concern in the United States and worldwide with an estimated prevalence of 1% among children [19, 21-25]. Peanuts are one of the most common food allergens and account for most cases of fatal and near fatal anaphylactic reactions to food [23]. Recent studies indicate a two-fold increase in the prevalence of peanut allergies in the past two decades [22]. Peanut allergy presents early in life and a minority of children, 20%, develop tolerance, the ability to consume peanuts without experiencing a type 1 hypersensitivity reaction [21, 23,27,35]. In highly sensitive people, trace quantities of this ubiquitous food can induce severe allergic reactions. Currently, the only available treatment is strict avoidance, recognition of early symptoms of a reaction and usage of emergency medication.

Knowledge of the percentage of children that develop tolerance and the factors associated with acquiring tolerance are imperative to understanding the natural history of disease and clinical management of patients. To date, the current research is limited and results conflicting. Therefore, a well-designed study that estimates the percentage of children who develop tolerance to peanuts and assesses the association between peanut-specific serum IgE level and tolerance is necessary. The research herein contributes to filling this gap in the literature.

The objective of this study was two-fold. First, the study aimed to estimate the percentage of children aged less than 18 years diagnosed with peanut allergy in Olmsted County, MN from 1999-2007 that develop tolerance to peanuts. Secondly, the study aimed to estimate the rate of peanut allergy tolerance associated with initial peanut-specific IgE level. The central hypothesis was that the lower the peanut-specific IgE level, the greater the rate of peanut allergy tolerance. The hypothesis was formulated based upon the pathophysiology of a type-1 hypersensitivity reaction and the current research regarding the natural history of peanut allergy [21, 23,27,35]. To test this hypothesis, the following specific aims were addressed:

1. To estimate the percentage of peanut allergic children, diagnosed from 1999-2007, residing in Olmsted County, MN that develop tolerance to peanuts.
2. To examine the association between peanut-specific IgE level and the rate of peanut allergy tolerance among children residing in Olmsted County, MN.

This study is important and the results significant as the research addresses an area in which data is limited and results conflicting. Furthermore, an understanding of the factors associated with the development of peanut allergy tolerance are important for clinicians and families in terms of patient management and counseling.

6.2 Background and Significance

6.2.1 Introduction

The current literature defines tolerance, the point at which a previously peanut allergic individual is able to consume peanuts without experiencing a type 1-hypersensitivity reaction, with varying terms and definitions. The most recent 2010 Guidelines for the Diagnosis and Management of Food Allergy in the United States refers

to the point at which a person is believed to be able to consume peanut safely as the development of tolerance. [65] In an effort to be consistent and clear, this document uses the term tolerance throughout to refer to the state at which other authors may refer to as remission, resolution, or outgrowing a peanut allergy.

The development of tolerance to many foods, especially milk, eggs and soy tends to increase with increasing age, whereas tolerance to peanut develops less frequently [13]. Approximately 20% of children whose peanut allergy was established under the age of two years develop tolerance by school age [21, 23, 27, 35]. In comparison, 85% of allergic children develop tolerance to milk and egg by age five [11]. Children diagnosed as having food allergy after three years of age are less likely to develop tolerance [21, 35]. Of those who develop tolerance to peanuts after being diagnosed with a peanut allergy, 9% may relapse and become intolerant again [11].

Why food allergy persists in some people and not others is unclear [21]. Research investigating the association between many factors including the size of the skin test wheal, level of peanut-specific IgE antibody, severity of reactions, number of additional atopic diseases including other food allergies, age at diagnosis and the development of tolerance have had conflicting results. [21, 35,36]

Well-researched and supported evidence as to whether serum IgE level is associated with the development of peanut tolerance would provide important information about the natural history of disease for clinicians, patients and their families. Such knowledge is a crucial piece to clinical management and patient counseling. Therefore, expanding upon the current research on this association in an effort to clarify whether IgE level is valuable in predicting a patient's clinical course is necessary. The

current research on this association is limited and conflicting and thus, warrants further investigation.

6.2.2 Etiologic Basis

Immunoglobulin E, IgE, is the antibody released by the immune system when a type-1 hypersensitivity reaction takes place after exposure to a food in which the individual is allergic [1]. People with a stronger Th2 immune response produce more IgE [1]. The etiologic basis for the association between peanut-specific IgE and tolerance relates to the probability that individuals who are more likely to produce high levels of IgE would have a decreased ability to acquire tolerance.

6.2.3 Review of the Literature

It was initially believed that peanut allergic individuals did not develop tolerance to peanuts. This belief was based on two studies that reported similar rates of peanut allergy among adults and children [29, 37]. However, over the last ten years, studies emerged that indicate a percentage of peanut allergic children do develop tolerance. Hourihane and colleagues first reported evidence that a subset of children with peanut allergy developed tolerance. The authors evaluated 230 children with a diagnosis of peanut allergy and performed oral food challenges in 120. Oral challenges were performed in those children with negative skin tests or a history of tolerating peanut after receiving an initial diagnosis of allergy. Twenty-two children pass their challenge, which was 18% of those challenged and 9.8% of the total group [23].

The researchers conducted a matched case control analysis among 15 children who developed tolerance as compared to 15 children who did not. Children underwent challenges based on the clinical profile of each patient and as deemed fit by their

physician. Children were believed to have passed the food challenge and developed tolerance if they had a clear history of a peanut reaction and subsequently passed an in-office food challenge. Failure of a challenge was poorly defined as any signs of an allergic reaction.

The analysis indicated no differences between children that developed tolerance as compared to those who did not with respect to age at initial reaction, severity of initial reaction or peanut-specific IgE levels determined at initial hospital visit [23]. However, the authors found that the development of tolerance was associated with a smaller skin test, determined at initial hospital visit, and fewer allergies to other foods compared with those with persistent peanut allergy. Specifically, allergies to other foods were less common in those with tolerance (2/15) than those without (9/15) ($p=0.02$) and all 13 with tolerance but only 3/14 allergic had a wheal of less than 6 mm ($p<0.0001$) [23]. The number of other atopic conditions was also lower in the tolerance group, though this difference was not significant [23].

Spergel and colleagues conducted a retrospective cohort study of 293 patients with a diagnosis of peanut allergy. All families were offered a peanut challenge and a total of 33 children with a convincing history of a reaction and a positive skin test underwent a challenge. Of these 33 children, 14 passed their challenge and were believed to have developed tolerance to peanut. A positive challenge was defined as any of the following symptoms following peanut ingestion; respiratory symptoms, vomiting, hives, faring of atopic dermatitis or anaphylaxis occurring within two hours of last ingestion. Interestingly, none (0%) of the 5 patients with a history of peanut anaphylaxis developed tolerance, compared with 9 of 17 (64%) with a history of urticaria (i.e. a mild reaction)

(p<. 05) [23]. In addition, those who developed tolerance had significantly smaller skin prick wheal sizes, as determined at the child's initial evaluation, than the 19 whose allergy persisted (5.8 mm versus 13.0 mm p<0.005) [23]. It appears that the severity of prior reactions and size of the skin test response may be important predictors in the development of tolerance. A personal or family history of atopy, existence of other food allergies or age of initial reaction were not significantly related to tolerance in this analysis [66].

Skolnick and colleagues studied 223 children with a diagnosis of peanut allergy in an attempt to understand the natural history of the disease. The children that were offered an oral food challenge had not had a reaction in the past year and had an IgE level less than 20 kU/L. If a child had a history of a severe reaction, the cut-off for a food challenge was reduced to less than 10 kU/L. Eighty-five children consented and underwent a peanut challenge of which forty-eight passed. A child was deemed to have passed the in office food challenge if they did not experience any subjective or objective signs of a reaction. Thus, 21.5% were reported to have developed tolerance to peanut in this study [27].

The children that developed tolerance were more likely to have had a mild initial reaction (involving skin only) (p=0.03) [27]. Those who did not develop tolerance were significantly more likely to have a reaction involving three systems (gastrointestinal, skin and respiratory) (p=0.03) [27]. Those who became tolerant to peanut had a lower rate of atopic dermatitis (p=0.04), were less likely to have tree nut allergy (p=0.058), had significantly lower peanut-specific IgE level (median 2.2. kU/L), as determined at their initial physician visit, than those that failed a challenge (median 2.91 kU/L), were unable

to be challenged (median >100 kU/L) and refused the challenge (median 6.27 kU/L) ($p<0.0001$) [27]. The authors concluded that a low initial IgE level at diagnosis may improve the odds of developing tolerance. This conclusion was based on the fact that only two out of fifty four patients with initial IgE levels greater than 10 kU/L developed tolerance [27].

Vander Leek and colleagues conducted a longitudinal study to examine the natural history of peanut allergy among young children. Eighty-five children with peanut allergy were studied, including 55 who were followed for at least 5 years. Four subjects developed tolerance to peanuts in this study as indicated by a negative in office food challenge which was defined loosely as the absence of any signs of an allergic reaction. All four children had low serum specific IgE levels (<2 kU/L) despite differing initial presentations of allergy. The authors conclude that a low serum peanut-specific IgE level in a child who has not had a reaction to peanuts for at least 1 to 2 years may be an indication of a potential to develop tolerance [62].

A prospective cohort study was conducted to determine early predictors of peanut tolerance among young children with peanut allergy in Australia. In this large cohort of children based at a single tertiary pediatric allergy center, 18% of study participants developed tolerance to peanuts by the age of 5 years. A peanut-specific IgE level of 3 kU/L or greater before the age of 2 years (HR 2.74 95%CI 1.13-6.61 p=0.025) was an independent predictor of persistent peanut allergy. [36]. Furthermore, the children that developed tolerance had a mean initial IgE level of 2.0 kU/L whereas those that did not had a mean IgE level of 12.2 kU/L. This difference of approximately 10.0 kU/L was

significant ($p<0.001$). In this study, factors including age at diagnosis, mode of presentation and severity of index reaction were not predictive of tolerance [36].

Fleischer and colleagues conducted a follow-up study from their earlier study lead by Dr. Skolnick. This retrospective cohort reviewed the medical records of children who underwent oral peanut challenges since previous study completion in an attempt to further describe the natural progression of peanut allergy. Patients with IgE levels of 5kU/L or less were offered a peanut challenge. Eighty patients underwent peanut challenges. In this study, 44 out of the 80 patients challenged passed the challenge and were considered to have developed tolerance. A positive challenge was loosely defined as the presence of any subjective or objective signs of an allergic reaction to peanut. The overall rate of tolerance cannot be estimated from this study because only children with IgE levels of 5 kU/L were challenged. The study found that initial IgE levels were not significantly different between those that did and did not develop tolerance however, data on initial IgE levels was available for only 34 of the 80 patients challenged [68]. Furthermore, severity of initial reactions and type and number of atopic diseases were not predictive of tolerance.

Rangaraj and colleagues evaluated the development of tolerance to peanuts in a general pediatric setting. They conducted double blind placebo controlled food challenges to peanut in children aged 4 to 16 years of age with a history of peanut allergy, no history of an adverse reaction in the previous 2 years, an IgE level less than 17.5 kU/L, and a SPT diameter less than 8 mm. Only 8 of the 54 children were eligible for a food challenge and 4 of the 8 challenges were negative. The authors report that 15% of the children achieved tolerance to peanut [69].

As can be seen, the percentage of peanut allergic children that develop tolerance ranges from 4.8% to 21.5% in the reported studies with an average of 15%-20% [23,27,36,69]. The research as to whether serum IgE level is associated with development of tolerance to peanuts is conflicting. The studies by Skolnick, Vander Leek and Ho, indicate that IgE level is significantly associated with tolerance however, studies by Fleisher and Hourihane indicate no association. Therefore, a study that utilizes diagnostic criteria based on medical guidelines is needed to accurately estimate the percentage of children that acquire tolerance and examine the association between serum IgE level and tolerance to peanuts among previously diagnosed peanut allergic children.

6.2.4 Limitations in the Literature

The current literature has several limitations that the study reported herein addresses. First, the diagnostic criteria for peanut allergy are not consistent among studies. Some studies rely solely on self-report, others rely on sensitization as indicated by diagnostic testing, whereas others use diagnostic testing in conjunction with confirmation of self-reported history and others utilize food challenges [70]. These details are important in that self-report of an adverse food reaction or evidence of sensitization does not equate with clinical allergy. Increasing public concern regarding peanut allergy may lead to increased case identification (i.e. children with sensitization) but this does not necessarily mean that these children will experience an adverse reaction to peanuts. Thus, the specific criteria used to diagnose food allergy may have a significant impact on the results of studies. This study utilizes criteria based upon the current medical guidelines to avoid these limitations [65].

Specifically, one study defines peanut allergy as a history of a reaction after exposure to peanut, which is self-reported [23]. Perceived food allergy has been shown to be higher than food allergy confirmed by medical diagnosis [11]. In addition, two studies include children in their cases that have positive results to diagnostic testing yet have no history of ingesting peanut, thus, have not had a type-1 hypersensitivity reaction to peanut.[27,68,70] These limitations may lead to inclusion of children into the study that may not truly be peanut allergic, which would inflate estimates of tolerance. In an effort to avoid the potential for bias and misclassification error, incident diagnosis of peanut allergy must be based upon medical guidelines, a physician's diagnosis of a type-1 hypersensitivity reaction in conjunction with diagnostic testing, as was done in this study.

The word used to refer to tolerance and the definition of tolerance varies among studies making comparability difficult due to inconsistencies. Many other studies refer to tolerance as remission, resolution, or outgrowing peanut allergy. Furthermore, the criteria to define tolerance are inconsistent. Depending on how stringent or loose the criteria, the study may capture more or less children with tolerance. For instance, the criteria in the Vander Leek study requires a child to have a serum IgE level of less than 2 kU/L whereas the Skolnick study challenged children with levels less than 20 kU/L and 10 kU/L depending on severity of past reaction.

In addition, some studies relied on patients to volunteer for food challenges in an effort to document the development of tolerance [27, 66]. Doing so may create a non-response bias, which will skew those that develop tolerance to milder cases of peanut allergy because they may represent a subset of children that experienced less severe

forms of peanut allergy. This may lead to an over estimation of the percentage of children that develop tolerance.

In this study, as in others reported in this protocol, children are selected to undergo food challenges based on medical best practice as decided by their physician, which also varies [23, 62-63,71]. For instance, Olmsted County adopts a fairly conservative approach in which a child is tested with a food challenge only when their diagnostic testing is negative, both serum IgE and skin prick tests, and the child has not experienced a reaction in the recent past.

Several of the studies have small sample sizes. In addition to concerns about sample sizes, in many instances serum IgE levels were obtained only on a fraction of patients [62, 68]. Lastly, longitudinal studies suffer from loss to follow-up, which may occur because a child has recognized their ability to consume peanuts and does not feel it necessary to return for a challenge. Loss to follow up may occur and introduce bias, for instance, if those that had a more extreme initial reaction do not agree to a challenge and/or the response differs by parental education [62,36].

6.2.5 Need in the Literature

The current research reporting the percentage of children that develop tolerance to peanuts and whether serum IgE level is associated with tolerance is limited, conflicting and fraught with limitations. As a result, research is needed that uses terminology and tolerance criteria consistent with that of the Guidelines for the Diagnosis and Management of Food Allergy in the United States to avoid the shortcomings of other studies including misclassification of patients as peanut allergic, non-response bias, and reliance on subjective criteria for food challenge eligibility [65]. Such research creates a

consistent and standardized approach based on guidelines that can be interpreted across populations. Furthermore, the results of the study will clarify an important question about predictors of tolerance and enhance our understanding of the natural history of disease.

6.3 Research Design and Methods

6.3.1 Overview

This retrospective cohort study was performed by identifying cases of peanut allergy among children aged 17 years or less residing in Olmsted County, Minnesota, between 1999 and 2007. All cases of peanut allergic children, as identified by a strict study definition, were followed forward in time in the medical records until they either developed or did not develop tolerance, as defined in this study. Specifically, to be considered allergic to peanuts, each patient must have met the following criteria [54].

1. Positive history of an IgE-mediated type 1 hypersensitivity reaction AND
2. A positive blood test result (>=0.35 kU/l) OR
3. If blood testing is negative, positive skin prick test (>-3.0 mm) OR
4. If both blood and skin prick testing are negative, a positive double blind placebo controlled food challenge

A child was considered to have become tolerant to peanuts if they met criterion one and one of criteria 2-4 below:

1. No recent reactions to peanuts upon ingestion AND one of the following:
2. Passed an in office food challenge

3. The child's IgE testing level becomes negative (IgE<0.35 kU/l), while their skin prick test (SPT) test is still positive, they did not have a skin text or their skin test is negative
4. SPT changes to a negative status, less than 3 mm*3mm wheal and flare, while IgE results were always negative

Time to event was calculated as date of first diagnosis to tolerance, loss to follow-up or end of study.

This population-based study made use of the Rochester Epidemiology Project (REP), a population based medical records linkage system in which medical diagnosis data from the primary providers of care in Olmsted County have been retained in a central bank and linked to individual patients [53]. Each REP health care provider uses a dossier type record containing both inpatient and outpatient data. All diagnosis and surgical procedures recorded in these records are entered into a computerized index. All patient diagnoses have been numerically coded with either the Hospital Adaptation of the International Classification of Diseases, Second Edition, codes or the International Classification of Diseases, Ninth Revision, coding systems. These codes are indexed for computerized retrieval. Using this retrieval system, a search was conducted for all patients who had a new diagnostic code related to peanut allergy from 1999 through 2007, were residents of Rochester, Minnesota and who had given permission for their medical records to be used for research.

A list of patients with a potential diagnosis of peanut allergy in the medical records from all three facilities from 1999-2007 was generated and the medical charts were retrieved and reviewed to confirm cases of peanut allergy. Specifically, all 547

patients with codes representing the following diagnoses were reviewed: allergy food, allergy food with dermatitis, allergy peanuts, allergy food personal history, allergy peanuts personal history, anaphylaxis shock peanuts, anaphylaxis, and allergic reaction. The unique REP patient identification number assigned to each person when they first sought care at the respective medical facility organized the list. Each patient's unique number was used to check and eliminate duplicate entries. Patients who met the criteria for a diagnosis of peanut allergy were included in the study.

The Mayo Foundation, Olmsted Medical Center and University of Minnesota institutional review boards approved the study.

6.3.2 Study Population

Subjects who did not provide research authorization, whose residency information was not available, who were not residents of Olmsted County at the time of diagnosis and/or who did not meet the diagnostic criteria for peanut allergy were excluded from the data set. The complete medical records of each potential subject were reviewed to confirm a case of peanut allergy and collect information regarding demographics, health status, presenting symptoms, and outcome. As seen in Figure 1, of the group of 547 potential cases, 171 (31.3%) met the criteria for an incident case of peanut allergy. 23 cases were eliminated due to missing data regarding the exposure of interest, IgE level. In this study, one more case was eliminated because the person had negative study days due to an error in data entry that could not be verified and thus, was not included in the analysis. The final analytic sample consists of 147 cases. All data were abstracted using an electronic data collection form.

6.3.3 Variables

The variables considered in the analysis are the following.

Peanut-specific IgE Level

IgE level is the exposure variable of interest and indicates the peanut-specific serum IgE class level determined at the patient's first visit to a physician following their first reaction. IgE was represented as a categorical variable with the break down of classes 1-3 and 4-6, which was decided upon due to sample size and clinical considerations. This classification allowed for sufficient cases to exist in each cell, which enabled a reliable statistical evaluation. Further, this categorization creates an even breakdown that enables an easier interpretation of results in terms of low versus high IgE levels.

Tolerance

Tolerance refers to the point at which a previously peanut allergic individual is able to consume peanuts without experiencing a type 1-hypersensitivity reaction. Tolerance, the outcome of interest, is a dichotomous variable, yes or no.

Age

Age represents the child's age at first diagnosis. Age was modeled as a categorical variable, 0-2 years and 3-17 years. Despite being a more powerful representation, age was not modeled as a continuous variable because the variable is not linear.

Year

Year represents the year of first diagnosis. Year was modeled as a categorical variable representing years 1999-2001, 2002-2004 and 2005-2007. Modeling year of first diagnosis as a categorical variable 1999-2007 led to large confidence intervals and

unreliable estimates, thus the years were grouped to allow for an adequate sample size in each strata enabling a more sound statistical evaluation. Further, a three-level categorization allows an even distribution of years in each group, which enables a more natural interpretation of data.

Gender

Gender was modeled as a dichotomous variable, male and female.

Symptom Severity

Symptom severity represents the severity of the child's first reaction to peanut. The variable is dichotomous, mild or moderate/severe.

Number of Atopic Diseases

The variable, number of atopic diseases, is a dichotomous variable, indicating a higher burden as compared to a lower burden of atopic disease. A low burden of disease is defined as peanut allergy only or peanut allergy and one other atopic disease, eczema, allergic rhinitis or asthma. A higher burden of disease is defined as a peanut allergy plus 2 or 3 other atopic conditions.

6.3.4 Statistical Analysis

Participant characteristics of all cases were descriptively summarized by means and standard deviations for continuous variables or by frequencies for categorical variables. These characteristics included IgE class, age of first diagnosis, year of first diagnosis, gender, number of atopic conditions, reaction severity and tolerance status. In addition, the difference between those that developed tolerance as compared to those that did not was compared statistically for all patient characteristics. Specifically, the median test, Wilcoxon-rank sum, was utilized to test the difference between two medians for

categorical variables and the t test was used to analyze the difference between two means for continuous variables. When testing the difference between two proportions or percentages for categorical characteristics, the chi square test was utilized.

Cox regression was used to estimate the hazard rate ratios for the development of tolerance according to IgE level determined at the patient's initial physician visit. Cox regression is the chosen analytical tool because it estimates the hazard rate ratio for disease, which is the preferred measure of association for longitudinal studies in which participants are entered over time and follow-up is variable. The following covariates were selected a priori for examination in the analysis: age at first diagnosis, year of first diagnosis, gender, severity of symptoms during first reaction, and number of other atopic diseases. Variables were selected based on previously published reports of independent associations with the development of tolerance.

In order to create the best fit model variables were sequentially added individually to the model along with the exposure variable of interest. Variables with significant independent associations with the outcome of interest, tolerance, were retained based on the *P* values. The potential for effect modification was explored by adding the variable, age, to the model as an interaction term. As each variable was added to the model, confounding was evaluated by comparing the parameter estimate for the exposure variable among the models run with and without the potential confounder. If the exposure parameter estimate differed by greater than 10%, the variable was considered a confounder. If the confounding variable was not a significant covariate, it was still retained in the model to account for its confounding effects.

Cox regression is modeled based upon the proportional hazards assumption which states that the rate of disease is not assumed constant over time, yet the rate ratio comparing rates of disease among the exposed and non-exposed is assumed constant over time. The proportional hazards assumption was tested by modeling an interaction between exposure and time at risk, t. Time to event was calculated from the child's date of initial diagnosis to the development of tolerance, date of last follow-up or end of study. Analyses were conducted using SAS v8 software.

6.4 Results

Twenty-eight of the 171 incident cases of peanut allergy acquired tolerance, which yields an estimate of 16.4%. As seen in Table 1, the median age of first diagnosis is 18 months or 1.5 years old. The majority of children are male (69.4%) and have two or three other atopic conditions in addition to peanut allergy (54.4%). The most common IgE class level at first diagnosis in this sample was a class 3 (33.3%). The majority of first reactions to peanut were mild reactions (76.2%). The number of incident cases of peanut allergy increased overall from 1999-2007 with 4.8% of new diagnoses in 1999 and 18.4% in 2007.

As seen in Table 2, which displays patient characteristics stratified by tolerance status, there exists a significant difference between those that did and did not develop tolerance according to IgE class levels 4-6 as compared to 1-3 ($p<0.01$). In examination of the univariate associations, as seen in Table 3, a higher IgE class level (HR=0.07 95% CI 0.01-0.51) indicated a significant association with tolerance. As compared to the reference years, 1999-2001, there is not a significant association between years 2002-2004 and tolerance (HR=2.10 95% CI 0.54-8.23). The data indicates a significant

relationship between the years 2005-2007 as compared to the reference years however, due to a small sample size and large confidence interval, this prediction is not stable (HR=8.42 95% CI 1.53-46.3). Therefore, year of diagnosis is not considered a significant covariate.

A test of the proportional hazards assumption indicates that the assumption is not violated. Therefore, the hazard rate ratio for tolerance among those with IgE class levels 4-6 as compared to those with IgE classes 1-3 does not vary over time. (HR=0.91 95% CI 0.50-1.63) Lastly, an assessment of confounding indicated that the number of atopic conditions confounds the association between IgE and tolerance, thus this variable was retained in the final model.

The multivariable analysis, as shown in Table 4, indicated a significant 91.0% reduced likelihood for the development of tolerance among IgE class levels 4-6 as compared to levels 1-3 after adjustment for number of atopic conditions. (HR=0.09 95% CI 0.01-0.68) Further, those children with a higher burden of atopic disease, 3-4 as compared to 1-2 conditions, were at a non-significant 55.0% decreased risk for tolerance after adjustment for IgE class level (HR=0.45 95%CI (0.17-1.17)).

6.5 Discussion

In this study, 16.4% of children developed tolerance to peanuts, which is consistent with reported estimates that range from 15.0%-20.0% [23,27,36,69]. Further, the results indicated that IgE level is associated with tolerance, which is consistent with the studies by Skolnick, Van der Leek and Ho [27,36,62]. Specifically, the analysis indicated that those children with an IgE class level at first diagnosis of 4-6 are 91% less likely to develop tolerance as compared to those with a level 1-3 after adjustment for number of

atopic conditions. The results of this study are in accordance with the etiologic assumption regarding IgE levels and tolerance.

In addition to studies corroborating a significant association between IgE level and tolerance, the literature also reports studies indicating no association [23, 68]. The findings of this study contribute to a conflicting literature and as such additional studies are needed for clinicians to be able to discuss allergy prognosis with confidence.

6.6 Strengths

This study has a number of strengths including foremost, the fact that it addresses an important unanswered question in our literature. Secondly, the study utilized the population based medical data available through the Rochester Epidemiology Project resources [53]. This linked medical records system enables access to accurate and detailed clinical and laboratory data over many years, which are not typically available in other databases or research settings. The REP captures the bulk of residents living in Olmsted County and the majority of patients receiving care at one of the REP facilities consented to inclusion of their medical records into the REP database. Thus, the patients captured in this medical record review are representative of the Olmsted County population.

In addition, retrospective cohort studies have several advantages including the ability to estimate the risk and/or rate of disease. Furthermore, the fact that a subject's history is followed forward in time making the temporal association between exposure and disease clear. Furthermore, retrospective studies are more efficient and less expensive than prospective cohort studies as they do not require investigators to wait until the outcome of interest has occurred which may take many years.

6.7 Limitations

There are a few potential limitations of this study. The quality of data were dependent upon the source of medical records. The Rochester Epidemiology Project provides a comprehensive source for patient medical record review which allows for proper categorization of both case and outcome status. However, the potential for misclassification bias exists as a result of errors in the categorization of either exposure or outcome status. Since data were collected from medical records, it is expected that any misclassification is non-differential and thus, would only increase the similarity between the two groups. Furthermore, the definitions of cases, peanut allergic individuals, are based upon current medical guidelines [65]. Identifying cases based on strict, evidence based diagnostic criteria enhances accuracy and avoids the potential for misclassification bias.

The initial study definition of tolerance included only those children that passed an in-office food challenge, which is in accordance with Food Allergy Guidelines [65]. However, only 7 children met this strict definition. The definition was expanded to include those children whose testing results became negative yet, may not have undergone a food challenge to confirm results. The original strict definition was believed to be too stringent and as result, missed several cases that experienced tolerance. It is believed that once testing results become negative several patients may not return for confirmatory testing. Expanding the original criteria to the current more comprehensive criteria captured an additional 10 cases that experienced tolerance.

Second, with only twenty-eight cases of tolerance, the model is technically powered for only one covariate in addition to IgE class. Despite this fact, all potential confounders were assessed as part of this dissertation with the aim to cautiously interpret

the results as hypothesis generating. The study utilized the results from the univariate associations as suggestive for the multivariable association. Furthermore, the potential for unknown confounding exists. The study aimed to collect information on all potential cofounding variables and controlled for them in the analysis.

In addition, there were not any cases of children that experienced recurrence of allergy after achieving tolerance in this sample. However, it is possible that cases of recurrence may have been missed thus, leading to an overestimation of tolerance. Recurrence is estimated to occur in approximately 9% of cases of tolerance, though research is still indecisive on this topic [11].

The analysis only included cases of peanut allergy that were diagnosed from 1999-2007 at a REP facility. This study excluded those patients in whom diagnosis occurred prior to this time frame or at an outside facility. There is the small possibility that serum IgE levels may differ between the patients included and excluded in the study. The potential for selection bias was checked by comparing the IgE level of cases that were included and excluded from the analysis. The median IgE class level for both sets of cases is the same (median 3.0 IQR 2-4).

As a retrospective cohort study, it is possible that cases that develop tolerance may have been lost to follow-up. As a result of becoming tolerant of peanuts, the patient may not have returned for a follow-up visit with their physician. This potential would lead to an underestimation of the percentage of cases that develop tolerance. Finally, Olmsted County is predominantly white and middle class, which may limit generalizability to other populations.

6.8 Conclusions

An understanding of whether peanut-specific IgE level has clinical relevance regarding tolerance is important to clinicians from two standpoints. First, elucidating this association will further the utility of the current diagnostic test, peanut-specific IgE testing, from solely being able to predict one's likelihood to react upon exposure. Secondly, clinicians will be able to advise and manage patients appropriately based on their serum IgE levels in regards to their likelihood to develop tolerance.

Physicians may counsel patients on their tolerance prognosis based upon peanut-specific IgE level in the context of discussing research limitations. Specifically, physicians may relay to their patients the data that 16.4% of children in Olmsted County and 15.0-20.0% in the United States are expected to acquire tolerance. Further, physicians can relay the news to patients with IgE class levels 1-3 that they are more likely than those with class levels 4-6 to acquire tolerance. Lastly, knowledge of one's potential to outgrow peanut allergy based on IgE levels will provide realistic expectations for patients and families.

6.9 Tables

Table 1: Characteristics of Children with Incident Diagnoses of Peanut Allergy from 1999-2007

Number of cases (n)	147
Age of diagnosis in months (median and IQR)	18 (13.0-24.0)
Gender n (%)	
Male	102 (69.4)
Female	45 (30.6)
Number of atopic conditions (0/1 vs. 2/3) n (%)	
0/1 (Just peanut or peanut plus 1 other)	67 (45.6)
2/3 (Peanut plus 2 or 3 other)	80(54.4)
Reaction severity n (%)	
Mild	112 (76.2)
Moderate/Severe	35 (23.8)
IgE Class Level n (%)	
Class 1	8 (5.4)
Class 2	41 (27.9)
Class 3	49 (33.3)
Class 4	21 (14.3)
Class 5	11 (7.5)
Class 6	17 (11.6)
Year of Diagnosis n (%)	
1999	7 (4.8)
2000	6 (4.1)
2001	17 (11.6)
2002	10 (6.8)
2003	9 (6.1)
2004	24 (16.3)
2005	21 (14.3)
2006	26 (17.7)
2007	27 (18.4)

Table 2: Patient characteristics stratified by tolerance status (n=147)

	Tolerance Status		
	Yes n=28, 16.4% *	**No n=153, 83.6%**	**p value**
Age of Diagnosis n (%)			p=0.30
0-2 years	16 (88.9)	101(78.3)	
25 months-204 months	2 (11.1)	28(21.7)	
Gender n (%)			p=0.17
Male	10 (55.6)	92 (71.3)	
Female	8 (44.4)	37 (28.7)	
Year diagnosis n (%)			p=0.98
1999-2001	4 (22.2)	26 (20.2)	
2002-2004	5 (27.8)	38 (29.5)	
2005-2007	9 (50.0)	65 (50.4)	
Number of atopic conditions n (%)			p=0.16
1-2	11 (61.1)	56 (43.4)	
3-4	7 (38.9)	73 (56.6)	
Reaction Severity n (%)			p=0.18
Mild	16 (88.9)	96 (74.4)	
Moderate/Severe	2 (11.1)	33 (25.6)	
IgE class n (%)			p<0.01 **
1-3	17 (94.4)	81(62.8)	
4-6	1 (5.56)	48 (37.21)	

* Calculation based on n=170 (number of total incident cases not including cases with missing IgE information (n=23) or inaccurate date information (n=1))
** Indicates p<0.01
Note: P value from chi-square

Table 3: Univariate Association between Potential Risk Factors and Tolerance (n=147)

	HR	95% CI
Age at diagnosis [1]	0.56	(0.13-2.43)
Gender [2]	1.32	(0.50-3.44)
Year of diagnosis [3]		
2002-2004	2.10	(0.54-8.23)
2005-2007	8.42	(1.53-46.3)*
Number of atopic conditions [4]	0.43	(0.17-1.12)
Reaction severity [5]	0.57	(0.13-2.57)
IgE [6]	0.07	(0.01-0.51) *

* Indicates $p<0.01$

[1] Reference group= 0-2 years

[2] Reference group= Male

[3] Reference group= 1999-2001

[4] Reference group= Only peanut allergic or peanut and one other atopic condition (allergic rhinitis, eczema, asthma)

[5] Reference group= Mild Reaction

[6] Reference group= IgE classes 1-3

Table 4: Multivariate Association between Risk Factors and Tolerance (n=147)

	HR	95% CI
Number of atopic conditions [1, a]	0.45	(0.17-1.17)
IgE [2]	0.09	(0.01-0.68) *

* Indicates $p<0.01$

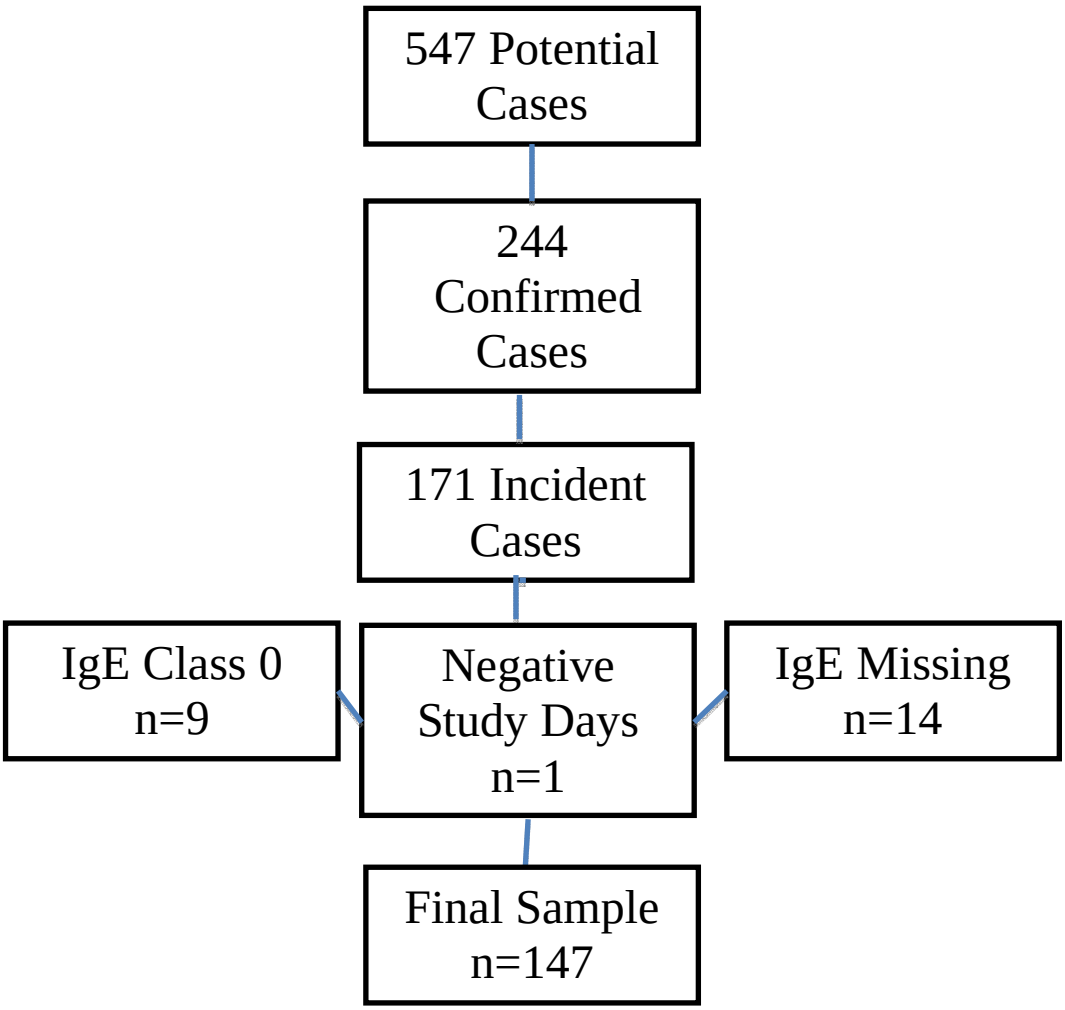
[1] Reference group= Only peanut allergic or peanut and one other atopic condition (allergic rhinitis, eczema, asthma)

[2] Reference group= IgE classes 1-3

[a] Non-significant covariate yet a confounder of the association

6.10 Figures

Figure 1: Sample Size Waterfall



7.0 Study 3: An analysis of the association between serum IgE Level and reaction severity among peanut allergic children aged less than 18 years residing in Olmsted County, MN between the years of 1999-2007

7.1 Specific Aims

Peanut allergy is a significant health concern in the United States and worldwide with an estimated prevalence of 1% among children [19, 21-25]. Peanuts are one of the most common food allergens and account for most cases of fatal and near fatal anaphylactic reactions to food [23]. Recent studies indicate a two-fold increase in the prevalence of peanut allergies in the past two decades [22]. Peanut allergy presents early in life and a minority, 20%, of children develop tolerance, the ability to consume peanuts without experiencing a type 1 hypersensitivity reaction [21, 23,27,35]. In highly sensitive people, trace quantities of this ubiquitous food can induce severe allergic reactions. Currently, the only available treatment is strict avoidance, recognition of early symptoms of a reaction and usage of emergency medication.

Many investigators have studied the ability of the diagnostic test for peanut allergy, peanut-specific serum IgE level, to predict a person's likelihood to experience a clinical reaction to peanuts. However, there is limited research on the association between peanut-specific serum IgE level and reaction severity, which indicates conflicting results. An understanding of the relationship between peanut-specific IgE level and severity of reaction will expand upon the utility of our current diagnostic test from solely predicting likelihood of clinical reactivity to an understanding of the association with reaction severity. Such knowledge will allow clinicians to advise

patients appropriately in regards to reaction likelihood as well as severity based on the results of diagnostic testing.

This cross-sectional study intends to fill this gap in the literature by examining the association between peanut-specific IgE level, taken at the patient's first physician visit, and reaction severity, defined as the patient's most severe reaction prior to visiting their physician. The objective of this proposal was to estimate the odds of reaction severity according to diagnostic testing level. The central hypothesis is that the lower the peanut-specific IgE level, the less severe the reaction. The hypothesis was formulated based upon the pathophysiology of a type-1 hypersensitivity reaction and the current research [66, 72, 74, 75]. To test this hypothesis, the following specific aim was addressed:

1. Examine the association between peanut-specific IgE levels, taken at the patient's first physician visit, and reaction severity, defined as the patient's most severe reaction prior to visiting their physician, among peanut allergic children residing in Olmsted County, MN.

This study is important as the current research is limited and conflicting. Clarification of this association is necessary to expand upon our understanding of the utility of peanut-specific IgE levels, which will enable more comprehensive clinical management and answers an important question for patients.

7.2 Background and Significance

7.2.1 Introduction

Type I Hypersensitivity Reaction

The term hypersensitivity denotes a condition in which an immune response results in an exaggerated or inappropriate reaction that is harmful to the host [1].

Hypersensitivity diseases are classified on the basis of the principle immunologic mechanism that is responsible for tissue injury and disease [1]. Food allergy is classified as one of four types of hypersensitivity conditions called type I or immediate hypersensitivity [2]. Type 1 hypersensitivity is a rapid, IgE antibody, mast cell mediated vascular and smooth muscle reaction provoked by re-exposure to a specific type of antigen referred to an allergen [1]. An initial contact is a necessary preliminary event that induces sensitization to that allergen [2].

Pathophysiology of a Type 1 Hypersensitivity Reaction

A type I hypersensitivity reaction against an allergen, encountered for the first time in an atopic individual, develops as a consequence of activation of T_h2 cells in response to protein antigens (allergens) [1]. Allergen specific T_h2 cells produce various cytokines, including interleukin-4 (IL-4) and interleukin-13 (IL-13). The action of these cytokines in conjunction with co-stimulatory signals from the T_h2 cells stimulate B cells specific for the foreign antigen to class switch to producing IgE antibodies [1,9].

The antigen specific IgE antibodies secreted from the B cells circulate in the blood and bind to high affinity receptors, FcεRI, expressed on the surface of immune cells called mast cells and basophils [1, 2]. Thus, in an atopic individual, mast cells and basophils are coated with IgE antibodies specific for the antigen(s) to which the individual is allergic. The process of coating the cells with IgE is called sensitization because coating with IgE specific for an antigen makes the mast cells sensitive to activation by subsequent encounter with that antigen [1].

Allergens are small proteins with a repeating structure to which many IgE antibodies can bind close together [7]. Upon a subsequent exposure to the same allergen,

the allergen cross-links the IgE molecules on the mast cell and basophils surfaces dragging the FcεRI receptors together. This clustering of FcεRI receptors signals activation of the sensitized cell[7]. Activated mast cells and basophils undergo a process called degranulation, during which biochemical signals form the signal transducing chains on the FcεRI cause rapid release of mediators [7]. The release of mediators is responsible for a variety of physiologic responses including the acute vascular and smooth muscle reactions and inflammation associated with hypersensitivity reactions.

Identifying Peanut as the Cause of a Type 1 Hypersensitivity Reaction

The primary tools available to identify peanuts as the cause of a type-1 hypersensitivity reaction include patient medical history in conjunction with allergy testing for peanut-specific IgE antibodies [21]. If history indicates a type-1 hypersensitivity reaction to peanut, specific IgE testing is the next step. [21, 47] A blood test is conducted to determine peanut-specific IgE antibodies in serum to confirm the allergy [21, 47]. The ImmunoCap by the company PHADIA is the current food specific IgE antibody blood test, which is used to diagnose peanut allergy [47]. The test involves drawing blood from the patient's arm and sending it to a laboratory to be analyzed for the presence of antibodies to specific allergens. The amount of allergen specific IgE contained in the patient's blood is calculated to determine how much antibody that person has to that specific allergen. The assays use a total serum IgE heterologous reference curve based on a World Health Organization IgE standard and quantitative results are reported in classes (class 1 through 6) or units of concentration that range from 0.35 to 100 kU/ml (see table 1). [21]

Prior to the development of the Immunocap in 1989, the RAST test was used to identify and quantify the level of peanut-specific IgE in serum. A few of the older laboratory results from Mayo patients and many of the less recent tests performed for Olmsted County patients were performed by an outside laboratory that uses the older RAST technology. The reported IgE values from RAST tests are based on a different scale than the Immunocap. The results from RAST testing are reported as peanut-specific IgE levels in a range from 0 to 40,000 mass units or greater. The levels have associated class ratings and similar to the ImmunoCap, increasing values represent greater levels of peanut-specific IgE in serum. Studies comparing serum analyzed by multiple laboratories and technologies indicate that the class ratings are comparable between the two technologies when a level of 750 is used as the cut-off for a positive result [48]. See section, Diagnosis of Peanut Allergy, and tables 1 and 2 for further explanation of the rating scales.

If blood test results are negative (<0.35 (ImmunoCap) or <751 (RAST)), yet the patient has a positive history of a type-1 hypersensitivity reaction from peanut, an allergy skin prick test is usually performed to double check the diagnosis [21, 47]. A serum blood test is preferable as the first diagnostic tool, however, for several reasons:

- The patient does not have to discontinue medications that may interfere with test results or cause medical complications
- In the event that a patient suffers from severe skin conditions such as widespread eczema or psoriasis
- A patient has such a high sensitivity level to the suspected allergen that any exposure may result in potentially serious side effects

Furthermore, the serum test may be a better way to follow children for the presence of food allergy since skin tests can remain positive even when clinical activity lessens as a child develops tolerance to the food [20].

Increasing peanut-specific IgE levels correlate with increasing probability of clinical reactivity yet, it is not clear from the current research if serum IgE level correlates with severity of reaction. An understanding of this relationship would expand the utility of our current diagnostic tools and enhance clinical management. Therefore, further research is necessary to clarify the association between diagnostic testing level and reaction severity to enable a more comprehensive interpretation of testing levels and physician advisement of patients.

Factors that Affect Peanut-Specific IgE Levels

IgE antibodies are made by the body's immune system in response to foreign substances including food proteins. Individuals with atopic disease have raised levels of IgE, which is measured with blood tests. Total IgE levels naturally increase from infancy to adolescence when they plateau and then slowly decrease with older age [81]. Specific types of allergy tend to be age-related as well. The production of IgE antibodies to food allergens has been reported to be high in young children and is often replaced by IgE antibodies to inhalant allergens as the child ages. For instance, allergy to cow's milk and egg are common in those under three years old and then tend to resolve naturally with time. However, peanut, nut and fish allergy tend to persist through life with many studies indicating that levels of peanut-specific IgE remain high for many sufferers.

It is not understood if peanut-specific IgE levels fluctuate and if they follow a similar age pattern. One study investigated the development of peanut hypersensitivity

among children with sensitization and clinical allergy to peanut from baseline in 1993-1995 to follow-up 5-6 years later [81]. Increased IgE level at follow-up was related to age [81]. Subjects aged 0-6 years at initial test occasion were significantly more likely to have a higher IgE class at follow-up than older children. Exposure and/or reaction to peanut during the 5-6 years since diagnosis did not affect IgE levels at follow-up [81]. The results of this study seem to follow the reported pattern of decreasing IgE levels with age however diagnostic limitations prevent a definitive conclusion.

In sum, it is not known if factors such as age, repeated exposure to peanut, subsequent reactions, or health status of the child affect peanut-specific IgE due to a lack of research in this area. As a result, physicians do not interpret IgE test results differently if they obtain them soon after a reaction, as there is no data to indicate that a recent reaction would affect a person's peanut-specific IgE level (personal communication, Dr. Matthew Rank).

7.2.2 Etiologic Basis

Immunoglobulin E, IgE, is the antibody released by the immune system when an allergic reaction takes place after exposure to a food in which the individual is allergic [1]. Antibodies are specific to each type of foreign substance [1]. An immunoglobulin test is conducted to determine what the person is allergic to and to measure the level of immunoglobulins in blood [1]. Researchers determined predictive values of IgE levels for certain foods including peanuts. If a person's level is higher than a certain value for that food, there is a 95% chance the person will have an allergic reaction if they ingest that food [65].

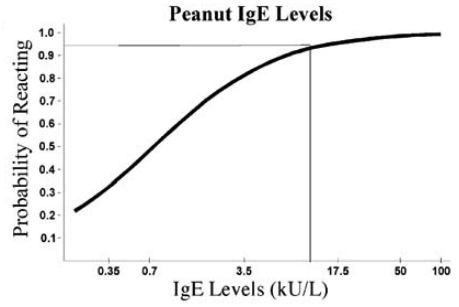
Individuals with a stronger Th2 immune response produce more IgE [1]. It is known that an individual's peanut-specific IgE level is associated with their likelihood to react upon exposure and individuals have an increasing probability of clinical reactivity with increasing level of peanut-specific IgE values. It would stand to reason that the elevated IgE in the blood would cause a more severe reaction because there is more IgE for the allergen to cross-link on mast cell cells and basophils leading to the release of the mediators responsible for a stronger and more robust physiological response.

7.3.3 Review of the Literature

Relationship between diagnostic test results and clinical reactivity

Research indicates that peanut-specific IgE antibody level is predictive of a patient's likelihood to experience a type-1 hypersensitivity reaction [77, 66]. Diagnostic decision points have been established for peanut based on the World Health Organization IgE standard [82]. Sampson and colleagues determined that a peanut-specific IgE level of 14 kU/L has a 95% positive predictive value [45, 72, 66]. Furthermore, patients have an increasing probability of clinical reactivity with increasing level of peanut-specific IgE values as seen in Figure 1 [45]. Thus, clinicians are able to advise patients based on their likelihood to react upon exposure to peanut based on their peanut-specific IgE level.

Figure 1. Predictive values of peanut-specific IgE Levels[45]



90. Increasing probability of clinical reactivity with increasing level of food-antigen specific IgE value; note: values <0.35 do not exclude allergic reactivity

Relationship between diagnostic test results and reaction severity

The current research indicates that serum IgE levels are able to predict the likelihood of clinical reaction to peanut for an individual patient. It is not known, however, if the diagnostic testing level, peanut-specific serum IgE, is associated with reaction severity. Clarification as to the relationship between testing level and reaction severity would significantly enhance the utility of the diagnostic test and enable more comprehensive clinical management.

Sampson and colleagues conducted a prospective study of 196 children over a 10-year period to determine the utility of serum IgE levels in the diagnosis of IgE-mediated food hypersensitivity. As part of this study, the researchers assessed the correlation between IgE level, as determined at their initial visit, and reaction severity as defined as the child's worst reaction prior to visit or recorded during an in office challenge. Reaction severity was categorized as mild, moderate or severe based on the authors' scoring system. The authors did not find an association between the level of peanut-specific IgE and severity of allergic reaction reported by history or during a food

challenge [73]. However, the reported values appear to follow a pattern of increasing median IgE level with increasing severity of reaction that is hypothesis generating [73].

Vander Leek and colleagues conducted a longitudinal study of 102 children to examine the natural history of peanut allergy among young children. The study found that the group of children with only skin symptoms during their first and any subsequent reactions had significantly lower levels of serum IgE, as taken at time of study, than those who experienced respiratory and/or gastrointestinal symptoms during their first reaction (median 1.25 kU/L versus 10.4 kU/L p=0.02) [62]. The authors state that it is tempting to suggest that serum specific IgE levels could predict future symptoms during adverse reactions however, due to the fact that the overlap of individual values between groups is so great, they can not suggest that these values have any prognostic value.

A cross sectional study was conducted of 1,000 nut (peanut and tree nut) allergic children and adults in the United Kingdom. The researchers examined the association between serum IgE level, assessed at the initial physician visit, and severity of the most severe reaction experienced before consulting the allergist. Severity was graded as mild, moderate and severe. The researchers did not find a correlation between SPT size or IgE level and severity of worst reaction from peanut. The authors were not able to exactly quantify the amount of nut ingested but were able to identify those that had eaten at least one nut as compared to those who ate a trace amount or had only skin contact in order to conduct a sub-analysis of the data. The results did not change. Thus, the authors concluded that the magnitude of SPT size or IgE level is not associated with clinical severity [67].

Hourihane and colleagues conducted a questionnaire study of 622 self-reported, peanut allergic children and adults to understand the patterns of clinical severity and symptom progression of allergic reactions to peanut. Serum IgE was collected in 346 subjects after receipt of their questionnaires. Reactions were categorized as mild versus severe. Overall reaction severity was classified based on the most severe reaction reported. The results indicate that peanut-specific IgE was not associated with clinical severity in this study [75].

A case control study was conducted in Sydney, Australia to determine the usefulness of peanut SPT and/or IgE level for predicting the risk of a severe reaction during an oral food challenge of up to 12 g of peanut in peanut sensitized children. The study involved 55 children; 34 who completed the challenge and did not react or reacted without anaphylaxis comprised the comparison group and 21 children who experienced anaphylaxis constituted the study group. The mean SPT wheal size and median peanut-specific IgE level were each tested for an association with the occurrence of anaphylaxis. The mean peanut SPT wheal size and specific IgE level were significantly associated with the severity of reactions on challenge. The mean SPT wheal size for the anaphylactic group was 10.2 mm compared with 6.7 mm in the control group ($p < 0.0001$). The median peanut-specific IgE level in the anaphylactic group was 19 kU/l compared with 0.68 kU/l in the control group ($p < 0.0001$). The authors concluded that provided a fixed amount of peanut is ingested, available tests for peanut allergy may assist in predicting the risk of anaphylaxis during challenge in peanut-sensitized children [77].

A study was designed to assist in developing future regulatory and industry guidelines aimed at improving food labeling by identifying no-observed-adverse-effect levels (NOAELs) and eliciting doses (EDs) for peanut in a group of peanut-sensitized children. A NOAEL is the level of exposure at which there is no increase in the frequency or severity of any adverse effects in the exposed population. A group of 27 children were evaluated by questionnaires, skin prick test, IgE, and double-blind placebo-controlled food challenge (DBPCFC) with peanut in an effort to identify these levels and to investigate the relationship between severity of the reaction during DBPCFC with peanut-specific IgE level. The results indicate that the severity of the reaction during DBPCFC was weakly correlated to SPT (r= 0.49; p=0 .021), but not to IgE levels [78].

A cross sectional assessment was conducted to compare a questionnaire evaluation of 40 patients' (adults and children) most recent reaction to peanut as a result of accidental exposure in the community with the results of a double blind placebo controlled food challenge. As part of this analysis, the authors assessed whether peanut-specific IgE and SPT wheal size, evaluated at the time of challenge, were associated with clinical severity in community and DBPCFC-induced reactions. The results indicate that only the most recent community reaction was associated with the severity of reaction in the DBPCFC, but this association was weak (r= 0.37, P=0.03). Furthermore, IgE and SPT wheal size were not associated with community reaction severity score but IgE level correlated well with the challenge score (r=0.6, P=0.001). Thus, this study highlights the fact that IgE level is associated with severity of reaction induced during peanut DBPCFC, as assessed by a scoring system that takes into account symptom severity and dose of allergen [79].

A retrospective chart review was performed to examine the risk and reaction severity of failed oral food challenges on children who underwent food challenges to milk, egg, peanut, soy, and/or wheat Johns Hopkins Pediatric Allergy Clinic from 1997 to 2003. The authors found that higher peanut-specific IgE levels, taken at the time of challenge, corresponded with more severe reactions. Specifically, those with mild reactions had a mean IgE level of 1.3, moderate reactions had a mean level of 2.1 and severe reactions had a mean level of 2.2 ($p<0.05$) [80].

A cross-sectional study of 363 children referred for an evaluation of suspected food hypersensitivity was conducted to evaluate diagnostic strategies. All participants underwent the same approach to diagnosis, which included a clinical history, a skin prick test (SPT), and a specific IgE assay. Double blind placebo controlled food challenges, DBPCFCs, were performed on all the children. The researchers compared the performance characteristics of the SPT and the specific IgE assay with those provided by the DBPCFCs. The analysis indicated no association between IgE level and the initial severity of the food allergy or the symptoms triggered by the DBPCFCs in subjects with peanut allergy [77].

7.2.4 Limitations in the Literature

The current literature has several limitations, which the study reported herein addresses. First, the definition of peanut allergy is not consistent among studies. Some studies rely solely on self-report, others rely on sensitization as indicated by diagnostic testing, whereas others use diagnostic testing in conjunction with confirmation of self-reported history and others utilize food challenges. These details are important in that a self-report of an adverse food reaction or evidence of sensitization does not equate with

the potential for a clinical reaction upon exposure to that food. Increasing public concern regarding peanut allergy may lead to increased case identification (i.e. children with sensitization) but this does not necessarily mean that these children will experience an adverse reaction to peanuts. Thus, the specific criteria used to diagnose food allergy may have a significant impact on the results of studies due to the potential for inaccurate data if a patient self-reports allergy yet, does not truly have a history of an type-1 hypersensitivity reaction to peanut and thus, is not truly a case. This study utilized criteria based upon current guidelines [65].

Specifically, one study identified cases based upon self-report responses to a questionnaire [75]. Perceived food allergy has been shown to be higher than food allergy confirmed by medical diagnosis [11] Reliance upon self-report data may lead to inclusion of children into the study that may not truly be peanut allergic which influences the results in regards to serum IgE levels and reaction severity. Severity of reaction was classified based upon self-report in three reported studies [66-67,75]. In an effort to avoid the potential for bias and misclassification error, diagnosis of peanut allergy must be based upon the Guidelines for the Diagnosis and Management of Food Allergy in the United States including a physician's diagnosis of an type-1 hypersensitivity reaction in conjunction with diagnostic testing, as was done in this study [65].

Additionally, the temporal relationship between IgE levels and reaction occur at varying time points in the published literature. The time point of the referent reaction varies among studies and includes the worst reaction experienced before the patient's first visit to the physician, most recent reaction, both of which occur prior to the time in which IgE levels are drawn, and lastly, an in-office challenge, which is completed at the same

time that IgE levels are drawn. Three of the reported studies refer to the referent reaction that occurred prior to, four refer to the reaction occurring concurrently with IgE levels being drawn and two studies employ both methods. The studies that analyzed the association with the time points occurring concurrently found an equal amount of significant and non-significant associations. The studies that analyzed data from a prior reaction with an IgE testing occurring after the reaction found two non-significant associations and two cases of non-significance with suggestive trends. The differences in timing of when IgE levels are determined and reactions occur will affect the interpretation of the association between IgE level and reaction severity and ability to draw temporal conclusions. It is not clear due to inconclusive results from the published literature how timing affects study results.

Lastly, the classification of reaction severity differs among the reported studies making comparisons of results difficult. Further, several of the studies have small sample sizes. In addition to concerns about sample sizes overall, in many instances serum IgE levels were obtained only on a fraction of patients [62]. In addition, one study analyzed the association between the nut that caused the most severe reaction and IgE level. The research presented results pertaining to all nuts in the aggregate and did not present data specific to the association between peanut-specific IgE level and the most severe reaction.

7.2.5 Need in the Literature

The current research indicates that serum IgE levels are able to predict the likelihood of clinical reaction to peanut for an individual patient. It is not known, however, if peanut-specific serum IgE level is associated with reaction severity. The

current research on the association between serum IgE level and reaction severity is limited, conflicting and fraught with limitations. Research is needed that relies on diagnostic criteria outlined by the Guidelines for the Diagnosis and Management of Food Allergy to avoid the potential for misclassifying patients as peanut allergic. Doing so will also avoid reliance on self-report of a reaction, which insures that reaction severity is not only accurate but indeed a type-1 hypersensitivity reaction. In sum, research is needed based on accurate and consistent data collection methods to enhance our understanding of this relationship which will further the clinical utility of the diagnostic test, serum peanut-specific IgE level and answer an important question for patients.

7.3 Research Design and Methods

7.3.1 Overview

This cross-sectional study was performed by identifying cases of peanut allergy among children aged 17 years or less residing in Olmsted County, Minnesota, between 1999 and 2007. This population-based study made use of the Rochester Epidemiology Project (REP), a population based medical records linkage system in which medical diagnosis data from the primary providers of care in Olmsted County have been retained in a central bank and linked to individual patients [53]. Each REP health care provider uses a dossier type record containing both inpatient and outpatient data. All diagnosis and surgical procedures recorded in these records are entered into a computerized index. All patient diagnoses have been numerically coded using either the Hospital Adaptation of the International Classification of Diseases, Second Edition, codes or the International Classification of Diseases, Ninth Revision, coding systems. These codes are indexed for computerized retrieval. Using this retrieval system, a search was conducted for all

patients who had a new diagnostic code related to peanut allergy from 1999 through 2007, were residents of Rochester, Minnesota and who had given permission for their medical records to be used for research.

A list of patients with a potential diagnosis of peanut allergy in the medical records from all three facilities from 1999-2007 was generated and the medical charts were retrieved and reviewed to confirm cases of peanut allergy. Specifically, all 547 patients with codes representing the following diagnoses were reviewed: allergy food, allergy food with dermatitis, allergy peanuts, allergy food personal history, allergy peanuts personal history, anaphylaxis shock peanuts, anaphylaxis, and allergic reaction. The list was organized by the unique REP patient identification number assigned to each patient when they first sought care at the respective medical facility. Each patient's unique number was used to check and eliminate duplicate entries. Patients who met the criteria for a diagnosis of peanut allergy, as defined below, were included in the study. Specifically, to be considered allergic to peanuts, each patient had to meet criterion 1, as outlined below, and at least one of criteria 2-4.

1. Positive history of an IgE-mediated type 1 hypersensitivity reaction AND
2. A positive blood test result (>=0.35 kU/l) OR
3. If blood testing is negative, positive skin prick test (>-3.0 mm) OR
4. If both blood and skin prick testing are negative, a positive double blind placebo controlled food challenge

The Mayo Foundation, Olmsted Medical Center and University of Minnesota institutional review boards approved the study.

7.3.2 Study Population

Subjects who did not provide research authorization, whose residency information is not available, who are not residents of Olmsted County at the time of diagnosis and/or who do not meet the diagnostic criteria for peanut allergy were excluded from the data set. The author reviewed the complete medical records of each potential subject to confirm a case of peanut allergy and collect information regarding demographics, health status, presenting symptoms, and outcome. 171 (31.3%) met the criteria for an incident case of peanut allergy out of a group of 547 potential cases. 23 cases were eliminated due to missing data regarding the exposure of interest, IgE level. The final analytic sample consists of 148 cases. All data were abstracted using an electronic data collection form.

7.3.3 Variables

The variables considered in the analysis are the following.

Serum IgE Level

IgE level is the exposure variable of interest and indicates the peanut-specific serum IgE class level determined at the patient's first visit to a physician following their first reaction. IgE was represented as a categorical variable with the break down of classes 1-3 and 4-6, which was decided upon due to sample size and clinical considerations. This classification allowed for sufficient cases to exist in each cell, which enabled a reliable statistical evaluation. Further, this categorization creates an even breakdown that enables a more natural interpretation of results in terms of low vs. high IgE levels.

Reaction Severity

Reaction severity, the outcome of interest, represents the severity of the child's worst reaction to peanut before consulting a physician. The variable is dichotomous, mild versus moderate/severe. A mild reaction is defined as one in which the child experienced cutaneous symptoms only. A moderate/severe reaction is defined as an anaphylactic reaction, which is a systemic type 1 hypersensitivity reaction in which a child may experience a range of potential symptoms from moderate to severe. There are three criteria each reflecting a different clinical presentation and range of potential symptoms, which constitute anaphylaxis[54]. Anaphylaxis is considered highly likely when any one of the following criteria is fulfilled:

Criterion 1- Acute onset of an illness involving the skin, mucosal tissue or both (i.e. generalized hives, pruritus or flushing, swollen lips, tongue, uvula) and respiratory compromise or reduced blood pressure.

Criterion 2- Two or more of the following that occur after exposure to a likely allergen

- Involvement of the skin-mucosal tissue (generalized hives, itch, flush, swollen lips, tongue, uvula)
- Respiratory compromise
- Reduced blood pressure
- Persistent gastrointestinal symptoms

Criterion 3- Reduced blood pressure after exposure to a known allergen for that patient

- In infants and children, reduced blood pressure is defined as low systolic blood pressure or greater than thirty percent decrease in systolic blood pressure

Age

Age represents the child's age at first diagnosis. Age was modeled as a

categorical variable, 0-2 years and 3-17 years. Despite being a more powerful representation, age was not modeled as a continuous variable because the variable is not linear.

Year

Year represents the year of first diagnosis. Year was modeled as a categorical variable representing years 1999-2001, 2002-2004 and 2005-2007. Modeling year of first diagnosis as a categorical variable 1999-2007 led to large confidence intervals and unreliable estimates, thus the years were grouped to allow for an adequate sample size in each strata enabling a more sound statistical evaluation. Further, a three-level categorization allows an even distribution of years in each group, which enables a more natural interpretation of data.

Gender

Gender was modeled as a dichotomous variable, male and female.

Number of Atopic Diseases

The variable, number of atopic diseases, is a dichotomous variable, indicating a higher burden as compared to a lower of atopic disease. A low burden of disease is defined as peanut allergy only or peanut allergy and one other atopic disease, eczema, allergic rhinitis or asthma. A higher burden of disease is defined as a peanut allergy plus 2 or 3 other atopic conditions.

7.3.4 Statistical Analysis

Participant characteristics of all cases were descriptively summarized by means and standard deviations for continuous variables or by frequencies for categorical variables. These characteristics included IgE class, age of first diagnosis, year of first

diagnosis, gender, number of atopic conditions and reaction severity. In addition, the difference between those that experienced moderate/severe as compared to mild reactions was compared statistically for all patient characteristics. Specifically, the median test, Wilcoxon-rank sum, was utilized to test the difference between two medians for categorical variables and the t test was used to analyze the difference between two means for continuous variables. When testing the difference between two proportions or percentages for categorical characteristics, the chi square test was utilized.

Logistic regression was used to estimate the odds of a moderate/severe versus a mild reaction, defined as the worst reaction prior to seeing a physician, according to peanut-specific serum IgE level, as determined at the patient's initial visit. The following covariates were selected a priori for examination in the analysis: age at diagnosis, year of first diagnosis, gender and number of other atopic diseases. Variables were selected based on previously published reports of independent associations with reaction severity.

In order to create the best fit model variables were sequentially added individually to the model along with the exposure variable of interest. Variables with significant independent associations with the outcome of interest, reaction severity, were retained based on the *P* values. The potential for effect modification was explored by adding the variable age to the model as an interaction term. As each variable was added to the model, confounding was evaluated by comparing the parameter estimate for the exposure variable among the models run with and without the potential confounder. If the exposure parameter estimate differed by greater than 10%, the variable was considered a confounder. If the confounding variable was not significant, it was still retained in the model to account for its confounding effects.

7.4 Results

As seen in Table 1, the median age of first diagnosis was 18 months or 1.5 years old. The majority of children were male (69.6%) and had two or three other atopic conditions in addition to peanut allergy (54.7%). The most common IgE class level at first diagnosis in this sample was a class 3 (33.1%). The number of incident cases of peanut allergy increased overall from 1999-2007 with 4.73% of diagnoses in 1999 and 18.9% in 2007.

As seen in Table 2, which displays case characteristics stratified by reaction severity, 75.7% of reactions were mild and 24.3% were moderate/severe. There exists a significant crude difference between reaction severity levels according to IgE class levels 4-6 as compared to 1-3 (p=0.02) and age of first diagnosis, 3-17 years as compared to 0-2 years (p=0.04).

In examination of the univariate associations, as seen in Table 3, a higher IgE class level (OR=2.5 95% CI 1.16-5.40) indicated a significant association with reaction severity. An assessment of confounding indicated that age of first diagnosis confounds the association between IgE and reaction severity and thus, the variable was retained in the final model.

The multivariable analysis, as shown in Table 4, indicated a non-significant 2.15 greater odds for a moderate/severe reaction among IgE class levels 4-6 at first diagnosis as compared to levels 1-3 after adjustment for age at first diagnosis (OR=2.15 95% CI 0.96-4.80). In addition, the multivariable analysis indicated a non-significant 1.97% greater odds for a moderate/severe reaction among those with an age at first diagnosis of 3-17 years months as compared to 0-2 years after adjustment for IgE class level at first

diagnosis. (OR=1.97 95% CI 0.81-4.79). In sum, neither the exposure variable, IgE level, nor the covariate, age at diagnosis, were significantly associated with the outcome of interest, reaction severity, in this sample.

7.5 Discussion

The results from study three indicated a 2.15 non-significant greater odds for a moderate/severe reaction among IgE class levels 4-6 at first diagnosis as compared to levels 1-3 after adjustment for age at first diagnosis (OR=2.15 95% CI 0.96-4.80). Thus, in this sample, peanut-specific IgE level is not significantly associated with reaction severity. The results are consistent with an inconclusive literature [66,67,72,73,74,75,76,77,79,80].

There are a few possible reasons that the results of study three are not in accordance with the etiologic assumption that higher IgE levels are associated with a more severe reaction. Severity of reaction may be dependent upon many factors including dose of allergen, and health status of the child, in particular, the presence of asthma at the time of ingestion. This has been studied with inconclusive results [73,74,75,80]. Due to the nature of this study and data collection, this information is not available and thus, the study was unable to account for these potential covariates in the analysis and thus, may have created bias.

Further, it is not understood, due to a paucity of research, if peanut-specific IgE levels fluctuate and if so, what causes the variations. Factors that have been considered, with inconclusive results, as a cause of fluctuations include age, repeated exposure to peanut, subsequent reactions and health related factors. These potential confounding variables were not considered in the analysis because the information has not been clarified.

As a cross sectional study, a temporal relationship cannot be established. Therefore, the results are only suggestive of a causal relationship. In this study, cases visited their physician and received an IgE test following the referent reaction, though this exact time period is not known. In examining the association between IgE and reaction severity, the timing of when the IgE test is taken in relation to the referent reaction affects interpretation of results. The literature reports studies that examine the association between IgE and reaction severity in which the reaction and testing occur on the same day and instances in which the referent reaction occurs prior to the test. The results of studies examining both scenarios produced inconclusive results [67,66,73,74,76,77,79 72,75,80].

Lastly, with 148 cases, study three is underpowered to detect a difference in those with moderate/severe as compared to mild reactions based on IgE test level. Thus, a statistical association may exist that was not identified due to the small number of cases in this study. In sum, as a result of the potential for unknown confounding, uncertainty regarding temporality and small sample size, the association between IgE level and reaction severity remains unclear.

7.6 Strengths

The study has a number of strengths. First, this is an important unanswered question in our literature. Secondly, the research takes advantage of the population-based medical data available through the Rochester Epidemiology Project resources. This linked medical records system enables access to accurate and detailed clinical and laboratory data over many years, which are not typically available in other databases or research settings. The REP captures the bulk of residents living in Olmsted County and the majority of patients receiving care at one of the REP facilities consented to inclusion

of their medical records into the REP database. Thus, the patients captured in this medical record review are representative of the Olmsted County population.

7.7 Limitations

There are a few limitations of this study. The potential for misclassification bias exists as a result of errors in the categorization of either exposure or outcome status. Since data were collected from medical records, it is expected that any misclassification is non-differential and thus, would only increase the similarity between the two groups. Furthermore, the definition of cases, peanut allergic individuals, and reaction severity, are based upon guidelines for the diagnosis and management of food allergy in the United States, which minimized the likelihood for misclassification [65].

Secondly, the potential for unknown confounding exists. Severity of reaction may be dependent upon many factors in addition to the exposure variable of interest, IgE level, including dose of allergen, and health status of the child at the time of ingestion, though this has been studied with inconclusive results [73,74,75,80]. Further, it is not understood, due to a paucity of research, if peanut-specific IgE levels fluctuate and if so, what causes the variations. Due to in uncertainty in the literature, these potential confounding variables were not included in the analysis.

The analysis only included cases of peanut allergy that were diagnosed from 1999-2007 at a REP facility. This study excluded those patients in whom diagnosis occurred prior to this time frame or at an outside facility. There is the small possibility that serum IgE levels may differ between the patients included and excluded in the study. This potential was checked by comparing IgE level of cases that were included and

excluded from the analysis. The median IgE class level for both sets of cases is the same (median 3.0 IQR 2-4).

As a cross sectional study, a temporal relationship between exposure and disease cannot be established. Therefore, the data are only suggestive of a causal relationship. Lastly, Olmsted County is predominantly white and middle class, thus the findings may not be generalizable to all other populations.

7.8 Conclusions

Study results indicate that IgE level is not significantly associated with reaction severity, which is consistent with several studies [72, 75,79] yet inconsistent with a number of other studies [62,67 ,73,74, 76,77,80]. The findings of this study contribute to a conflicting literature and as such additional research that addresses the study limitations are needed to clarify this relationship. If future studies support the notion of an association between IgE level and reaction severity, clinicians may be able to use IgE level as indicator for reaction severity. However, at this time, this study does not support this notion.

An understanding of whether peanut-specific IgE level has clinical relevance regarding reaction severity is important to clinicians from two standpoints. First, elucidating this association will further the utility of the current diagnostic test, peanut-specific IgE testing, from solely being able to predict one's likelihood to react upon exposure. Secondly, clinicians will be able to advise and manage patients appropriately in regards to the association between IgE level and reaction severity. Specifically, physicians may discuss the inconclusive results regarding the relationship between IgE level and reaction severity in the context of study limitations. For instance, clinicians

may mention that this study indicated a 2.0-fold non-significant greater likelihood for a severe reaction among those with IgE levels 4-6.

7.9 Tables

Table 1: Characteristics of Children with Incident Diagnoses of Peanut Allergy from 1999-2007

Number of cases (n)	148
Age of diagnosis in months (median and IQR)	18 (13.0-24.0)
Gender n (%)	
Male	103 (69.6)
Female	45 (30.4)
Number of atopic conditions (0/1 vs. 2/3) n (%)	
0/1 (Just peanut or peanut plus 1 other)	67 (45.3)
2/3 (Peanut plus 2 or 3 other)	81 (54.7)
Reaction severity n (%)	
Mild	112 (75.7)
Moderate/Severe	36 (24.3)
IgE Class Level n (%)	
Class 1	8 (5.41)
Class 2	41 (27.7)
Class 3	49 (33.1)
Class 4	21 (14.2)
Class 5	11 (7.43)
Class 6	18 (12.2)
Diagnosis Year	
1999	7 (4.73)
2000	6 (4.05)
2001	17 (11.5)
2002	10 (6.76)
2003	9 (6.08)
2004	24 (16.2)
2005	21 (14.2)
2006	26 (17.6)
2007	28 (18.9)

Table 2: Patient characteristics stratified by reaction severity (n=148)

	Reaction Severity		
	Mild n=112, 75.7%	Moderate/Severe n=36, 24.3%	p value
Age n (%)			p=0.04*
0-2 years	93 (83.0)	24 (66.7)	
3-17 years	19 (17.0)	12 (33.3)	
Gender n (%)			p=0.69
Male	35 (31.3)	10 (27.8)	
Female	77 (68.8)	26 (72.2)	
Year Diagnosis n (%)			p=0.82
1999-2001	22 (19.6)	8 (22.2)	
2002-2004	34 (30.4)	9 (25.0)	
2005-2007	56 (50.0)	19 (52.8)	
Number of atopic conditions n (%)			p=0.38
1-2	53 (47.3)	14 (38.9)	
3-4	59 (52.7)	22 (61.1)	
IgE n (%)			p=0.02*
1-3	80 (71.4)	18 (50.0)	
4-6	32 (28.6)	18 (50.0)	

* Indicates significant difference between groups
Note: P value from chi-square test

Table 3: Univariate Association between Potential Risk Factors and Reaction Severity (n=148)

	OR	95% CI
Age at diagnosis [1]	1.97	(0.81-4.79)
Gender [2]	1.11	(0.48-2.59)
Number of atopic conditions [3]	1.14	(0.51-2.54)
Year of diagnosis [4]		
2002-2004	0.78	(0.26-2.39)
2005-2007	1.25	(0.45-3.46)
IgE [5]	2.5	(1.16-5.40) *

* Indicates $p<0.01$
[1] Reference group= 0-2 years
[2] Reference group= Male
[3] Reference group= Only peanut allergic or peanut and one other atopic condition (allergic rhinitis, eczema, asthma)
[4] Reference group= 1999-2001
[5] Reference group= IgE classes 1-3

Table 4: Multivariable Association between Risk Factors and Reaction Severity (n=148)

	OR	95% CI
Age [1,a]	1.97	(0.81-4.79)
IgE [2]	2.15	(0.96-4.80)

* Indicates $p<0.01$

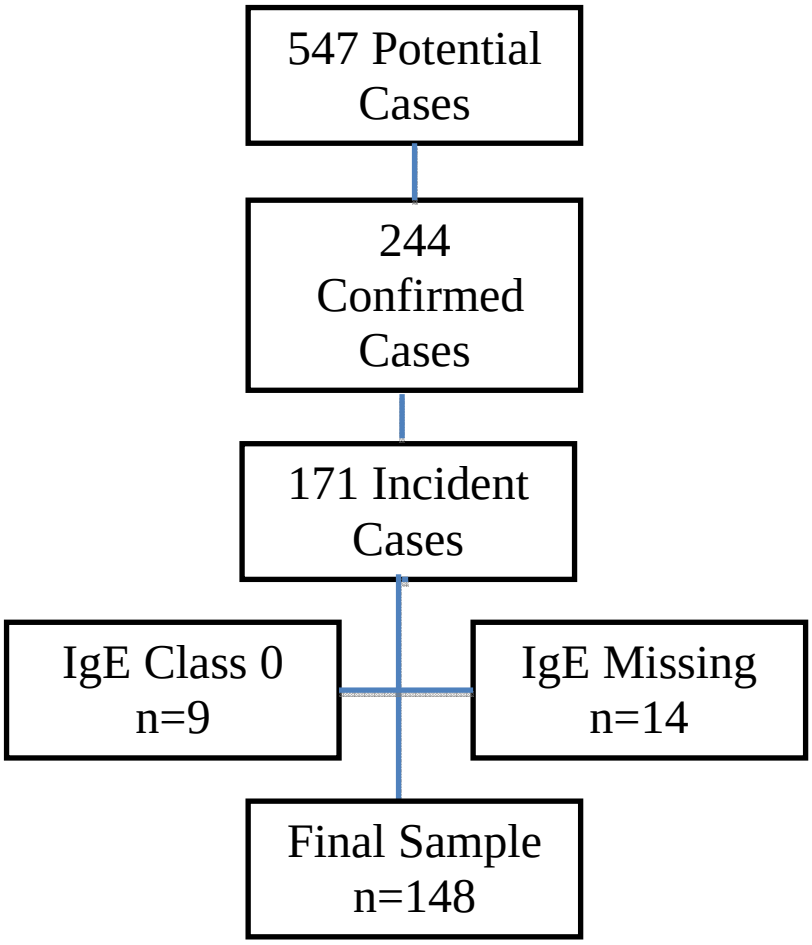
[1] Reference group= 0-2 years

[2] Reference group= IgE classes 1-3

[a] Non-significant covariate yet, a confounder of the association

7.10 Figures

Figure 1: Sample Size Waterfall



8.0 Overall Summary

Synopsis: Current state of knowledge regarding peanut allergy

Peanut allergy is a major health concern worldwide, particularly in developed countries such as the United States in which 1% of the population is allergic. Reactions to peanuts are often severe and the allergy tends to be life-long [1]. Peanut allergy accounts for the majority of fatal and near-fatal anaphylactic reactions to food. Peanut allergy causes a significant burden for the allergic individual and their families. Unfortunately, there are not any known treatments to prevent or treat the disease.

Prevalence estimates and the question as to whether the number of children suffering from peanut allergy is increasing over time are up for debate due to conflicting published reports. Despite this fact, several prevalence studies suggest a dramatic increase in estimates over the last two decades. Specifically, studies from the United States report a significant increase in sensitization to peanut from 0.4% in 1997 to 1.4% in 2008 [60]. A study in the United Kingdom reports a significant increase in clinical allergy from 1989 (0.5%) to 1996 (1.4%) and a non-significant decrease in 2003 (1.2%) [58,59]. Further, a study conducted in Canada reports a non-significant increase in clinical allergy from 2002 to 2007 from 1.34% to 1.62%. Lastly, an Australian study reports a significant 2.5- fold increase in incidence rates from 1995 (0.47%) to 2007 (1.15%) [61].

The results from these studies are difficult to compare and interpret in the aggregate due to reliance on differing criteria to define peanut allergic cases as a result of reliance on self-report of allergy or evidence of sensitization alone. In addition to the difficulty in obtaining accurate prevalence estimates and subsequently evaluating a

potential increase in disease, the reasons for the reported rise in prevalence estimates are not understood. Many potential factors have been investigated with inconclusive results including food processing methods, maternal/infant diet, hygiene hypothesis, non-oral exposures and an increase in diagnoses of mild allergy. Adding to the stress created by not knowing what is causing our children to become allergic to a potentially deadly allergen at increasing numbers is the fact that the only available treatment is vigilant avoidance of peanut-containing foods and educating caregivers on how to recognize and treat acute reactions. Further, there are not any proven techniques to reverse the allergy. Treatments are under investigation with exciting yet inconclusive results including most notably oral immunotherapy.

Synopsis: How this dissertation contributes to the current state of knowledge

This dissertation provides an estimation of the prevalence of peanut allergy in 2007 and incidence rates of peanut allergy diagnoses from 1999-2007 among Olmsted County children. The definition of peanut allergic cases was based upon the current medical guidelines to reduce the potential for misclassification. The study utilized the Rochester Epidemiology Project as a means to identify peanut allergic cases by confirming history of a physician-documented type 1-hypersensitivity reaction in conjunction with positive diagnostic testing. This is significant because many of the published studies relied on self-report of allergy, which is known to result in inflated estimates. In sum, the ability to collect all necessary data to make a diagnosis based on medical guidelines through a complete medical records database is a major strength in this study.

Secondly, there is only one published study that calculates the incidence rate of peanut allergy diagnoses over time. The other studies estimate the prevalence at one time point and in some instances conduct follow-up studies to assess the change in prevalence over time. Study one of this dissertation contributes to the literature by estimating the incidence rate of new peanut allergy diagnoses over time, 1999-2007, and evaluating whether the increase is statistically significant. Further, studies that assess changes in prevalence or incidence over time, discuss the fact that it is unclear whether the reported increase is accurate or just a reflection of an increase in diagnoses of mild diagnoses in recent years. This study examines this question by assessing whether the number of incident diagnoses over time differs by reaction severity.

Studies two and three contributed to the literature by examining whether peanut-specific IgE level has clinical relevance regarding tolerance acquisition and reaction severity, which are two of the most frequently asked questions regarding a child's allergy. The data from these studies contributes to a conflicting literature base and will aid in providing clinicians with the data to help counsel patients in regards to reaction severity and their likelihood for developing tolerance with confidence based on peanut specific serum-IgE levels.

Synopsis: How these studies contribute to the literature from an etiologic, public health and clinical perspective

Etiologic Basis for Study 1

There are numerous theories attempting to answer the question as to why allergic conditions, including peanut allergies, are more prevalent in children today as compared

to a generation ago. It is likely that both genetic and environmental influences affect the development of peanut allergy. Theories under investigation include the hygiene hypothesis, maternal and infant diet, including increased consumption of peanuts by mothers and young children, early feeding when the immune system is immature, sensitization in utero or through breast milk, food processing methods, in particular the allergenicity of roasted forms, and non-oral exposure including the use of topical ointments containing peanut. There also exists a debate whether there truly is an increase in peanut allergy diagnoses or if more cases of mild reactions are being captured as a result of heightened awareness, improved surveillance, changes in diagnostic criteria, health insurance, or food availability and not truly a reflection of an increase in the rate of disease.

The Hygiene Hypothesis, a theory that receives much support, postulates that a lack of early childhood exposure to infectious agents increases susceptibility to allergic diseases by modulating immune system development [28, 38,30]. The United States, among other westernized countries, is an industrialized society concerned with cleanliness and germ prevention. These assumptions regarding germ prevention and the concurrent rise in the incidence rate of diagnoses in the U.S. provides support for the hygeine hypothesis. Although intriguing, no cause has been proven at this time.

In addition to an increase in rates over time, it is established that rates of atopic diseases differ according to gender and age [42, 62]. Gender differences in atopic disease, assessed as total serum IgE levels, have been reported to be consistent across the lifespan, with levels in females being lower than those in males [62]. The physiological pathway

leading to such gender differences is reported to be a result of immune dimorphism, the term given to differences in immune responses and regulation between the sexes [62].

Lastly, children aged 0-2 years are more susceptible to food allergies as a result of their immature immune system, which is more likely than an older child's to deem certain food proteins as foreign and launch an allergic reaction [42]. Further, the introduction of new foods typically begins in this age range and if a reaction were to occur, it would be expected to surface at this time. [42, 62]

Study 1 Results

The results from study one indicate that the prevalence of peanut allergy in 2007 is 0. 59% and the number of incident cases of peanut allergy increased overall from 1999-2007 with 5.9% of new diagnoses in 1999 and 17.7% in 2007. The overall, age and gender adjusted and standardized, rates increased 1.7-fold from 3.84 cases per 10,000 children in 1999-2001 to 6.53 cases per 10,000 children in 2005-2007. Further, females had a statistically significant 82% lower rate of peanut allergy diagnosis as compared to males. As compared to children aged 0-2 years old at first diagnosis, older children aged 3-17 years had a 99.9% statistically significantly lower rate of peanut allergy diagnosis. Lastly, there is not a significant difference in the number of children diagnosed having had mild as compared to moderate/severe reactions over time, 1999-2007 (p=0.91).

The increase in rates of peanut allergy diagnosis among Olmsted County children experiencing both mild and moderate/severe reactions over time confirms that the increase is likely accurate and not a result of an increase in diagnoses of mild cases over time and therefore, provides support for the investigation into potential causes for this

increase. It is unlikely that factors such as heightened awareness, improved surveillance, changes in diagnostic criteria, health insurance or food availability are solely responsible for the increase in rates. Lastly, the higher rates among males and children aged 0-2 are consistent with biological theory, timing of age of first introduction of foods and other reported studies.

Etiologic Basis for Studies 2 and 3

Immunoglobulin E, IgE, is the antibody released by the immune system when an allergic reaction takes place after exposure to a food in which the host is allergic [1]. Antibodies are specific to each type of foreign substance [1]. An immunoglobulin test is conducted to determine what the person is allergic to and to measure the level of immunoglobulins in blood [1]. Researchers determined predictive values of IgE levels for certain foods including peanuts. If a person's level is higher than a certain value for that food, there is a 95% chance the person will have an allergic reaction if they ingest that food [65].

Individuals with a stronger Th2 immune response produce more IgE [1]. It is known that an individual's peanut-specific IgE level is associated with their likelihood to react upon exposure and individuals have an increasing probability of clinical reactivity with increasing level of peanut-specific IgE values. It would stand to reason that the elevated IgE in the blood would cause a more severe reaction because there is more IgE for the allergen to cross-link on mast cell cells and basophils leading to the release of the mediators responsible for a stronger and more robust physiological response. Further, it

seems probable that individuals who are more likely to produce high levels of IgE would have a decreased ability to outgrow the disease.

Study 2 Results

The results from study two indicate that 16.4% of children developed tolerance to peanuts in this sample, which is consistent, yet slightly less than reported estimates that range from 15.0%-20.0% [23,27,36,69]. As a retrospective cohort study, it is possible that cases that develop tolerance may have been lost to follow-up. Further, results from study two indicate lower IgE levels (1-3) are associated with tolerance. Specifically, the analysis indicates that those children with an IgE class level at first diagnosis of 4-6 are 91% less likely to develop tolerance as compared to those with a level 1-3 after adjustment for number of atopic conditions. The results of this study are in accordance with the etiologic assumption regarding IgE levels and tolerance.

Study 3 Results

The results from study three indicated a 2.15 non-significant greater odds for a moderate/severe reaction among IgE class levels 4-6 at first diagnosis as compared to levels 1-3 after adjustment for age at first diagnosis (OR=2.15 95% CI 0.96-4.80). Thus, in this sample, peanut-specific IgE level is not significantly associated with reaction severity, which is consistent with some yet not all studies [67,66,73,74,76,77,79 72,75,80].

The results of study 3 are not in accordance with the etiologic assumption that higher IgE levels are associated with a more severe reaction. There are a few possible reasons for this discrepancy. Severity of reaction may be multi-factorial and dependent upon many factors including dose of allergen, and health status of the child, in particular,

the presence of asthma at the time of ingestion. This has been studied with inconclusive results [73,74,75,80]. Due to the nature of this study and data collection, this information is not available and thus, the study was unable to account for these potential covariates in the analysis and thus, may have created bias.

Further, it is not understood, due to a paucity of research, if peanut-specific IgE levels fluctuate and if so, what causes the variations. Factors that have been considered, with inconclusive results, as a cause of fluctuations include age, repeated exposure to peanut, subsequent reactions and health related factors. As a result, these potential confounding variables were not included in the analysis.

Lastly, with 148 cases, study 3 is underpowered to detect a difference in those with moderate/severe as compared to mild reactions based on IgE test level. Thus, a statistical association may exist that was not identified due to the small number of cases in this study.

Clinical and Public Health: Study 1

As the numbers of peanut allergic children rise, public health plays an increasingly important role in awareness, safety, and educational efforts in high-risk setting such as schools and restaurants. Education of teachers, children and families on prevention of an inadvertent reaction and proper management of symptoms is vital to child safety. Further, public health policy plays a pivotal role in regards to labeling laws and bans of peanuts in high-risk setting such as planes. Lastly, confirmation of an increase in rates provides support for further research into etiology, prevention and therapeutic techniques, all of which public health expertise is paramount.

In regards specifically to this dissertation, utilization of the REP allowed an accurate estimation of the burden of disease in Olmsted County children, which enables medical providers and public health officials to adequately prepare for patient care for the 0.59% of children with peanut allergy in the population and the potential increase in numbers over time, as indicated by the increase in rates since 1999.

Clinical and Public Health: Studies 2 and 3

Studies two and three examined whether peanut-specific IgE level has clinical relevance regarding tolerance and reaction severity. An understanding of these associations is important to clinicians from two standpoints. First, elucidating these associations will further the utility of the current diagnostic test, peanut- specific IgE testing, from solely being able to predict one's likelihood to react upon exposure. Secondly, clinicians will be able to advise and manage patients appropriately based on their serum IgE levels in regards to reaction severity and likelihood to develop tolerance. Further, this information is important to patients as an understanding of factors influencing one's reaction severity is important. Lastly, knowledge of one's potential to outgrow peanut allergy based on IgE levels will provide realistic expectations for patients and families.

Clinical Summary: Studies 1, 2 and 3

The results from all three studies provide important information to clinicians in terms of counseling. Physicians can inform patients that their condition, peanut allergy, is becoming more common and that 0.59% of children in Olmsted County and 1.0% in the United States are estimated to have a peanut allergy. Further, 16.4% in Olmsted

County and 15.0-20.0% in the United States are expected to acquire tolerance. In addition, younger age, 0-2 years, and male gender are risk factors for disease. Physicians may counsel patients on their tolerance prognosis based upon peanut-specific IgE level in the context of discussing research limitations. Specifically, physicians can relay the news to patients with IgE class levels 1-3 that they are more likely than those with class levels 4-6 to acquire tolerance. Further, physicians may discuss the inconclusive results regarding the relationship between IgE level and reaction severity in the context of study limitations. For instance, clinicians may mention that this study indicated a 2.0-fold non-significant greater likelihood for a severe reaction among those with IgE levels 4-6.

Clinical and Public Health: A Screening Program

A screening test is a test performed in the absence of symptoms to separate those with a high probability of developing or having a condition from those with a low probability of having or developing the condition [83]. Due to the increasing numbers of children who are diagnosed with peanut allergy and the potential severity of a reaction, it is reasonable to consider whether a screening program to identify children at risk of having and/or developing peanut allergy prior to the first time they eat peanuts and subject to a reaction is a prudent idea.

There are several factors to consider in determining whether early detection of sensitization to peanuts, defined as a positive peanut-specific serum IgE test, is beneficial for patients [83]. A child may become sensitized to peanut without any known peanut ingestion through several potential means including in utero, via skin or ingestion through cross-contamination. To be considered allergic to peanuts, a child must have

evidence of sensitization through positive testing and have experienced a type-1 hypersensitivity reaction from peanuts. A child without a history of a reaction, but who has evidence of sensitization is considered sensitized to peanut, not necessarily allergic.

It is believed by clinicians that evidence of sensitization, in the absence of a history of peanut ingestion, indicates a greater likelihood for a child to be allergic and/or develop peanut allergy (personal communication, Dr. Matthew Rank)[84]. For instance, when considering two children, both of whom have limited or no known exposure to peanut, one who has detectable serum IgE to peanut and one who does not, the child with detectable antibody is thought to be at an increased risk to develop peanut allergy. This relationship has not been elucidated clearly in the literature and the increase in risk has not been quantified.

As a result of the lack of empirical support, screening practices vary among clinicians because there is not one standard approach at this time. Many clinicians test children considered high risk, those with older siblings with peanut allergy, for sensitization. A positive test, however, does not equate with clinical allergy. Thus, if the child's serum IgE level is low and as result, not clear-cut, an in-office food challenge is considered to clarify the diagnosis.

In addition to making decisions regarding screening on an individual level, would it be wise to institute an overall screening program for children? First, peanut allergy is a rare disease, affecting on average 1% of children. A large proportion of children who screen positive will have false positive results even if the test has high specificity and sensitivity due to the rarity of the disease [83]. In addition, children would not benefit from

early detection in the sense of having access to medical treatments that may help in curing or preventing the development of peanut allergy as none exist.

However, early identification of sensitization would alert the child and their caregivers to the child's allergic state and/or propensity to become allergic upon ingestion, thereby warning the individuals of the potential danger of consuming peanuts and likely preventing an adverse reaction. This benefit is significant due to the potentially severe nature of reactions to peanut.

In sum, a screening program for peanut allergy implemented among all children is not sensible as the yield would be low, as a result of the rarity of the disease, and the false-positives high. A peanut allergy diagnoses has a significant impact on the quality of life of patients and families due to the need for vigilance regarding exposure to peanut, the potential for cross-contamination and awareness of proper medical management in the event of a reaction. Furthermore, evidence of sensitization does not equate with clinical peanut allergy and the literature indicates that estimates of peanut allergy based on sensitization are much higher than those based on clinical allergy. For these reasons, a screening program resulting in a large number of false diagnoses would be detrimental.

However, there are clear benefits associated with screening for sensitization among children considered high-risk, a subset of children in which the prevalence of peanut allergy is much higher than the general population, including the ability to confirm allergy through an in-office food challenge, as indicated by the clinician, and not through a potentially severe reaction in the community.

Synopsis: Future Directions

Additional studies that utilize diagnostic criteria based on current clinical guidelines are needed to validate the prevalence and incidence rate estimates and to confirm that the incidence rates are increasing among children with true clinical peanut allergy regardless of severity of initial reaction. Further, research must continue to explore the cause for the increase in peanut allergies. Once the etiology of the increase is clarified, steps can be made towards prevention of disease. Lastly, research relating to therapeutic techniques must continue with the goal of finding a cure for the increasing number of children suffering from peanut allergies.

The findings of studies two and three, the association between IgE level, tolerance and reaction severity, contribute to a conflicting literature and as such additional studies are needed that address the limitations of these studies and others. Specifically, research assessing the association between IgE level and tolerance must define tolerance criteria based on current guidelines and aim for complete follow-up to ensure proper classification and enumeration of tolerance status. In addition, studies with epidemiologic designs that make the temporal relationship between the timing of IgE testing and the referent reaction clear are needed to elucidate the association between IgE level and reaction severity.

Further, little is known as to factors that may affect peanut-specific IgE level and reaction severity. These factors may affect the association between IgE level and reaction severity and thus, must be evaluated as part of the analysis. Therefore, future studies to identify these factors, if any, are necessary. If these limitations are addressed and results support the association between IgE level, tolerance and/or reaction severity,

clinicians may be able to discuss reaction severity and/or tolerance prognosis with confidence and provide important answers to patients.

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